116. P. D. McCrary, L. A. Flores, G. Chatel, and R. D. Rogers, "Evaluating Ionic Liquids as Hypergolic Fuels: From Reactive Nanomaterials to Trigger Additives," Presented by P. D. McCrary before the Energetic Ionic Liquid Mini-Symposium (May 21-22, 2013), Air Force Research Laboratory, Edwards Air Force Base, CA, No Abstract.
117. L. A. Flores, P. D. McCrary, G. Chatel, O. Andreea Cojocaru, and R. D. Rogers, "Molecular Characteristics and Interactions Leading to Liquid Clathrate Behavior," Presented by L. A. Flores before the Energetic Ionic Liquid Mini-Symposium (May 21-22, 2013), Air Force Research Laboratory, Edwards Air Force Base, CA, No Abstract.
118. R. D. Rogers and S. P. Kelley, "Supramolecular chemistry in the liquid state: What can halogen bonding offer ionic liquids?" Presented by R. D. Rogers before the 49th Midwest Regional Meeting of the American Chemical Society (November 12-15, 2014), Columbia, MO, Abstract 384.
119. R. D. Rogers, "Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists," Presented by R. D. Rogers before the 3rd Annual *Sustainable Innovation through Green Chemistry* Workshop and Case Competition Schedule (January 16-17, 2015), McGill University, Montreal, QC, Canada, No Abstract (Invited Keynote).
120. R. D. Rogers, "Innovation is the Gateway to the Biomass Biorefinery and Ultimately A Sustainable Bio-based Economy," Presented by R. D. Rogers before the Quebec-Ontario Biotech Meeting (May 21-22, 2015), McGill University, Montreal, QC, Canada, No Abstract (Invited Keynote).
121. R. D. Rogers, "What is an Appropriate Academic Business Model to Drive Commercialization of Sustainable Technology?" Presented by R. D. Rogers before the Science for a Sustainable Society Symposium (January 26-27, 2016), McGill University, Montreal, QC, Canada, No Abstract (Invited Keynote).

E. Seminars:

1. "C_1 Chemistry in Group IVB - Some Structural Aspects," Presented by R. D. Rogers at Bell Laboratories, Murray Hill, NJ, on 8/15/83.
2. "Structural Investigations of New Pentamethylcyclopentadienyl Derivatives of Group IVB," Presented by R. D. Rogers at Northwestern University, Evanston, IL on 2/3/84.
3. "Early Transition Metal Chemistry: A Structural Point of View," Presented by R. D. Rogers at the Instituto Venezolano de Investigaciones Cientifica, Caracas, Venezuela on 8/15/84.
4. "Structural Organometallic Chemistry of the Early Transition Metals," Presented by R. D. Rogers at Fisk University, Nashville, TN on 3/18/85.
5. "Structural Organometallic Chemistry of the Early Transition Metals," Presented by R. D. Rogers at Tuskeegee Institute, Tuskeegee, AL on 3/20/85.
6. "Structural Organometallic Chemistry of the Early Transition Metals," Presented by R. D. Rogers at The University of Alabama, Tuscaloosa, AL on 3/21/85.
7. "Crown Ether Coordination in the f-Element Series," Presented by R. D. Rogers at the University of Illinois at Chicago, Chicago, IL on 2/18/86.
8. "Early Transition Metal Chemistry - A Structural Point of View," Presented by R. D. Rogers at Marquette University, Milwaukee, WI on 3/21/86.
9. "f-Element/Crown Ether Complexes, Structural Effects of Solvent and Water of Hydration Hydrogen Bonding," Presented by R. D. Rogers at Victoria University, Wellington, New Zealand on 7/24/87.
10. "f-Element/Crown Ether Complexes," Presented by R. D. Rogers at the University of Hawaii, Honolulu, HA on 8/26/87.
11. "f-Element/Crown Ether Complexes," Presented by R. D. Rogers at the University of Toledo, Toledo, OH on 10/14/87.
12. "Hydrogen Bonding in f-Element Complexes of Crown Ethers," Presented by R. D. Rogers at Ripon College, Ripon, WI, on 11/22/88.
13. "Crown Ether Complexation Chemistry of the Lanthanides," Presented by R. D. Rogers at Albany State College, Albany, GA, on 2/10/89.
14. "Crown Ether Complexation Chemistry of the Lanthanides," Presented by R. D. Rogers at Tuskeegee University, Tuskeegee, AL, on 2/13/89.
15. "f-Element/Crown Ether Complex Chemistry," Presented by R. D. Rogers at The University of Alabama, Tuscaloosa, AL, on 2/14/89.
16. "Crown Ether Chemistry of the Lanthanides," Presented by R. D. Rogers at Saint Mary's University, Halifax, Canada on 3/3/89.
17. "Coordination versus Hydrogen Bonding in Crown Ether Complexes of Hydrated f-Element Salts," Presented by R. D. Rogers at Dalhousie University, Halifax, Canada, on 3/3/89.
18. "Macrocycle Complexation Chemistry: The Toxic Metals (Cd, Hg, Tl, Pb, Bi) and Their Removal from the Environment," Presented by R. D. Rogers at Western Michigan University, Kalamazoo, MI, on 10/23/89.
19. "Macrocycle Complexation Chemistry: The Toxic Metals (Cd, Hg, Tl, Pb, Bi) and Their Removal from the Environment," Presented by R. D. Rogers at Rockford College, Rockford, IL, on 3/27/90.
20. "Structural Characterization of Light Atom Structures via X-ray Crystallography," Presented by R. D. Rogers at The University of Mississippi, Oxford, MS, on 4/10/90.
21. "The Toxic Metals and Their Removal from the Environment," Presented by R. D. Rogers at Illinois Benedictine College, Lisle, IL, on 4/19/90.
22. "Crown Ether vs. Polyethylene Glycol Complexation of Lanthanide Chlorides," Presented by R. D. Rogers at Indiana University, Bloomington, IN, on 2/28/91.
23. "Polyethylene Glycols as Ionizable Complexing Agents of Bi^{+3}," Presented by R. D. Rogers at Indiana University, Bloomington, IN, on 3/1/91.
24. "Investigations of Polyethylene Glycols as Complexing Agents and Liquid/Liquid Extraction Diluents for Bismuth," Presented by R. D. Rogers at Loyola University of Chicago, Chicago, IL, on 9/19/91.
25. "Polyethylene Glycols and Metal Ions: Structural Chemistry to Aqueous Biphasic Extraction," Presented by R. D. Rogers at the Universität Bayreuth, Bayreuth, Germany, on 6/23/92.
26. "Polyethylene Glycols: From Coordination Chemistry of Metal Cations to Unique Systems for Dissolved Metal Ion Separations," Presented by R. D. Rogers at the University of Groningen, Groningen, The Netherlands, on 7/2/92.
27. "Macrocycle Complexation Chemistry: Toxic Metals and Their Removal from the Environment," Presented by R. D. Rogers at Elmhurst College, Elmhurst, IL, on 11/18/92.
28. "Aqueous Biphasic Systems: New Systems for Metal Ion Extraction," Presented by R. D. Rogers at Los Alamos National Laboratory, Los Alamos, NM, on 5/26/93.
29. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Systems for Novel Metal Ion Separations," Presented by R. D. Rogers at the University of New Mexico, Albuquerque, NM, on 9/24/93.
30. "Structural Investigation of Cyclic and Acyclic Polyether Complexes - Cation Control of Coordination," Presented by R. D. Rogers at Valparaiso University, Valparaiso, IN, on 12/10/93.
31. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Systems for Novel Metal Ion Separations," Presented by R. D. Rogers at Loyola University of Chicago, Chicago, IL, on 4/14/94.

32. "The Effects of Polyethylene Glycol on the Coordination Sphere of Strontium: Are PEGs Useful in Sr^{2+} Extraction Technologies?" Presented by R. D. Rogers at Oak Ridge National Laboratory, Oak Ridge, TN, on 5/16/94.
33. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Systems for Novel Metal Ion Separations," Presented by R. D. Rogers at Union Carbide Corporation, South Charleston, WV, on 6/3/94.
34. "The Effects of Polyethylene Glycol on the Coordination Sphere of Strontium: Are PEGS Useful in Sr^{2+} Extraction Technologies?" Presented by R. D. Rogers at The University of Alabama, Tuscaloosa, AL, on 6/22/94.
35. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Ways to Separate Metal Ions," Presented by R. D. Rogers at Western Michigan University, Kalamazoo, MI, on 10/10/94.
36. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Ways to Separate Metal Ions," Presented by R. D. Rogers at the University of Wisconsin-Oshkosh, Oshkosh, WI, on 11/10/94
37. "Polyethylene Glycols: Coordination Chemistry of Metal Cations to Unique Systems for Metal Ion Separations," Presented by R. D. Rogers at the University of Sevilla, Sevilla, Spain, on 6/16/95.
38. "Polyethylene Glycols: Coordination Chemistry of Metal Cations to Unique Systems for Metal Ion Separations," Presented by R. D. Rogers at The University of Alabama, Tuscaloosa, AL, on 7/12/95.
39. "Polyethylene Glycols: Coordination Chemistry of Metal Cations to Unique Systems for Metal Ion Separations," Presented by R. D. Rogers at The University of Iowa, Iowa City, IA, on 9/13/95.
40. "Polyethylene Glycols: Coordination Chemistry of Metal Cations to Unique Systems for Metal Ion Separations," Presented by R. D. Rogers at The University of Wisconsin at Milwaukee, Milwaukee, WI, on 10/9/95.
41. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Technologies for Metal Ion Separations," Presented by R. D. Rogers at Argonne National Laboratory, Argonne, IL, on 1/29/96.
42. "Polyethylene Glycol-Based Aqueous Biphasic Systems: New Technologies for Metal Ion Separations," Presented by R. D. Rogers at Monash University, Clayton, Victoria, Australia, on 3/19/96.
43. "ABEC Resins: From Aqueous Biphasic Novelties to Selective Aqueous Biphasic Extraction Chromatography Resins for Metal Ions," Presented by R. D. Rogers at Mississippi State University, Starkville, MS, on 1/24/97.
44. "Green Chemistry in Separation Science," Presented by R. D. Rogers at the March meeting of the Alabama Section of The American Chemical Society, Birmingham, AL, on 3/20/97.
45. "ABEC Resins: From Aqueous Biphasic Novelties to Selective Aqueous Biphasic Extraction Chromatography Resins for Metal Ions," Presented by R. D. Rogers at the University of Alabama at Huntsville, Huntsville, AL, on 3/28/97.
46. "The SMART System at The University of Alabama: Experiences, Reflections, and Data," Presented by R. D. Rogers at the Siemens Area Detector Users Group Meeting (SADUG97), Athens, GA, on 4/19/97.
47. "Coordination Chemistry and Separations of Actinides," Presented by R. D. Rogers at Florida State University, Tallahassee, FL, on 4/24/97.
48. "Polyethylene Glycol-Based Aqueous Biphasic Systems and ABEC Resins for the Selective Removal and Recovery of Metal Ions," Presented by R. D. Rogers at the University of Birmingham, Birmingham, England, UK, on 5/21/97.
49. "Polyethylene Glycol-Based ABEC Resins for the Selective Removal of Technetium from Hanford Tank Wastes," Presented by R. D. Rogers at British Nuclear Fuels, Ltd., Preston, England, UK, on 5/22/97.
50. "Aqueous Biphasic Systems: New Technologies for Metal Ion Separations," Presented by R. D. Rogers at Queen's University, Belfast, Northern Ireland, UK, on 5/27/97.
51. "Clean Separation Technologies," Presented by R. D. Rogers at the University of New Hampshire, Durham, NH, on 7/24/97.
52. Clean Separation Technologies," Presented by R. D. Rogers at the University of Marburg, Marburg, Germany, on 9/26/97.
53. "Green Separation Science: Traditional Polymeric Supports to Crystal Engineered Inorganic Polymers," Presented by R. D. Rogers at Clemson University, Clemson, SC, on 10/1/97.
54. "Utilization of Polyethylene Glycol in Industrially and Environmentally Important Separations," Presented by R. D. Rogers at Union Carbide, South Charleston, WV, on 10/3/97.
55. "Clean Separation Technologies," Presented by R. D. Rogers at The University of Alabama (Chemical Engineering Department), Tuscaloosa, AL, on 10/9/97.
56. "Polyethylene Glycol-Based Aqueous Biphasic Systems and ABEC Resins for the Selective Removal and Recovery of Metal Ions," Presented by R. D. Rogers at the University of Tennessee at Knoxville, Knoxville, TN, on 2/5/98.
57. "Green Separation Science: Traditional Polymeric Supports to Crystal Engineered Inorganic Polymers," Presented by R. D. Rogers at Oak Ridge National Laboratory, Oak Ridge, TN, on 2/6/98.
58. "Green Separation Science: Traditional Polymeric Supports to Crystal Engineered Inorganic Polymers," Presented by R. D. Rogers at Tennessee Technological University, Cookeville, TN, on 2/19/98.
59. "Coordination Chemistry to Crystal Engineering," Presented by R. D. Rogers at the University of Puerto Rico, San Jaun, PR, on 4/6/98.
60. "Clean Separations Using Non-Toxic Aqueous Polymers: In Support of Vision 2020," Presented by R. D. Rogers in the J. Clarence Karcher Lecture series at the University of Oklahoma, Norman, OK, on 4/23/98.
61. "Environmentally Benign Liquid/Liquid Extraction Media for Metal Ion Separations: Aqueous Biphasic Systems and Room Temperature Ionic Liquids," Presented by R. D. Rogers at the University of Mississippi, Oxford, MS, on 12/4/98.
62. "Green Separation Science and technology: Using Environmentally Benign Liquid/Liquid Extraction Media for Metal Ion Separations: Aqueous Biphasic Systems and Room temperature Ionic Liquids," Presented by R. D. Rogers at the Exxon Research and Development Laboratories, Baton Rouge, LA, on 5/7/00.

63. "Green Separation Science and Technology: Using Environmentally Benign Liquid/Liquid Extraction Media, Aqueous Biphasic Systems and Room Temperature Ionic Liquids," Presented by R. D. Rogers at the University of South Alabama, Mobile, AL, on 5/21/99.
64. "Environmentally Benign Liquid/Liquid Extraction Media: Aqueous Biphasic Systems and Room Temperature Ionic Liquids," Presented by R. D. Rogers at Pacific Northwest National Laboratory, Richland, WA, on 10/7/99.
65. "Environmentally Benign Liquid/Liquid Extraction Media: Aqueous Biphasic Systems and Room Temperature Ionic Liquids," Presented by R. D. Rogers at Washington State University, Pullman, WA, on 10/8/99.
66. "A Toolbox Approach to Green Separations Science & Technology: Crystal Engineering, Aqueous Biphasic Systems, and Room Temperature Ionic Liquids," Presented by R. D. Rogers at The University of Kentucky, Lexington, KY, on 10/28/99.
67. "Room Temperature Ionic Liquids as VOC Solvent Replacements," Presented by R. D. Rogers at Mercer University, Macon, GA, on 11/9/99.
68. "Ionic Liquids in Separations," Presented by R. D. Rogers at Queen's University, Belfast, Northern Ireland, UK, during Ionic Liquid Week, 1/31/00-2/4/00.
69. "Green Chemistry and Ionic Liquids: Sustainable Industrial Development from Academic Challenges," Presented by R. D. Rogers at Birmingham Southern College, Birmingham, AL, on 2/29/00.
70. "Ionic Liquids in Separations," Presented by R. D. Rogers as the 2nd Queen's University Ionic Liquid Laboratory Lecture, Queen's University, Belfast, Northern Ireland, UK on 4/3/00.
71. "Ionic versus Molecular Solvents: Challenges in Adopting Ionic Liquids as Alternative Reaction Media," Presented by R. D. Rogers at the University of Florida, Gainesville, FL on 5/3/00.
72. "The Role of the Sugar Industry in the New Green Chemistry & Engineering Paradigm of Sustainable Industry," Presented by R. D. Rogers at the Sugar Cane Growers Cooperative of Florida, Belle Glade, Fl on 5/4/00.
73. "Ionic Liquids & Their Application to Separation Processes," Presented by R. D. Rogers at Union Carbide, South Charleston, WV on 5/9/00.
74. "Crystal Engineering of Coordination Polymers," Presented by R. D. Rogers at Université Louis Pasteur, Strasbourg, France on 6/7/00 (Visiting Professor Lecture).
75. "Green Chemistry and Applications of Ionic Liquids as Solvents," Presented by R. D. Rogers at Université Louis Pasteur, Strasbourg, France on 6/16/00 (Visiting Professor Lecture).
76. "How Green Chemistry can Shape the Future of the Chemical Industry," Presented by R. D. Rogers at the Green Chemical Processes –Issue, Challenges, Innovations, Technical Symposium, BP Amoco Chemicals Central Technology, Naperville, IL on 7/11/00.
77. "Engineering Tetrapyridylporphyrin Coordination Complexes for Metal Ion Recognition in Crystalline Materials or on Surfaces," Presented by R. D. Rogers at Emory University on 9/28/00.
78. "Ionic Liquids as Alternatives to Organic Solvents" Presented by R. D. Rogers at North Carolina State University, Raleigh, NC on 10/5/00.
79. "Ionic Liquids as Alternatives to Organic Solvents" Presented by R. D. Rogers at Kennedy Space Center, Cape Canaveral, FL on 10/6/00.
80. "Ionic Liquids as 'Green' Alternatives to Organic Solvents," Presented by R. D. Rogers at Dow Agrosciences LLC, Indianapolis, IN on 10/30/00.
81. "Ionic Liquids," Presented by R. D. Rogers at University of Massachusetts at Boston, Boston, MA on 11/28/00.
82. "Room Temperature Ionic Liquids as Alternative Reaction Media," Presented by R. D. Rogers at Tulane University, New Orleans, LA on 12/5/00.
83. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at Louisiana State University, Baton Rouge, LA on 1/31/01.
84. "Ionic Liquids as 'Green' Alternatives to Organic Solvents," Presented by R. D. Rogers at Dow Corning, Midland, MI on 2/5/01.
85. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at University of South Florida, Tampa, FL on 4/19/01."Ionic Liquids as 'Green' Alternatives to Organic Solvents," Presented by R. D. Rogers at Cognis Corporation, Cincinnati, OH on 5/16/01.
87. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at the U.S. Environmental Protection Agency, Washington, DC on 5/23/01.
88. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at Tennessee State University, Nashville, TN on 10/18/01.
89. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at the University of Illinois at Urbana-Champaign, Urbana, IL on 2/12/02.
90. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at Wesleyan University, Middletown, CT on 2/15/02.
91. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers at Kansas State University, Manhattan, KS on 2/22/02.
92. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to Stellenbosch University, Stellenbosch, South Africa on 3/20/02.
93. "Non-Sugar Products from Sugarcane for the New Millennium: Green Pathways to a Carbohydrate Economy?" Presented by R.

D. Rogers to the Sugar Milling Research Institute, Durban, South Africa on 3/26/02.

94. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to Monsanto Company, St. Louis, MO on 6/6/02.
95. "Ionic Liquids: What are They? Are They Useful? And the Case of the Missing Data," Presented by R. D. Rogers to The Dow Chemical Company, Midland, MI on 6/19/02.
96. "Ionic Liquids: What are They? Are They Useful? And the Case of the Missing Data," Presented by R. D. Rogers to The Lubrizol Corporation, Cleveland, OH on 7/24/02.
97. "Ionic Liquids: What are They? Are They Useful? And the Case of the Missing Data," Presented by R. D. Rogers to Honeywell Corporation, Buffalo, NY on 8/14/02.
98. "Ionic Liquids: What are They? Are They Useful? And the Case of the Missing Data," Presented by R. D. Rogers to Eastman Corporation, Kingsport, TN on 10/28/02.
99. "Ionic Liquids: What are They? Are They Useful? And the Case of the Missing Data," Presented by R. D. Rogers to Savannah River Technical Center, SC on 11/13/02.
100. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to Auburn University, Auburn, AL on 1/16/03.
101. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to GE Global Research Center, Schenectady, NY on 2/3/03.
102. "Challenges and Opportunities in the Use of Ionic Liquids: Separations, Extractions, and the Choice of Ionic Liquid," Presented by R. D. Rogers to AstraZeneca, Loughborough, United Kingdom on 3/28/03.
103. "Green Chemistry" in Pursuit of Traditional Chemical Research, Education, and Service: A Path Forward for the University of Massachusetts- Boston?" Presented by R. D. Rogers to the University of Massachusetts-Boston, Boston, MA on 5/5/03.
104. "Radiochemistry in the Rogers Group at The University of Alabama," Presented by R. D. Rogers at The University of Alabama, Tuscaloosa, AL on 8/28/03.
105. "Challenges and Opportunities in the Use of Ionic Liquids: Separations, Extractions, and the Choice of Ionic Liquid," Presented by R. D. Rogers to Los Alamos National Laboratory, Los Alamos, NM on 9/18/03.
106. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to Mississippi State University, Starkville, MS on 10/17/03.
107. "Ionic Liquids as Green Solvents: Engineering New Bio-Based Materials," Presented by R. D. Rogers at the University of Alabama at Huntsville, Huntsville, AL on 1/16/04.
108. "Green Chemistry and Applications of Ionic Liquids as Solvents: Synergies and Ironies," Presented by R. D. Rogers to Sun Yat-Sen University, Guangzhou, China on 2/23/04.
109. "A Burnum Legacy: Red Chemistry, Green Chemistry, and My Road from Alabama to Alabama" Presented by R. D. Rogers to The University of Alabama (Burnum Award Address), Tuscaloosa, AL on 4/6/04.
110. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids for Extreme Environments (i.e. NASA)?" Presented by R. D. Rogers to Marshall Space Flight Center, Huntsville, AL on 4/8/04.
111. "Green Chemistry" in Pursuit of Traditional Chemical Research, Education, and Service: A Path Forward for the University of Central Florida?" Presented by R. D. Rogers to the University of Central Florida, Orlando, FL on 4/14/04.
112. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids?" Presented by R. D. Rogers to BASF Corporation, Ludwigshafen, Germany on 4/26/04.
113. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids?" Presented by R. D. Rogers to Merck KGaA, Darmstadt, Germany on 4/27/04.
114. "Advanced Materials from Direct Dissolution of Cellulose," Presented to by R. D. Rogers to Gulf States Paper Corporation, Tuscaloosa, AL on 6/7/04.
115. "Applications of Ionic Liquid Technologies to f-Element Separations," Presented by R. D. Rogers to the E. O. Lawrence Berkeley Laboratory, Berkeley, CA on 6/16/04.
116. "Ionic Liquids: An Overview," Presented by R. D. Rogers to Stepan Company, Northfield, IL on 8/20/04.
117. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids?" Presented by R. D. Rogers to the U. S. Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH on 9/1/04.
118. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids?" Presented by R. D. Rogers to Davidson College, Davidson, NC on 9/2/04.
119. "Ionic Liquids: An Overview," Presented by R. D. Rogers to The Proctor & Gamble Company, Cincinnati, OH on 11/10/04.
120. "Green Chemistry and Applications of Ionic Liquids as Solvents: Enabling Sustainable Technologies for New Advanced Materials," Presented by R. D. Rogers to the Swiss Federal Institute of Technology at Lausanne, Switzerland, on 10/13/04.
121. "Ionic Liquid Processing of Cellulose," Presented by R. D. Rogers to Lenzing AG, Lenzing, Austria, on 10/18/04.
122. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids?" Presented by R. D. Rogers to the University of South Dakota, Vermillion, SD on 11/1/04.
123. "Green Chemistry and Applications of Ionic Liquids as Solvents: Enabling Sustainable Technologies for New Advanced Materials," Presented by R. D. Rogers to the Naval Research Laboratory, Washington, DC on 11/9/04.
124. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between" Presented by R. D. Rogers to the University of Missouri, Columbia, MO on 11/11/04.
125. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between" Presented by R. D. Rogers to Howard

University, Washington, DC on 12/3/04.

126. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between" Presented by R. D. Rogers to the University of Bucharest, Bucharest, Romania on 12/13/04.
127. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between" Presented by R. D. Rogers to Wake Forest University, Winston, NC on 1/12/05.
128. "Supramolecular Chemistry in Alternative Solvents: Are Nonvolatile Ionic Liquids Effective Crystallization Solvents or Fodder for Co-Crystals?" Presented by R. D. Rogers to the University of Bucharest, Bucharest, Romania on 3/16/05.
129. "Green Chemistry – An Overview," Presented by R. D. Rogers to the University of Bucharest, Bucharest, Romania on 3/17/05.
130. "Ionic Liquids: Solvents for Cellulose," Presented by R. D. Rogers to the U.S. Bureau of Engraving and Printing, Washington, DC on 4/29/05.
131. "Ionic Liquids: Applications are Coming; Get Ready Now!," Presented by R. D. Rogers to NIEHS, Raleigh, NC, on 5/4/05.
132. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between" Presented by R. D. Rogers to the Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China on 5/26/05.
133. "Ionic Liquids: Solvents for Green Chemistry or Advanced Technological Fluids? (R&D, Trends, and Practical Application)," Presented by R. D. Rogers to Merck KGaA, Darmstadt, Germany on 6/16/05.
134. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents for Crystal Engineering to Advanced New Materials," Presented by R. D. Rogers to The University of Tokyo, Tokyo, Japan on 7/20/05.
135. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to Tokyo University of Agricultural and Technology, Tokyo, Japan on 7/21/05.
136. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents for Crystal Engineering to Advanced New Materials," Presented by R. D. Rogers to Kyoto University, Kyoto, Japan on 7/22/05.
137. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to Eastman Kodak Company, Rochester, NY on 8/9/05.
138. "Energetic Ionic Liquids: Fundamental Studies Relating Target Structures and Key Physical Properties," Presented by R. D. Rogers to the Air Force Research Laboratory, Edwards Air Force Base, CA on 8/11/05.
139. "A Platform Strategy Using Ionic Liquids to Dissolve and Process Cellulose for Advanced New Materials," Presented by R. D. Rogers to FMC BioPolymer, Princeton, NJ on 9/13/05.
140. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to the Changchung Institute of Applied Chemistry, Chinese Academy of Sciences, Changchung, China on 9/27/05.
141. "Energetic Ionic Liquids: Fundamental Studies Relating Target Structures and Key Physical Properties," Presented by R. D. Rogers to the American Pacific/Georgia Tech. Roundtable, Atlanta, GA on 10/6/05; (also Panel Member for the Energetic Materials Panel Discussion).
142. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to the University of South Carolina, Columbia, SC on 11/18/05.
143. "Green Chemistry and Applications of Ionic Liquids as Solvents: Enabling Sustainable Technologies for New Advanced Materials," Presented by R. D. Rogers to the University of Southern Mississippi, Hattiesburg, MS on 12/2/05.
144. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to the DuPont 2005 Discovery Chemistry Seminar Series, DuPont Central Research and Development, Wilmington, DE on 12/7/05.
145. "Green Chemistry and Applications of Ionic Liquids as Solvents," Presented by R. D. Rogers to Jackson State University, Jackson, MS on 1/27/06.
146. "Designer Ionic Liquids Enabling Sustainable Technologies," Presented by R. D. Rogers to Millennium Chemical/Lyondell, Baltimore, MD on 2/28/06.
147. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers as the Arnold C. Ott Lectureship in Chemistry (research presentation), Grand Valley State University, Allendale, MI on 4/5/06.
148. "Green Chemistry: Can Society and the Chemical Industry Co-Exist?" Presented by R. D. Rogers as the Arnold C. Ott Lectureship in Chemistry (public lecture), Grand Valley State University, Grand Rapids, MI on 4/5/06.
149. "Ionic Liquids," Presented by R. D. Rogers to Albion College, Albion, MI on 4/7/06.
150. "How the Center for Green Manufacturing Can Impact Alabama," Presented by R. D. Rogers to the Tuscaloosa League of Women Voters, Tuscaloosa, AL on 4/20/06.
151. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at the 7th Annual Science Symposium *The Science of Sustainability, A Balance for the Future*, St. Olaf College, Northfield, MN on 5/5/06. (Invited Keynote Lecture)
152. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at the University of Cologne, Cologne, Germany on 5/16/06.
153. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at IReS Chimie Nucleaire Strasbourg, France on 6/14/06.
154. "Green Chemistry, Ionic Liquids, Advanced Materials, and Everything in Between," Presented by R. D. Rogers at the Institute Le Bel, Université Louis Pasteur, Strasbourg, France on 6/15/06 (Visiting Professor Lecture).
155. "Strategies Toward the Design of Energetic Materials," Presented by R. D. Rogers at the Institute Le Bel, Université Louis Pasteur, Strasbourg, France on 6/16/06 (Visiting Professor Lecture).

156. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at the Stamford Seminar Series, Cytec Industries, Inc., Stamford, CT on 10/18/06.
157. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at the University of Texas San Antonio, San Antonio, TX on 10/20/06.
158. "Green Chemistry and Applications of Ionic Liquids as Solvents: Enabling Sustainable Technologies For New Advanced Materials," Presented by R. D. Rogers at the University of Texas Arlington, Arlington, TX on 11/10/06.
159. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers at the University of Toledo, Toledo, OH on 1/17/07.
160. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers to Lyondell Chemical Co., Newton Square, PA on 2/13/07.
161. "The Past, Present, and Future of Ionic Liquids: From Designer Solvents to Advanced New Materials," Presented by R. D. Rogers to Colgate-Palmolive, Piscataway, NJ on 9/10/07.
162. "Applications and The Third Evolution of Ionic Liquids: Physical to Chemical to Biological Properties," Presented by R. D. Rogers to Colgate-Palmolive, Piscataway, NJ on 9/10/07.
163. **"**The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Technische Universiteit Eindhoven, Eindhoven, The Netherlands on 9/24/07.
164. "Ionic Liquids as Transformational Technologies," Presented by R. D. Rogers to Nippon Chemical Industrial Company, Tokyo, Japan, on 4/21/08.
165. "The Evolution of Ionic Liquids – From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers at the Danish Technical University (2008), Copenhagen, Denmark, on 6/16/08.
166. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Brookhaven National Laboratory, Upton, NY on 7/14/08.
167. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Abbott, Waukegan, IL on 8/14/08.
168. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to the University of Alabama at Birmingham, Birmingham, AL on 9/8/08.
169. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to AMGEN, South San Francisco, CA on 9/10/08.
170. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples for the Fragrance Industries," Presented by R. D. Rogers to Givaudan, Ashford, United Kingdom on 10/01/08.
171. "Green Chemistry and the Industrial Revolution," Presented by R. D. Rogers to the Royal Institution of Great Britain as an invited Friday Evening Discourse, London, United Kingdom on 11/14/08. (Invited)
172. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to The U.S. Army Research Office/U.S. Army Research Laboratory Ionic Liquids in Eletroactive Devices MURI Annual Review, Philadelphia, PA on 12/16/08. (Invited Guest Speaker)
173. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Rutgers University, New Brunswick, NJ on 1/20/09. (Invited Colloquium Speaker)
174. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Abbott, Waukegan, IL on 2/20/09. (Invited Abbott Seminar Series)
175. "Ionic Liquids and the Green Industrial Revolution," Presented by R. D. Rogers to The Queen's University of Belfast, Belfast, United Kingdom on 3/2/09. (Inaugural Lecture)
176. "The 'Ionic Liquid Talk'," Webinar presented by R. D. Rogers to the American Chemical Society Publications Division from Belfast, Northern Ireland to Washington, DC on 4/24/09.
177. "Ionic Liquid Cracking of Biomass: Beyond Cellulose to Biorefineries," Presented by R. D. Rogers to the Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China on 8/22/09.
178. "From Green Chemistry to a 'Green' Industrial Revolution: Are Ionic Liquids Pointing the Way?" Presented by R. D. Rogers to the Foster Colloquium University of Buffalo, Buffalo, NY on 10/30/09. (Invited Colloquium Speaker)
179. "Ionic Liquid Cracking of Biomass: Beyond Cellulose to Biorefineries," Presented by R. D. Rogers to Tuskegee Institute, Tuskegee, AL, on 11/30/09.
180. "Ionic Liquid Cracking of Biomass: Beyond Cellulose to Biorefineries," Presented by R. D. Rogers to The Westerveld Company, Tuscaloosa, AL, on 12/16/09.
181. "Ionic Liquid Cracking of Biomass: Beyond Cellulose to Biorefineries," Presented by R. D. Rogers to The University of Colorado, Boulder, CO, on 1/12/10.
182. "Ionic Liquid Advances and Retreats as Solvents and Materials," Presented by R. D. Rogers to Colgate-Palmolive, Piscataway, NJ on 1/27/10.
183. "Ionic Liquids with or without Biological Activity for use in Personal Care Products," Presented by R. D. Rogers to Colgate-Palmolive, Piscataway, NJ on 1/27/10.

184. "Crystallization Process in Ionic Liquids," Presented by R. D. Rogers to Nippon Chemical Industrial, Tokyo, Japan on 2/8/10.
185. "Ionic Liquids Laboratory to Commercialization," Presented by R. D. Rogers to the Institute of Process Engineering, Chinese Academy of Sciences, Beijing, China on 04/28/10.
186. "Ionic Liquids: Introduction, Brief History, Evolution, and Potential Applicability to Your Projects," Presented by R. D. Rogers to the Massachusetts Institute of Technology, Cambridge, MA on 06/10/10.
187. "Ionic Liquids: Introduction, Brief History, Evolution, and Potential Applicability to Your Projects," Presented by R. D. Rogers to the Arch Chemicals Inc., Innovation Committee, Atlanta, GA on 09/15/10.
188. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to the Joint Bioenergy Research Institute, Lawrence Berkeley National Laboratory, Emeryville, CA on 10/05/10.
189. "Ionic Liquids: Laboratory to Commercialization Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Lanzhou University, Lanzhou, China on 10/28/10.
190. "Ionic Liquids: Laboratory to Commercialization," Presented by R. D. Rogers to The Chinese Academy of Sciences Institute of Chemical Physics, Lanzhou, China on 10/29/10.
191. "Ionic Liquids: Laboratory to Commercialization Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Jiaotong University, Xi'an, China on 11/01/10.
192. "Ionic Liquids: Laboratory to Commercialization Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Northwest University, Xi'an, China on 11/01/10.
193. "Ionic Liquids: Introduction, Brief History, Evolution, and Potential Applicability to Your Projects," Presented by R. D. Rogers to Monsanto, St. Louis, MO on 11/11/10.
194. "Ionic Liquids: Introduction, Brief History, Evolution, and Potential Applicability to Your Projects," Presented by R. D. Rogers to Frontier Scientific and Echelon, Logan, UT on 12/09/10.
195. "Vignettes of Ionic Liquids Strategies in the Rogers Group," Presented by R. D. Rogers to Tokyo University of Agricultural and Technology, Tokyo, Japan on 1/13/11.
196. "Vignettes of Ionic Liquids Strategies in the Rogers Group," Presented by R. D. Rogers to Nippon Chemical Industrial Company, Tokyo, Japan, on 1/14/11.
197. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to University of Guelph on 1/24/11.
198. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Tennessee Technological University, Cookeville, TN on 2/8/11.
199. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Oak Ridge National Laboratory, Oak Ridge, TN on 2/9/11.
200. "An Editor's Perspective on Contentious Issues Arising During the Peer Review Process," Presented by R. D. Rogers to the National Chemical Laboratory, Pune, India, on 6/24/11.
201. "An Editor's Perspective on Contentious Issues Arising During the Peer Review Process," Presented by R. D. Rogers to the Indian Institute of Science, Bangalore, India, on 6/27/11.
202. "Ionic Liquids: Unique Environments for f-Element Chemistry," Presented by R. D. Rogers to the Changchung Institute of Applied Chemistry, Chinese Academy of Sciences, Changchung, China on 07/26/11.
203. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Merck, Summit, NJ on 09/09/11.
204. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to Loyola University, New Orleans, LA on 11/21/11.
205. "The Evolution of Ionic Liquids - From Solvents and Separations to Advanced Materials and Pharmaceuticals: Examples from the Ionic Liquid Cookbook," Presented by R. D. Rogers to Ruhr Universität Bochum, Bochum, Germany on 12/01/11.
206. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to the Fraunhofer Institute for Wood Research Wilhelm Klauditz Institute, Braunschweig, Germany on 12/05/11.
207. "Ionic Liquids: Solvents and Materials," Presented by R. D. Rogers to Reliance Industries Limited, Mumbai, India on 03/09/12.
208. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to the Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India on 03/15/12.
209. "Ionic Liquids in Support of the Pharmaceutical Industries," Presented by R. D. Rogers to Novartis, Basel, Switzerland on 05/07/12.
210. "Green Chemistry, Technology, & Innovation (on the road to 'Shrimp Bandages')," Presented by R. D. Rogers to the Mobile Kiwanis Club, Mobile, AL on 6/27/12.
211. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to the University of Mississippi, Oxford, MS on 11/01/12.

212. "Unique Roles for Ionic Liquids in a Biorefinery: Extraction, Separation, and Processing of Lignin, Cellulose, Hemicellulose, and Chitin" Presented by R. D. Rogers to the U.S. Army ERDC Environmental Laboratory, Vicksburg, MS on 11/02/12.
213. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to McGill University, Montreal, Quebec, Canada on 11/06/12.
214. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to The University of Tennessee at Martin, Martin, TN on 02/18/13.
215. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to The University of Aveiro, Averio, Portugal on 04/29/13.
216. "A study of Ionic Liquids in the pharmaceutical sector - How can the liquid state help us master the solid state?" Presented by R. D. Rogers to Instituto de Technologia Quimica e Biologica (ITQB), Lisbon, Portugal on 04/30/13.
217. "Ionic Liquid Solvents for the Grand Challenge Inherent in a Biorefinery: Extraction and Separation of Lignin, Cellulose, and Hemicellulose," Presented by R. D. Rogers to the Sugar Milling Research Institute, Council for Scientific and Industrial Research Forestry and Forest Products Research Centre, University of KwaZulu-Natal Chemical Engineering Department, and Durban University of Technology, Durban, South Africa on 07/03/13.
218. "A study of Ionic Liquids in the pharmaceutical sector - How can the liquid state help us master the solid state?" Presented by R. D. Rogers to McGill University, Montreal, Quebec, Canada on 08/21/13.
219. "Fine Tuning Double Salt Ionic Liquids and Their Applications in the Pharmaceutical Industry," Presented by R. D. Rogers at Novartis, Basel, Switzerland on 09/11/13.
220. "A study of Ionic Liquids in the pharmaceutical sector" Presented by R. D. Rogers to Nova University, Ft. Lauderdale, FL on 10/11/13.
221. R. D. Rogers, "Liquid Engineering to Crystal Engineering: How Ionic Liquids Can Help Us Master the Pharmaceutical Solid State," Presented by R. D. Rogers to the University of Cologne, Cologne, Germany, 11/28/13.
222. R. D. Rogers, "Liquid Engineering to Crystal Engineering: How Ionic Liquids Can Help Us Master the Pharmaceutical Solid State," Presented by R. D. Rogers to the University of Bochum, Bochum, Germany, 11/29/13.
223. "Unique Roles for Ionic Liquids in a Biorefinery: Extraction, Separation, and Processing of Lignin, Cellulose, Hemicellulose, and Chitin" Presented by R. D. Rogers to Mississippi State University, Starkville, MS on 02/14/14.
224. R. D. Rogers, "Liquid Engineering to Crystal Engineering: How Ionic Liquids Can Help Us Master the Pharmaceutical Solid State," Presented by R. D. Rogers to the University of Rostock, Rostock, Germany, 04/07/14.
225. R. D. Rogers, "Liquid Engineering: Ionic Liquids for the Pharmaceutical Sector in Drug Development, Drug Delivery, and as Drugs," Presented by R. D. Rogers to Takeda Millennium, Cambridge, MA, 05/09/14.
226. R. D. Rogers, "Ideality vs. Reality of Green Chemistry in the Development of Advanced Materials from Renewable Polymers," Presented by R. D. Rogers before the North Alabama Section of the American Chemical Society, Huntsville, AL, 09/08/14.
227. R. D. Rogers, "Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists," Presented by R. D. Rogers to Iowa State University, Ames, IA on 11/03/14.
228. R. D. Rogers, "Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists," Presented by R. D. Rogers to McGill University Macdonald Campus, Montreal, QC Canada on 04/16/15.
229. R. D. Rogers, "Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists," Presented by R. D. Rogers to Institut für Technische und Makromolekulare Chemie, RWTH Aachen, Aachen, Germany on 04/30/15.
230. R. D. Rogers, "Sustainability, from Ideas to Implementation: Can Ionic Liquids Help?" Presented by R. D. Rogers to L'Oréal, Aulnay sous Bois, France on 05/11/15.
231. R. D. Rogers, "Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists," Presented by R. D. Rogers to the University of Calgary (Department of Chemistry), Calgary, AB, Canada on 07/07/15.
232. R. D. Rogers, "Is 'Sustainability' a new paradigm for the future chemical industry? Cross border perspectives and what we need to train the next generation to face," Presented by R. D. Rogers to Alberta Innovates Technology Futures, Calgary, AB, Canada on 07/09/15.
233. "Utilization of Ionic Liquids in Support of Continuous Pharmaceutical Manufacturing: Fine Tunability of Double Salt Ionic Liquids," Presented by R. D. Rogers at Novartis, Basel, Switzerland on 09/14/15.
234. R. D. Rogers, "Liquid Engineering: Ionic Liquids for the Pharmaceutical Sector in Drug Development, Drug Delivery, and as Drugs," Presented by R. D. Rogers to the Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada on 11/09/15.
235. R. D. Rogers, "Innovation is the Gateway to the Biomass Biorefinery and Ultimately A sustainable Bio-based Economy," Presented by R. D. Rogers to Concordia University, Montreal, QC, Canada on 11/13/15.
236. R. D. Rogers, "Innovation is the Gateway to the Biomass Biorefinery and Ultimately A sustainable Bio-based Economy," Presented by R. D. Rogers as a Waterloo Institute for Nanotechnology (WIN) Distinguished Lecture to the University of Waterloo, Waterloo, ON, Canada on 11/19/15.

237. R. D. Rogers, “Green Chemistry and Advanced Materials from Renewable Polymers: Education, Research, and Entrepreneurship to Motivate the Next Generation of Scientists,” Presented by R. D. Rogers to West Virginia University (Department of Chemical Engineering), Morgantown, WV on 12/04/15.
238. “Before Applications You Need Understanding: Does the Nature of the Bonding in Double Salt Ionic Liquids ‘Prove’ a Difference Between Ionic Liquids and Molecular Liquids?,” Presented by R. D. Rogers to Reliance Industries Limited, Mumbai, India on 01/19/16.
239. “Millions of New Ionic Liquids are Hiding in Plain Sight: Understanding the Nature of the Bonding in Double Salt Ionic Liquids (aka Ionic Liquid Mixtures),” Presented by R. D. Rogers to the PATH Workshop, University of Aveiro, Aveiro, Portugal on 05/09/16.

F. Theses and Dissertations Directed:

1. M. M. Benning, "Actinide/Crown Ether Chemistry," Ph.D., Northern Illinois University, 1988.
2. L. Nuñez, "Structural, Magnetic, and Superconducting Properties of $YBa_2Cu_{3-x}Fe_xO_{7-\delta}$ Single Crystals," Ph.D., Northern Illinois University, 1990.
3. R. F. Henry, "Synthesis and Characterization of Novel Macrocycles and Their Complexes," M. S., Northern Illinois University, 1990.
4. A. N. Rollins, "Controlling the Primary Coordination Sphere: Complexation of the 4-f Elements by Crown Ethers as Models for Potential Extraction Systems," Ph.D., Northern Illinois University, 1993.
5. A. H. Bond, "Heavy Main Group Metal Ions: Structural Chemistry of Polyether Complexes and Aqueous Biphasic Separations," Ph.D., Northern Illinois University, 1995.
6. C. B. Bauer, "Polyether Complexation Chemistry of Hard Metal Ions: Structural Investigation and Partitioning Behavior in Aqueous Biphasic Systems," Ph.D., Northern Illinois University, 1995.
7. J. Zhang, "Polyethylene Glycol (PEG) Chemistry: Partitioning of Chaotropic Ions in PEG-Based Aqueous Biphasic Systems and Structural Investigation of Lanthanide Isothiocyanate/PEG Complexes," Ph.D., Northern Illinois University, 1997.
8. K. S. Granger, non-thesis option, M.S., The University of Alabama, 2000.
9. H. D. Willauer, "Fundamentals of Phase Behavior and Solute Partitioning in ABS and Applications to the Paper Industry," Ph.D., The University of Alabama, 2002.
10. A. E. Visser, "Metal Ion Separations in Aqueous Biphasic Systems and Room Temperature Ionic Liquids," Ph.D., The University of Alabama, 2002. (Recipient of The University of Alabama Award for Excellence in Research by a Doctoral Student)
11. G. A. Broker, non-thesis option, M.S., The University of Alabama, 2003.
12. S. T. Griffin, "The Development and Applications of ABEC Resins," Ph.D., The University of Alabama, 2004.
13. M. Dilip, non-thesis option, M.S., The University of Alabama, 2004.
14. M. A. Klingshirn, "Relating Ionic Liquids and Polyethylene Glycols to Green Chemistry, Organometallic Catalysis, and Materials Science," Ph.D., The University of Alabama, 2005.
15. M. B. Turner, "Ionic Liquids in the Life Sciences: Are Ionic Liquids Useful in the Manipulation of Biomolecules?," Ph.D., The University of Alabama, 2005.
16. W. M. Reichert, "The Effects of Cation-Anion Interactions on the Properties of Ionic Liquids," Ph.D., The University of Alabama, 2005.
17. R. P. Swatloski, "Ionic Liquids as Green Solvents: Enabling New Materials and Technologies," Ph.D., The University of Alabama, 2005.
18. G. A. Broker, "Crystal Engineering Studies of some Nitrogen Containing Multifunctional Ligands," Ph.D., The University of Alabama, 2006.
19. V. A. Cocalia, "Separations, Solvation, and Coordination of Actinides in Ionic Liquids," Ph.D., The University of Alabama, 2006.
20. K. E. Gutowski, "Computational Thermodynamic Studies of the Formation and Stability of Ionic Liquids and Actinide-Ligand Complexes," Ph.D., The University of Alabama, 2006. (Recipient of The University of Alabama Award for Excellence in Research by a Doctoral Student)
21. N. J. Bridges, Ph.D., "Ionic Liquids and Water: An Investigation of Solvation," The University of Alabama, 2007.
22. C. C. Hines, "Ionic Liquids for Crystallization: Echoes of Solvation in the Solid State," M.S., The University of Alabama, 2007 (Recipient of The University of Alabama's Award for Excellence in Research by a Masters Student)
23. M. L. Moody, "A Study of the Influence of Water on Polyethylene Glycol Solutions," Ph.D., The University of Alabama, 2007
24. M. Smiglak, "A Modular "Ionic Liquid" Platform for the Custom Design of Energetic Materials," Ph.D., The University of Alabama, 2007. (Recipient of The University of Alabama Award for Excellence in Research by a Doctoral Student)
25. M. Dilip, "Towards Greener Separations: Role of water in Aqueous Biphasic Systems," Ph.D. The University of Alabama, 2008.
26. W. L. Hough, "Functional Ionic Liquids for Use in Pharmaceutical Applications," Ph.D. The University of Alabama, 2010.
27. N. Sun, "Dissolution and Processing of Cellulosic Materials with Ionic Liquids: Fundamentals and Applications," Ph.D. The University of Alabama, 2010.
28. D. M. Drab, "A Versatile Design Platform for Multi-Heterocyclic Ionic Liquid Synthesis," Ph.D. The University of Alabama, 2011.
29. M. L. Maxim, "Ionic Liquids Platform for Biomass Dissolution Leading to Advanced Biocomposite Materials," Ph.D. The University of Alabama, 2012.
30. P. A. Beasley, "Understanding the Effects of Molecular Additions in Energetic Ionic Liquids," M.S. The University of Alabama, 2013.
31. P. M. McCrary, "Controlling the Properties of Energetic Ionic Liquids by Stabilizing Reactive Nanomaterials," Ph.D. The University of Alabama, 2014.
32. Kelley, S. P., "Isolation of Soft Donor Complexes of d- and f-Block Metals Using Ionic Liquids," Ph.D. The University of Alabama, 2015.

Solid-State Chemistry of Drugs

SECOND EDITION

Stephen R. Byrn
Ralph R. Pfeiffer
Joseph G. Stowell

SSCI, Inc. • West Lafayette, Indiana
www.ssci-inc.com

10

Polymorphs

As discussed in Chapter 1, polymorphs exist when two crystals have the same chemical composition but different internal structure, including different unit cell dimensions and different crystal packing. Compounds that crystallize as polymorphs can show a wide range of different physical and chemical properties, including different melting points and spectral properties. Polymorphs can also differ in their solubility, density, hardness, and crystal shape. While some compounds may exist in only two polymorphs, others may exist in many polymorphs (*e.g.*, progesterone has five polymorphs and water has nine polymorphs). Control of polymorphism is particularly important for pharmaceuticals where changing the polymorph can alter the bulk properties, dissolution rate, bioavailability, chemical stability, or physical stability of a drug. The clearest indication of the existence of polymorphs comes from the X-ray crystallographic examination of single crystals of the various samples that are known to have the same composition. Often, however, X-ray powder diffraction is sufficient to establish the existence of polymorphs.

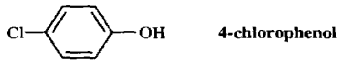
There is, unfortunately, no standard numbering system for polymorphs. In the literature, the various polymorphs have been designated by Roman numerals (preceded by the word "Form," *e.g.*, Form I), Greek letters (with the suffix "-form," *e.g.*, α-form), or in some cases, capital letters (similar to the Roman numeral system). To add to the confusion, some of numbering schemes of polymorphs also include solvates (*e.g.*, the α- and γ-forms of indomethacin are anhydrates, yet the β-form is the benzene solvate). Furthermore, some polymorphs have been identified only by their crystallographic classification (*e.g.*, the two polymorphs of ($\pm$)-β-promedol are designated the monoclinic form and the rhombohedral form). It has been suggested that polymorphs be numbered consecutively in the order of their stability at room temperature or by their melting point. This of course would lead to confusion upon the discovery of a new polymorph having intermediate stability or melting point and thus requiring renumbering of the existing polymorph system. It has also been suggested that polymorphs be numbered consecutively in the order of discovery, but this requires knowledge of their history and a timely access to that information. Whatever the numbering system, it is imperative that it be consistent. Thus, when a new polymorph is discovered and characterized, the designation of the new polymorph should be the next increment in the

previous system. However, this is not always practical when more than one laboratory is involved in the development process at the same time.

10.1 CLASSIC EXAMPLES OF POLYMORPHISM

This section summarizes several classic examples of polymorphism which have appeared in the chemical literature.

A. 4-CHLOROPHENOL



4-chlorophenol

The crystal structure of both the thermodynamically stable (α) and unstable (β) forms of 4-chlorophenol have been determined (Perrin and Michel, 1973a–b). Both forms belong to the same space group ($P2_1/c$); they both have the same number of molecules per unit cell ($Z = 8$) and nearly identical densities, yet they have different cell parameters (see Table 10.1). The crystal structure of the β-form projected on the (100) plane is shown in Figure 10.1. The packing consists of tetramers of molecules connected by hydrogen bonding. The crystal packing of the α-form (shown in Figure 10.2) also consists of tetramers connected by hydrogen bonds, but the arrangement of the rings is slightly different than that of the β-form. Although the β-form converts to the α-form, no detailed studies of this transformation have been reported.

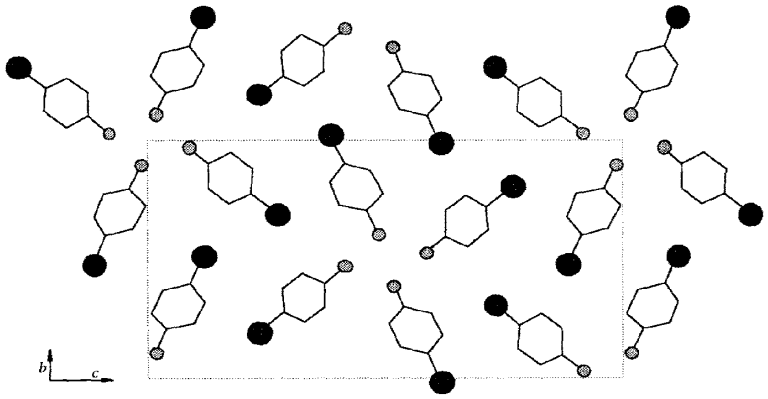


Figure 10.1 Projection of the crystal structure of the β-form of 4-chlorophenol (● chlorine atom, ◍ hydroxyl group) (Perrin and Michel, 1973b).

Table 10.1 Crystallograp
4-Chlorophen

Parameter	α-
Space Group	
a (Å)	
b (Å)	
c (Å)	
β	9
Z	
ρcalc (g cm^{-3})	
V (Å^3)	122

a Perrin and Michel, 1973a.

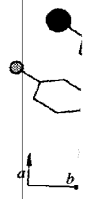
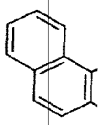


Figure 10.2 Projection of t
◍ hydroxyl gr

B. DIBENZ[*a*,*h*]ANTHRA



In an early study of polyr
[*a*,*h*]anthracene (1,2:5,6-
1947; 1956). Although th
groups (Table 10.2) and

Table 10.1 Crystallographic Parameters for Two 4-Chlorophenol Polymorphs

Parameter	α-Form[a]	β-Form[b]
Space Group	$P2_1/c$	$P2_1/c$
a (Å)	8.84	4.14
b (Å)	15.726	12.85
c (Å)	8.790	23.20
β	92.61°	93.00°
Z	8	8
ρ_{calc} (g cm^{-3})	1.40	1.38
V (Å^3)	1220.7	1232.5

a Perrin and Michel, 1973a. *b* Perrin and Michel, 1973b.

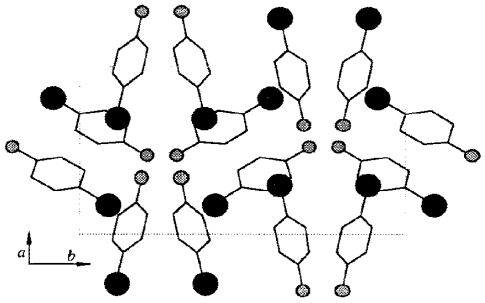
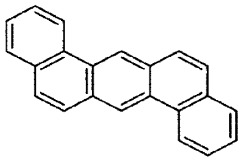


Figure 10.2 Projection of the crystal structure of the α-form of 4-chlorophenol (● chlorine atom, ◍ hydroxyl group) (Perrin and Michel, 1973a).

B. Dibenz[*a*,*h*]anthracene



dibenz[*a*,*h*]anthracene
(1,2:5,6-dibenzanthracene)

In an early study of polymorphism, the crystal structures of Forms I and II of dibenz-[*a*,*h*]anthracene (1,2:5,6-dibenzanthracene) were determined (Robertson and White, 1947; 1956). Although the forms have the same density, they belong to different space groups (Table 10.2) and have quite different packing. The crystal packing of Form I

(orthorhombic form) is shown in Figure 10.3 and the crystal packing of Form II (monoclinic form) is shown in Figure 10.4.

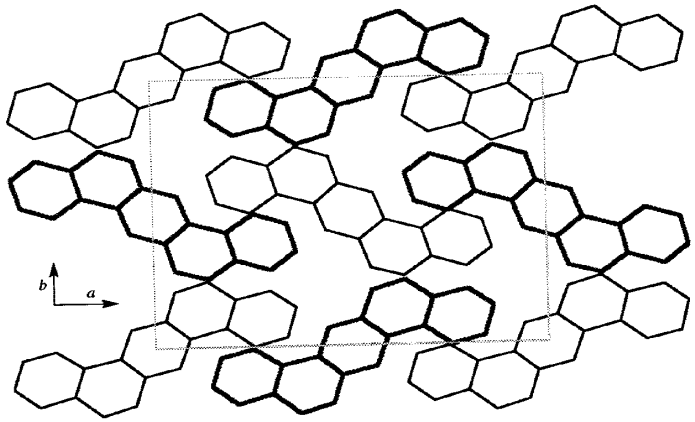


Figure 10.3 Crystal packing of Form I (orthorhombic form) of dibenz[*a*,*h*]anthracene (Robertson and White, 1947).

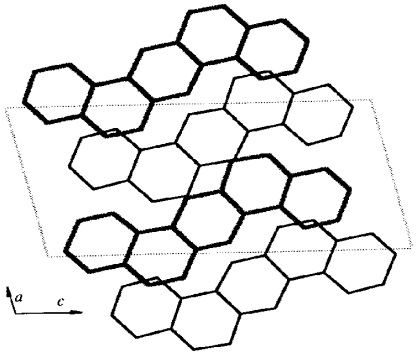


Figure 10.4 Crystal packing drawing of Form II (monoclinic form) of dibenz[*a*,*h*]anthracene (Robertson and White, 1956).

Table 10.2 Crystallographic I
Dibenz[*a*,*h*]anthra

Parameter	Fc
Space group	F
a (Å)	
b (Å)	1
c (Å)	1
β	9
Z	
ρ_{calc} (g cm^{-3})	
V (Å^3)	141
V/molecule	35

Robertson and White, 1947; R

C. Acridine

Acridine crystallizes in fi
Schmidt, 1955). The cry
and are shown in Figures
forms appear to be quite si

Table 10.3 Crystal Parameter

Parameter	α-For
Space group	$P2_1/c$
a (Å)	16.18
b (Å)	18.88
c (Å)	6.08
β	95.67°
Z	8
ρ_{calc} (g cm^{-3})	1.27
V (Å^3)	1848.2
V/*Z* (Å^3)	231.0
Habit	Needle

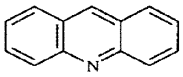
Herbstein and Schmidt, 1955

Table 10.2 Crystallographic Parameters for Two Dibenz[*a*,*h*]anthracene Polymorphs

Parameter	Form I	Form II
Space group	*Pcab*	$P2_1$
a (Å)	8.22	6.59
b (Å)	11.39	7.84
c (Å)	15.14	14.17
β	90.0°	103.5°
Z	4	2
ρ_{calc} (g cm^{-3})	1.29	1.29
V (Å^3)	1417.5	711.9
V/molecule	354.4	355.9

Robertson and White, 1947; Robertson and White, 1956.

C. Acridine



acridine

Acridine crystallizes in five polymorphs as shown in Table 10.3 (Herbstein and Schmidt, 1955). The crystal structures of the α- and γ-forms have been determined and are shown in Figures 10.5 and 10.6, respectively. The crystal packing of these forms appear to be quite similar although the cell parameters are obviously different.

Table 10.3 Crystal Parameters of the Various Polymorphs of Acridine

Parameter	α-Form	β-Form	γ-Form	δ-Form	ε-Form
Space group	$P2_1/a$	*Aa*	*Pnab*	$P2_12_12_1$	$P2_1/n$
a (Å)	16.18	16.37	17.45	15.61	11.37
b (Å)	18.88	5.95	8.89	6.22	5.98
c (Å)	6.08	30.01	26.37	29.34	13.64
β	95.67°	141.33°	90.00°	90.00°	98.67°
Z	8	8	16	12	4
ρ_{calc} (g cm^{-3})	1.27	1.29	1.15	1.24	1.29
V (Å^3)	1848.2	1826.3	4090.8	2848.7	918.2
V/*Z* (Å^3)	231.0	228.3	255.7	237.4	229.5
Habit	Needles	Plates	Laths	Laths	Prisms

Herbstein and Schmidt, 1955

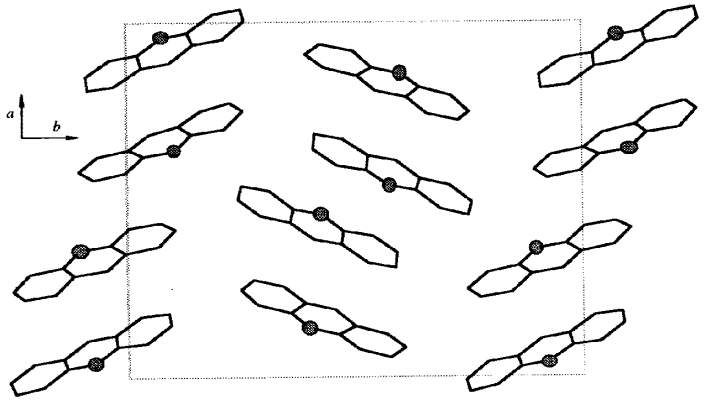


Figure 10.5 Crystal packing of acridine α-form with ● representing the nitrogen atom of the acridine ring (Phillips, 1956).

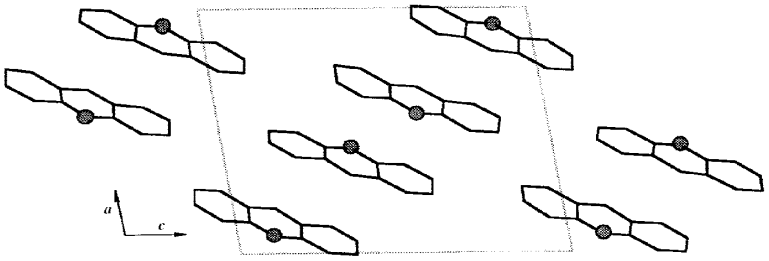


Figure 10.6 Crystal packing of acridine γ-form with ● representing the nitrogen atom of the acridine ring (Phillips *et al.*, 1960).

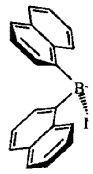
10.2 Conformational and Configurational Polymorphism

In this section, two special types of polymorphism will be discussed. *Conformational polymorphism* occurs when a molecule adopts a significantly different conformation in different crystal polymorphs (Bernstein, 1987). (The term "significantly different" is open to interpretation.) This term does not adequately describe cases where different types of isomers crystallize in different forms. Thus an additional term—*configurational polymorphism*—is defined. Configurational polymorphism exists when different

configurations (*i.e.*, *cis*,
forms.

Crystallization of *ci*
occurs whenever the pu
forms in separate crystal
The crystallization of equ
cantly more interest. W
phism can be used to is
crystalline form.

A. Tri-α-Naphthylb



tri-α-naphth
For

Brown and Sujishi (1948
with the following obser

1. Two crystalli
2. The metastab
room temper
3. The dissociat
stable form.
4. Removal of N
naphthylboro

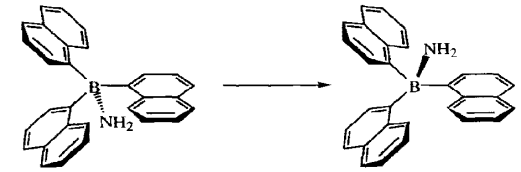
Based on these results,
above. In these forms, th
that the NH_3 is connected
and the less hindered side
ence in dissociation press
the same conformer of tri-
being the most sterically h

Unfortunately, while
formational polymorphism
The example, nevertheles
polymorph formation.

configurations (*i.e.*, *cis,trans* isomers or tautomers) crystallize in separate crystalline forms.

Crystallization of *cis,trans* isomers in different crystalline forms is well known and occurs whenever the pure isomer is crystallized. Crystallization of pure tautomeric forms in separate crystals leads to what may be called *tautomerizational polymorphism*. The crystallization of equilibrating isomers in configurational polymorphs is of significantly more interest. When this occurs, the phenomenon of configurational polymorphism can be used to isolate and study the individual isomers provided they exist in crystalline form.

A. Tri-α-Naphthylboronamine



tri-α-naphthylboronamine
Form A

tri-α-naphthylboronamine
Form B

Brown and Sujishi (1948) reported an early example of conformational polymorphism with the following observations:

1. Two crystalline forms of tri-α-naphthylboronamine are found.
2. The metastable Form A is converted to the stable Form B slowly at room temperature and rapidly above 100 °C.
3. The dissociation pressure of the metastable form is higher than the stable form.
4. Removal of NH_3 from either form gives identical samples of tri-α-naphthylboron.

Based on these results, the two forms were suggested to have structures depicted above. In these forms, the conformation of the tri-α-naphthylboron is the same except that the NH_3 is connected to the boron on the more hindered side for the unstable form and the less hindered side for the stable form. Thus these structures explain the difference in dissociation pressures of the two forms and the fact that removal of NH_3 gives the same conformer of tri-α-naphthylboron. They also explain why the unstable form, being the most sterically hindered, can be converted to the stable form.

Unfortunately, while tri-α-naphthylboron was one of the first suggestions of conformational polymorphism, it was never confirmed by X-ray crystallographic analysis. The example, nevertheless, points out some of the molecular factors that influence polymorph formation.

B. Ethyl 2-[(phenylmethyl)amino]-2-butenoate

ethyl Z-2-[(phenylmethyl)-amino]-2-butenoate

ethyl E-2-[(phenylmethyl)-amino]-2-butenoate

Infrared studies (Dabrowski, 1963) and NMR studies (Dudek and Volpp, 1963) indicate that the Schiff base ethyl 2-[(phenylmethyl)amino]-2-butenoate (ethyl β-benzylaminocrotonate) exists in configurational polymorphs; the low-melting form (mp 23 °C) has the *cis*- or *Z*-conformation and the high-melting form (mp 75–80 °C) has the *trans*- or *E*-conformation. These conformers equilibrate in solution, but upon crystallization, the configurations shown are "frozen" out in their respective polymorphic structures.

The crystal structure of the *E*-isomer has been determined in our laboratory (Shieh *et al.*, 1983). Crystals of the *E*-isomer belong to space group $P2_12_12_1$ with a = 19.655 Å, b = 5.778 Å, and c = 10.632 Å. Figure 10.7 shows the structure of this isomer, and indeed it has the structure of the *E*-isomer suggested by spectroscopic evidence (Dudek and Volpp, 1963).

The NMR and IR spectra of ethyl 2-[(phenylmethyl)amino]-2-butenoate are completely consistent with this assignment. A solution-NMR spectrum of the low-melting form (prepared by dissolving crystals at low temperature) indicates that it is indeed the *Z*-isomer (Dudek and Volpp, 1963). In this experiment the isomer present in the solid state predominates in solution because of the low temperature. In our laboratory we have studied the isomerization rate of the *Z*-isomer to the *E*-isomer at ambient temperature in DMSO where it is relatively rapid. Measurement of the rate of this reaction at various temperatures gives an activation energy of 56.9 kJ/mol.

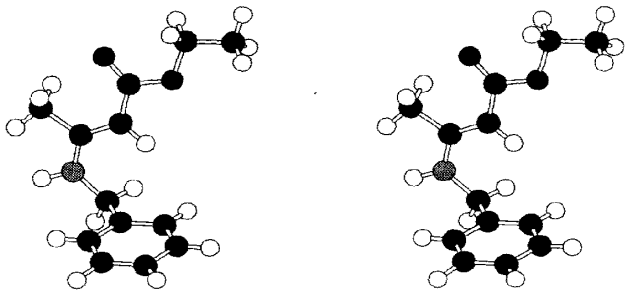
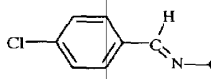


Figure 10.7 Stereoview of ethyl 2-[(phenylmethyl)amino]-2-butenoate in the high-melting *E*-isomer: H ○, C ●, N ◎, O ● (Shieh *et al.*, 1983).

10.

The energies in kJ/mol
been calculated using the C
employs semiempirical pote
each rotamer. These calcu
determined by X-ray cryst
although the *E*- and Z-isome

C. 4-(*N*-Chlorobenzylii



The Schiff base 4-(*N*-chlc
morphs (Bernstein and Hagl
disordered, it can be seen tha
the two polymorphs. Hen
Conformational polymorphi
10.11. In the stable (triclini
(orthorhombic) form the phe
with respect to the H—C=I
these two forms is shown ir

Molecular orbital and l
for conformational polymo
stein and Hagler, 1978).

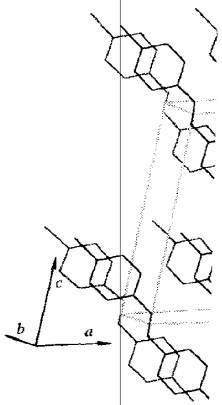
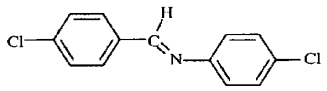


Figure 10.8 Stereoview of 4
and Hagler, 1978)

The energies in kJ/mol for a number of rotamers of the *E*- and *Z*-isomers have been calculated using the *CAMSEQ* program (Weintraub and Hopfinger, 1975) which employs semiempirical potential and electrostatic functions to calculate the energies of each rotamer. These calculations indicate that the conformation of the *E*-isomer as determined by X-ray crystallography is one of the lowest energy conformations, although the *E*- and *Z*-isomers have nearly the same energy in a vacuum.

C. 4-(*N*-Chlorobenzylidene)-4-chloroaniline



4-(*N*-chlorobenzylidene)-4-chloroaniline

The Schiff base 4-(*N*-chlorobenzylidene)-4-chloroaniline crystallizes in two polymorphs (Bernstein and Hagler, 1978). Although the structures of both polymorphs are disordered, it can be seen that the conformation of the molecule is strikingly different in the two polymorphs. Hence, these forms are termed conformational polymorphs. Conformational polymorphism of drugs is discussed in more detail later in Section 10.11. In the stable (triclinic) form, the molecules are planar, whereas in the unstable (orthorhombic) form the phenyl rings are rotated by equal but opposite amounts (24.8°) with respect to the H—C=N least-squares plane of the imine. The crystal packings of these two forms is shown in Figures 10.8 and 10.9.

Molecular orbital and lattice energy calculations were used to analyze the reasons for conformational polymorphism of 4-(*N*-chlorobenzylidene)-4-chloroaniline (Bernstein and Hagler, 1978). Quantum-mechanical calculations for a single molecule

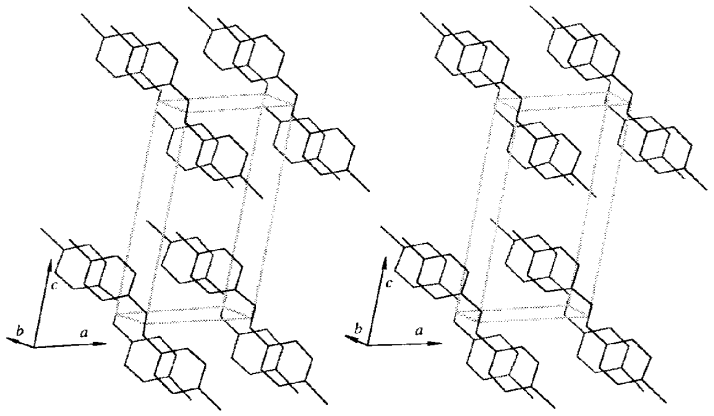


Figure 10.8 Stereoview of 4-(*N*-chlorobenzylidene)-4-chloroaniline triclinic polymorph (Bernstein and Hagler, 1978).

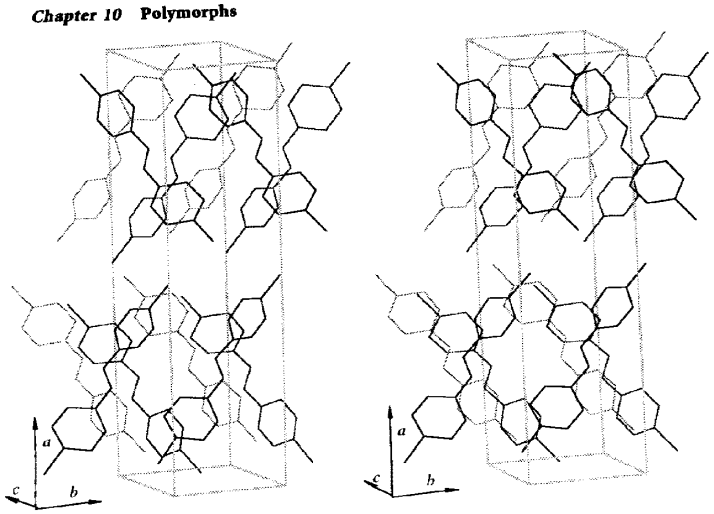
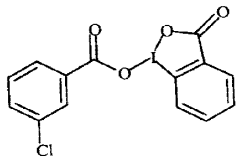


Figure 10.9 Crystal packing stereoview of 4-(*N*-chlorobenzylidene)-4-chloroaniline orthorhombic form. (Bernstein and Hagler, 1978).

showed that the nonplanar conformation was energetically favored by perhaps 2.09–6.28 kJ/mol but the lattice-energy calculations, using semiempirical potential functions, showed that the planar structure (triclinic form) gave a lower lattice energy by about 4.19 kJ/mol. These calculations explain why the triclinic polymorph is the stable crystalline polymorph even though it contains the less stable (planar) conformer.

Programs that calculate the packing energy are now available, for example, *Cerius*[2] (Molecular Simulations, Inc., 1997). These programs alone or in combination with structure elucidations based on powder diffraction data will provide new approaches to the structure analysis of materials when suitable single crystals are not available.

D. 3-Oxo-3*H*-2,1-benzoxiodol-1-yl 3-Chlorobenzoate



3-oxo-3*H*-2,1-benzoxiodol-1-yl 3-chlorobenzoate

As part of their extensive study of the crystal chemistry of iodoperoxides, Gougoutas and Lessinger (1974) determined the crystal structure of two polymorphs of 3-oxo-3*H*-2,1-benzoxiodol-1-yl 3-chlorobenzoate. This compound crystallizes in α- and β-forms that both belong to the monoclinic crystal system (Table 10.4).

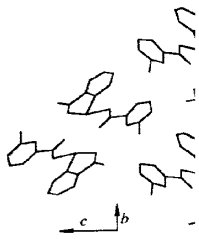


Figure 10.10 The crystal pa
(Gougoutas and

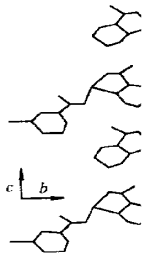


Figure 10.11 The crystal
(Gougoutas and

Table 10.4 Crystallographic
2,1-benzoxiodol-

Parameter
Space Group
a (Å)
b (Å)
c (Å)
β
Z
ρ_{calc} (g cm^{-3})
V (Å^3)

Gougoutas and Lessinger, 19

The α-form is essent
rings make an angle of a
two forms is also quite d

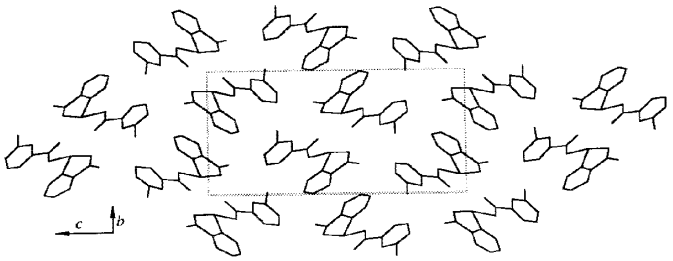


Figure 10.10 The crystal packing of 3-oxo-3*H*-2,1-benzoxiodol-1-yl 3-chlorobenzoate α-form (Gougoutas and Lessinger, 1974).

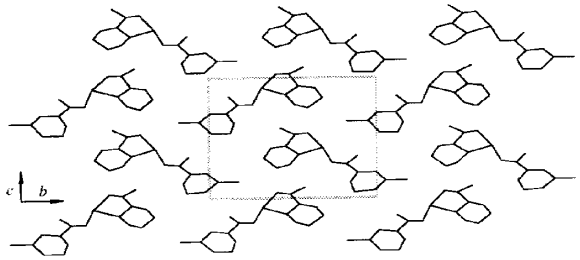


Figure 10.11 The crystal packing of 3-oxo-3*H*-2,1-benzoxiodol-1-yl 3-chlorobenzoate β-form (Gougoutas and Lessinger, 1974).

Table 10.4 Crystallographic Unit Cell Parameters for 3-Oxo-3*H*-2,1-benzoxiodol-1-yl 3-Chlorobenzoate

Parameter	α-Form	β-Form
Space Group	$P2_1/n$	Pc
a (Å)	6.376	5.057
b (Å)	10.547	13.035
c (Å)	20.066	10.339
β	92.0°	99.5°
Z	4	2
ρ_{calc} (g cm^{-3})	1.984	2.009
V (Å^3)	1348.6	672.2

Gougoutas and Lessinger, 1974.

The α-form is essentially planar in the crystal while in the β-form the two phenyl rings make an angle of approximately 55° with each other. The crystal packing of the two forms is also quite different as shown in Figures 10.10 and 10.11. These two

forms have different solid-state infrared spectra (see Figure 10.12), as expected since the molecule is in different conformation in the two crystal forms.

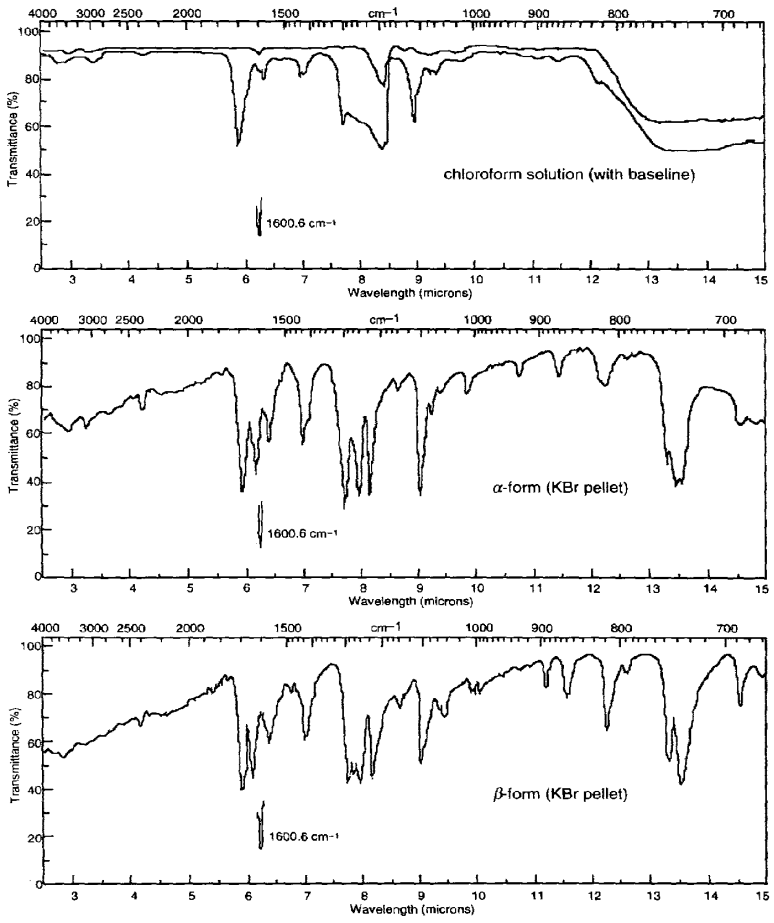
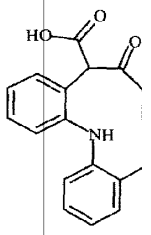


Figure 10.12 Infrared spectra of 3-oxo-3*H*-2,1-benzoxiodol-1-yl 3-chlorobenzoate (Gougoutas and Lessinger, 1974).

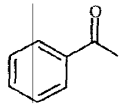
E. TAUTOMERIZATION



keto for
3-(4-chlorop
2-[2-(2-(methoxyca
amino)phenyl]-3-oxo

Schulenberg (1968) ha
phenyl)amino)phenyl]-3
form has a melting point
consistent with the
phenyl)amino)phenyl]-3
110–122 °C and upon di
(4-chlorophenyl)-3-hydr
acid. Addition of triethy
ing 70% of the keto forn

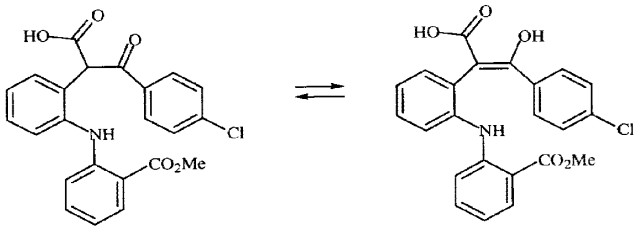
Although the crysta
mined, this study illustr
containing an individual
phism (*cf.* p. 143).



E-conformer of t
1,3-diphenylprop

Several other case
enol of 1,3-diphenylpro
the *E*-isomer and the ot
there are numerous exa
isomer or tautomer out
(1972).

E. Tautomerizational Polymorphism

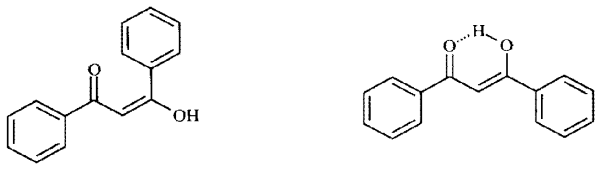


keto form
3-(4-chlorophenyl)-2-[2-(2-(methoxycarboxyphenyl)-amino)phenyl]-3-oxopropanoic acid

enol form
3-(4-chlorophenyl)-3-hydroxy-2-[2-(2-(methoxycarboxyphenyl)-amino)phenyl]propenoic acid

Schulenberg (1968) has reported that 3-(4-chlorophenyl)-2-[2-(2-(methoxycarboxyphenyl)amino)phenyl]-3-oxopropanoic acid crystallizes in two tautomeric forms. One form has a melting point of 93–99 °C that upon dissolution in $CDCl_3$ gave NMR spectra consistent with the keto form, 3-(4-chlorophenyl-2-[2-(2-(methoxycarboxyphenyl)amino)phenyl]-3-oxopropanoic acid. The other form had a melting point of 110–122 °C and upon dissolution gave NMR spectra consistent with the enol form, 3-(4-chlorophenyl)-3-hydroxy-2-[2-(2-(methoxycarboxyphenyl)amino)phenyl]propenoic acid. Addition of triethylamine to either solution gave an equilibrium mixture containing 70% of the keto form and 30% of the enol form.

Although the crystal structures of the keto and enol forms have not been determined, this study illustrates a case in which two different crystalline forms exist, each containing an individual tautomer. This situation is termed tautomerizational polymorphism (*cf.* p. 143).



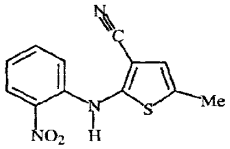
E-conformer of the enolate of
1,3-diphenylpropane-1,3-dione

Z-conformer of the enolate of
1,3-diphenylpropane-1,3-dione

Several other cases of tautomerizational polymorphism exist. For example, the enol of 1,3-diphenylpropane-1,3-dione crystallizes in two forms. One form contains the *E*-isomer and the other contains the *Z*-isomer (Eistert *et al.*, 1952). In addition, there are numerous examples of the crystallization process freezing one configurational isomer or tautomer out of solution. These cases are reviewed by Curtin and Engelmann (1972).

F. Polychromism

One of the most striking differences in physical properties among polymorphs is **polychromism** (*i.e.*, different colors). Polychromism has been reported for only a limited number of cases. Dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate, for example, crystallizes in yellow, light-yellow, and white polymorphs (Byrn *et al.*, 1972; Fletton *et al.*, 1986; Yang *et al.*, 1989; Richardson *et al.*, 1990). The colors of these three polymorphs are attributed to differences in orientation of the carboxylate group with respect to the aromatic ring (see also Sections 10.7E and 20.1A).



5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile
(ROY)

5-Methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile is a dramatic example of polychromism. Crystallization of this compound from ethanol yields a mixture of yellow and red prisms, whereas crystallization from methanol yields orange needles; hence the alias ROY for the red, orange, and yellow forms (Borchardt, 1997). Crystals of the red form also appear to be **pleochroic**, displaying both red and orange colors under polarized illumination.

The three polymorphs are free of solvent and stable at room temperature. The red, orange, and yellow forms are similar in energy with melting points of 106.2, 114.8, and 109.8 °C, respectively (Yu, 1998). The red and orange forms undergo solution-mediated transformation to the yellow form at room temperature, indicating the latter is the most stable at room temperature. The yellow and orange forms are related enantiotropically, with yellow being more stable at low temperature. Between room temperature and the melting point, the red form is always less stable than the yellow form. The heats of melting, as measured by DSC, confirmed these stability relationships. Solid-state phase transitions from red to yellow and from red to orange have been observed between 70—90 °C in a solvent free environment. The transition from red to yellow (at temperatures greater than 90 °C) results in a dramatic change in color but no apparent change in crystal morphology, whereas the transition from red to orange leads to the growth of orange needles from the initial red crystals.

The crystal structures of red, orange, and yellow forms have been determined by single-crystal X-ray diffraction and show that the molecule adopts a dramatically different conformation in each of the forms. Subsequent studies show that these different conformations are the reasons for the different colors. Hydrogen bonding in the polymorphs is exclusively intramolecular—between the adjacent amine and nitro substituents. The heteroatom-to-heteroatom distances of the hydrogen bond in red, orange, and yellow are 2.636(2), 2.607(3), and 2.625(3) Å, respectively. The conformations of the molecule in the three polymorphs are significantly different (Figure 10.13). In the yellow and orange forms, the nitro group is essentially co-planar with the phenyl ring, whereas in the red form it is twisted out-of-plane by 18°. The color of the polymorphs may be related to the degree of electron delocalization, which is related to the angle between the planes of the phenyl and the thiophene moieties (red 46°,

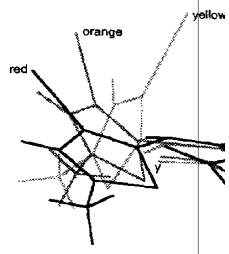


Figure 10.13 Conformations
crystalline form

orange 54°, and yellow 1(
order of the expected w
Section 8.1). Studies ha
direct result of the differer
1998; Yu, 1998). The ol
those calculated from the
^{13}C CP/MAS solid-st
tinguish the polymorphs.
reported for polymorphic
shifts of C3 (the carbon in
97.9, 105.2, and 109.3
covering a range of 11
104.41 ppm in solution.)
red form with respect to t
conjugation effect. Smitl
(total suppression of spin
shift anisotropy (CSA) o
increases in magnitude by
ric as the coplanar angle
electrons between the tw
site.

This parallels the re
quency are 2211, 2223,
tively (see Section 8.1).
the red form from a highe
vations confirm the signi
pronounced color change

A number of deriva
nitrile were synthesized
nitrophenylaminothiophe
Me) crystallized in three
the gold form were un
polymorph" class. How

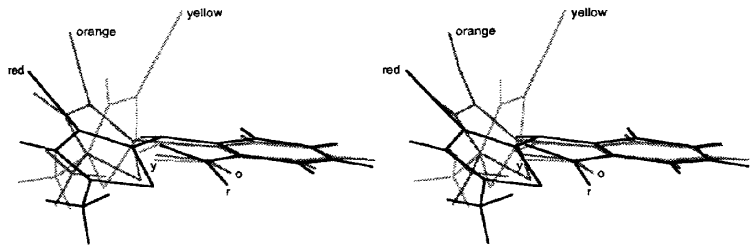


Figure 10.13 Conformations of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile in three crystalline forms.

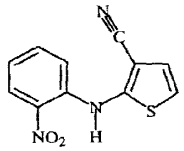
orange 54°, and yellow 106°). The order of these angles appears to correlate with the order of the expected wavelengths of absorption by the colored polymorphs (see Section 8.1). Studies have shown that the different colors of the polymorphs are a direct result of the difference in molecular conformation (Borchardt, 1997; Smith *et al.*, 1998; Yu, 1998). The observed XRPD patterns of the three polymorphs agree with those calculated from the single-crystal structures.

^{13}C CP/MAS solid-state NMR, solid-state FT-IR, and XRPD can be used to distinguish the polymorphs. The observed spectral differences are among the largest reported for polymorphic organic compounds. For example, the ^{13}C NMR chemical shifts of C3 (the carbon in the thiophene ring to which the nitrile group is attached) are 97.9, 105.2, and 109.3 ppm for the red, orange, and yellow forms, respectively, covering a range of 11.4 ppm. (For comparison, the chemical shift of C3 is 104.41 ppm in solution.) This indicates an increase in the electron density of C3 in the red form with respect to the yellow and orange forms, possibly a result of an increased conjugation effect. Smith and coworkers (1998) have used a two-dimensional TOSS (total suppression of spinning sidebands) pulse sequence to investigate the chemical-shift anisotropy (CSA) of C3. These studies show that the extent of the CSA for C3 increases in magnitude by 30 ppm and the line shape appears to become more asymmetric as the coplanar angle increases. This was taken to reflect a greater transfer of π electrons between the two ring systems and hence a greater electron density at the C3 site.

This parallels the results from IR spectroscopy in which the nitrile stretching frequency are 2211, 2223, and 2231 cm^{-1}, for the red, orange, and yellow forms, respectively (see Section 8.1). This shift is indicative of the decreased nitrile bond strength in the red form from a higher degree of conjugation with the aromatic ring. These observations confirm the significant changes in the electronic structure, as demonstrated by pronounced color changes among different polymorphs.

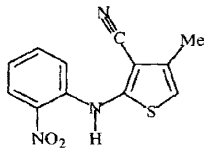
A number of derivatives of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile were synthesized in order to determine the extent of the color polymorphism of nitrophenylaminothiophenes. 2-[(2-Nitrophenyl)amino]-3-thiophenecarbonitrile (NorMe) crystallized in three forms: red, orange, and gold. Numerous attempts to obtain the gold form were unsuccessful thus placing the gold from in the "disappearing polymorph" class. However, crystallization of a newly synthesized lot of NorMe gave

the gold form once again only to disappear when the material was subjected to further crystallization and handling. As with other disappearing polymorphs, this behavior is due to the presence of impurities and the fact that the gold polymorph is unstable in the presence of seeds of the other forms (Dunitz and Bernstein, 1995).

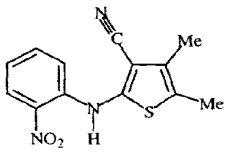


2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (NorMe)

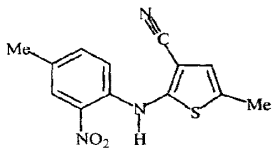
The XRPD patterns of the three forms of NorMe are different from the parent compound. The crystal structure of the red form NorMe was determined (Borchardt, 1997). The red form is nearly coplanar further substantiating the concept that the red color is associated with planarity. The IR spectra of the NorMe polymorphs are quite similar to ROY. The red form has a nitrile stretching absorption at 2210 cm^{-1}, the orange is a 2222 cm^{-1}, and the yellow at 2230 cm^{-1}.



4-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (4-Me)



4,5-dimethyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (4,5-DiMe)



5-methyl-2-[(4-methyl-2-nitrophenyl)amino]-3-thiophenecarbonitrile (4'-Me)

The conformation of the red form of 4-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (4-Me) is the most coplanar of the structures determined (see Figure 10.14). 4,5-Dimethyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (4,5-DiMe) crystallized in two polymorphs: red and orange. As with the previous derivatives, the conformation of the red form as determined by single-crystal X-ray methods is rather coplanar (see Figure 10.14). 5-Methyl-2-[4-methyl-2-nitrophenyl)amino]-3-thiophenecarbonitrile (4'-Me) was crystallized in red, dark red, light red, and orange forms. Only the red form gave crystals suitable for structure determination. As with the previous derivatives, this red form has a nearly coplanar conformation. Figure 10.14 compares the conformation of the various red forms in this nitrophenylaminothiophene series. In all cases, the red form has the most coplanar conformation of the polymorphs. This further supports the conclusion that the conformation of the nitrophenylaminothiophene determines the color of the polymorph.

Griesser and He (1998) have carried out a preliminary study of the solubilities and interconversions of the four forms of 4'-Me and found that all four forms are within 4 kJ/mol or less of each other in energy. These studies allowed the development of the energy–temperature diagram (see Section 5.2) shown in Figure 10.15. Such diagrams

are extremely useft
polymorphs.

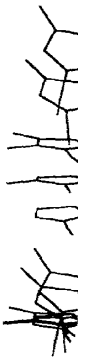


Figure 10.14 Stereovie
the thiop
Hydroger

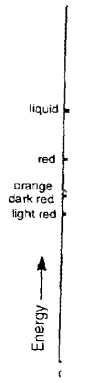


Figure 10.15 Energy–t
phenyl)a

are extremely useful in visualizing the energy–temperature relationships between polymorphs.

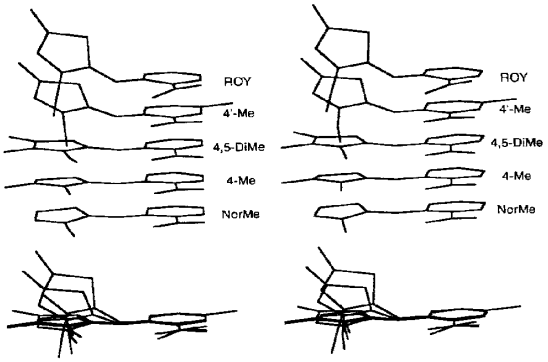


Figure 10.14 Stereoview showing a comparison (both stacked and overlayed) of the conformations of the thiophene and phenyl rings in the nitrophenylaminothiophene series red forms. Hydrogens were omitted for clarity.

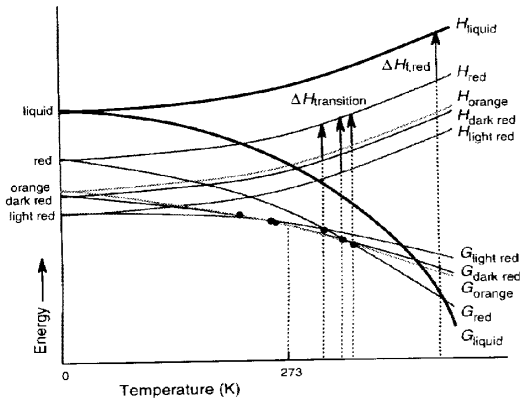


Figure 10.15 Energy–temperature diagram for the four forms of 5-methyl-2-[4-methyl-2-nitrophenyl)amino]-3-thiophenecarbonitrile (Griesser and He, 1998).

10.3 SULFONAMIDES

The polymorphism of sulfonamides has been investigated and reviewed by Kuhnert-Brandstätter (1971). These studies were carried out using microscopy on a Kohfler hot stage (see Section 4.4). Sulfonamides exhibited behavior expected of polymorphs, including successive melting points as the temperature is raised and changes in color under crossed Nicol gratings (crossed polarizers). Table 10.5 summarizes the results of Kuhnert-Brandstätter's (1971) studies on these compounds.

Although all of these studies have not been confirmed by crystallographic data, the crystal structures of several polymorphs of sulfonamides have been determined and will

Table 10.5 Polymorphism of Sulfonamides and Related Compounds[a]

Compound	Melting Point of Form (°C)						
	I	II	III	IV	V	VI	VII
Acetazolamide	258-260	248-250					
Acetyl Sulfisoxazole	190-195	176-177	173-174				
Chlorthalidone	212-224	188-189					
Clofenamide	210-215	203-207	183-185	168-170			
Diphenylmethane-4,4'-disulfonamide	185-187	172-174					
Mafenide HCl	250-260	235-240	220-225	210-212			
4'-(Methylsulfamoyl)-sulfanilanilide	148-151	144-146					
Phthalylsulfathiazole	260-274	230					
Sulfachlorpyridazine	196-197	178-181					
Sulfadicramide	176-180	174-176					
Sulfadimethoxine	194-198	176-177	156-158				
Sulfaethidole	188	181	149				
Sulfaguanidine	187-191	174-176	143-145				
Sulfameline	210-212	197-199	181-183	179-181	176-177	155	
Sulfamerazine	235-238	228					
Sulfamethazine	206-208	199	178	~175			
Sulfamethizole	209	193					
Sulfamethoxazole	169	168	166				
Sulfamethoxypyridazine	180-182	158-159	153-154				
Sulfamidochrysoidine	224-228	217-219	212				
Sulfamoxole	200-204	188-195	177-180				
Sulfanilamide	165	156	153				
N-Sulfanilyl-3,4-xylamide	215-218	208	203	196			
Sulfapyridine	192	185	179	176	174	167	149
Sulfathiazole	202	175	162	158			
Sulfathiourea	178-180	168-171					
Sulfatriazine	158-166	132-135					
Sulfazamet	182-185	176-178					
Sulfisoxazole	190-195	131-133					
Tolbutamide	127	117	106				

[a] Kuhnert-Brandstätter (1971).

be discussed next. In gen
polymorphs. Thus, in th
ble for polymorphism.

A. SULFANILAMIDE

NH

Sulfanilamide exists in th
ters shown in Table 10.6
(O'Conner and Maslen,
phenyl rings. In each sta
···amino···sulfonamide··
substituent in each stack.

The crystal packing
the α-form (Allcaume an
but the order of the s
···amino···sulfonamide··
stack.

The crystal packing
10.18 appears, in genera
sulfonamide amino grou
successive rings in a st
which resembles that of t

The density of the
(see Table 10.6). The p
sulfanilamide have been
diagram constructed. It i
group is similar in all for
plane of the phenyl ring
relationships between th
depicted in Figures 10.1
10.19.

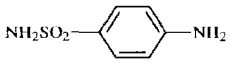
Table 10.6 Crystallographi

Parameter	
Space group	
a (Å)	
b (Å)	
c (Å)	
β	
Z	
ρ_{calc} (g cm^{-3})	
V (Å^3)	

O'Conner and Maslen, 1965

be discussed next. In general, the conformations of the drug are similar in the different polymorphs. Thus, in these cases, differences in crystal packing are mainly responsible for polymorphism.

A. Sulfanilamide



sulfanilamide

Sulfanilamide exists in three crystalline forms which have the crystallographic parameters shown in Table 10.6. The α-form has the crystal packing shown in Figure 10.16 (O'Conner and Maslen, 1965). The crystal packing of this form contains layers of phenyl rings. In each stack, the order of the substituent groups on successive rings is ···amino···sulfonamide···sulfonamide···amino···, etc., resulting in alternating pairs of substituent in each stack.

The crystal packing of the β-form shown in Figure 10.17 is quite different from the α-form (Alleaume and Decap, 1965). There are, again, columns of phenyl rings but the order of the substituent groups on successive rings is ···sulfonamide··· ···amino···sulfonamide···amino···, etc., resulting in alternating substituents in the stack.

The crystal packing of the γ-form (Alleaume and Decap, 1966) shown in Figure 10.18 appears, in general, to be similar to the α-form with layers of phenyl rings and sulfonamide amino groups. In these columns, the order of substituent groups on successive rings in a stack is ···amino···sulfonamide···amino···sulfonamide···, etc., which resembles that of the β-form.

The density of the β-form (the most thermodynamically stable form) is greatest (see Table 10.6). The polymorphic interconversions and thermodynamic properties of sulfanilamide have been investigated by Burger (1973a–b) and an energy-temperature diagram constructed. It is interesting to note that the conformation of the sulfanilamide group is similar in all forms, with the nitrogen atom being the atom furthest out of the plane of the phenyl ring. A comparison of the α-, β-, and γ-forms showing the relationships between the arrangement of the substituents in successive molecules depicted in Figures 10.16, 10.17, and 10.18 is illustrated in a stereoview in Figure 10.19.

Table 10.6 Crystallographic Data for the Polymorphs of Sulfanilamide

Parameter	Form α	Form β	Form γ
Space group	*Pbca*	$P2_1/c$	$P2_1/c$
a (Å)	5.65	8.98	7.95
b (Å)	18.51	9.01	12.95
c (Å)	14.79	10.04	7.79
β	90.00°	111.43°	106.50°
Z	8	4	4
ρ_{calc} (g cm^{-3})	1.47	1.51	1.49
V (Å^3)	1547.1	755.2	768.7

O'Conner and Maslen, 1965

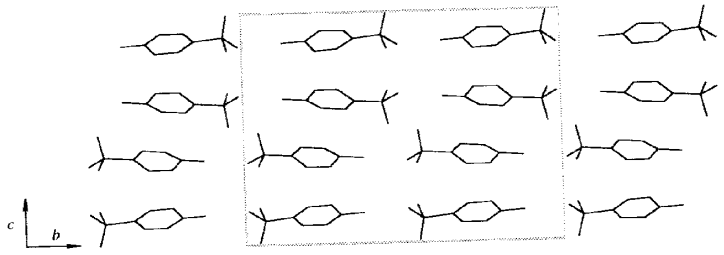


Figure 10.16 Molecular packing of the α-form of sulfanilamide (O'Conner and Maslen, 1965).

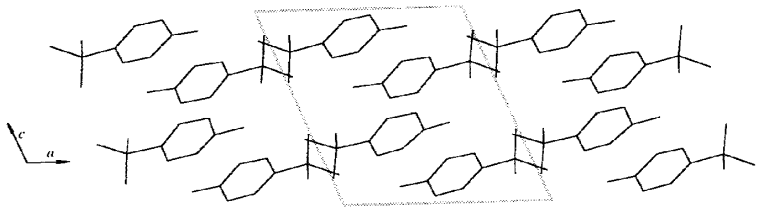


Figure 10.17 The crystal packing of the β-form of sulfanilamide (Alleaume and Decap, 1965).

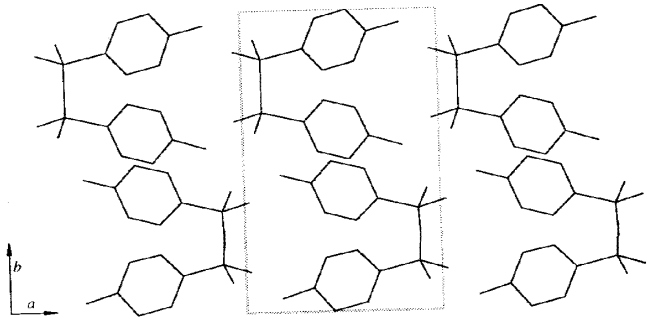


Figure 10.18 Crystal packing of the γ-form of sulfanilamide (Allcaume and Decap, 1966).

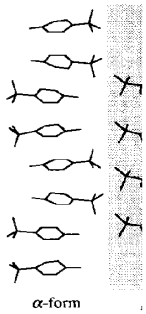


Figure 10.19 Stereovie
α-, β-, ar

B. Sulfathiazole

NH_2

Table 10.7 indicates
(1983) have studied
the four polymorphs.
dynamically stable at
of all three polymorp
mide group is the at
This is in marked si
molecule in all three
between these forms

Table 10.7 Crystallogr

Parameter
Space Group
a (Å)
b (Å)
c (Å)
β
Z
ρ_{meas} (g cm^{-3})
V (Å^3)
Habit
Melting point
Transition point

a Kruger and Gafner, 19

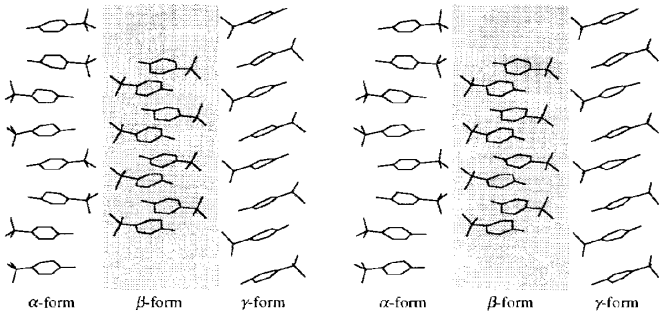
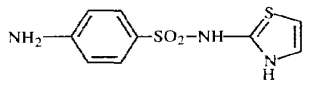


Figure 10.19 Stereoview showing the molecular arrangement of sulfanilamide columns in the α-, β-, and γ-forms.

B. Sulfathiazole



sulfathiazole

Table 10.7 indicates that sulfathiazole exists in four polymorphs. Burger and Dialer (1983) have studied this system and have produced an energy-temperature diagram of the four polymorphs. Form I is the least stable of the four forms; Form III is thermodynamically stable at room temperature. Figures 10.20–10.22 show packing drawings of all three polymorphs of sulfathiazole. It is obvious that the nitrogen of the sulfonamide group is the atom that is the greatest distance from the plane of the phenyl ring. This is in marked similarity to sulfanilamide. In addition, the conformation of the molecule in all three forms is very similar. The major crystallographic difference between these forms is the nature and type of hydrogen bonds.

Table 10.7 Crystallographic Parameters for the Polymorphs of Sulfathiazole

Parameter	Form I[a]	Form II[b]	Form III[a]
Space Group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a (Å)	10.554	8.235	17.570
b (Å)	13.220	8.550	8.574
c (Å)	17.050	15.558	15.583
β	108.06°	93.67°	112.93°
Z	8	4	8
ρ_{meas} (g cm^{-3})	1.50	1.55	1.57
V (Å^3)	2261.7	1093.2	2162.0
Habit	Rods	Hexagonal prisms	Hexagonal plates
Melting point	200-202	200-202	173-175 (or 200-202)
Transition point	...	173-175	173-175

a Kruger and Gafner, 1971a. *b* Kruger and Gafner, 1971b.

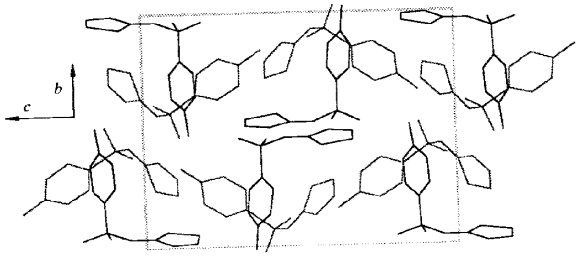


Figure 10.20 Crystal packing of sulfathiazole Form I (Kruger and Gafner, 1971a).

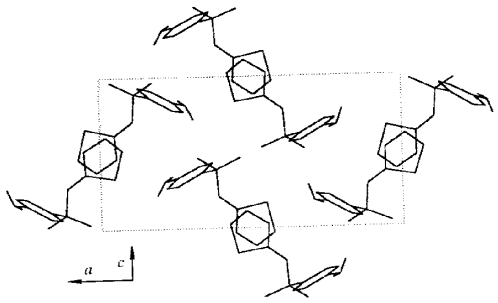


Figure 10.21 Crystal packing of sulfathiazole Form II (Kruger and Gafner, 1971b).

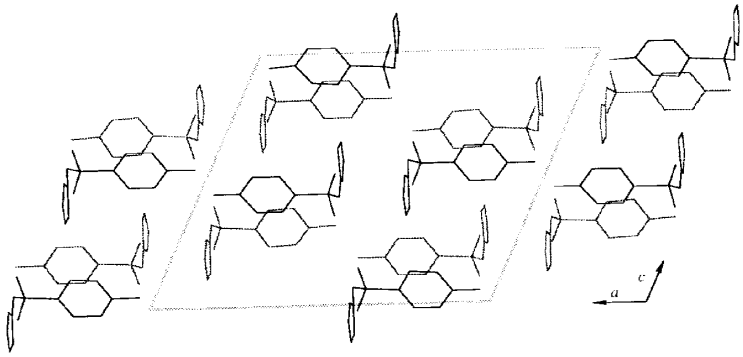


Figure 10.22 Crystal packing of sulfathiazole Form III (Kruger and Gafner, 1971a).

Table 10.8 Dissolution Rate

Temperature (°C)	Form (mg cm^{-2}
59.1	0.18
48.8	0.10
39.4	0.05
29.6	0.03
24.1	0.02
20.4	0.02

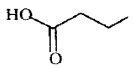
Milosovich, 1964.

The crystallographic
morphs of sulfathiazole; I
polymorphism of this dru
Kuhnert-Brandstätter rep
stage microscopy. In th
lory (1967), and Higuchi
Shenouda (1970) also ir
Mesley (1971) using IR,
of three polymorphs. H
with mixtures of the three
these findings and charac
microscopy, solubility, a

To avoid prolonged
involve separation of hal
each habit. X-ray powd
crystal X-ray data and
approach would make su

The physical propert
and Eisen, 1971; Miloso
the dissolution rate under
results in Table 10.8 sho
solubility than Form I. T
II should have a slower d

C. Succinylsulfathi



In early studies of succi
and Higuchi, 1963) a lar

Table 10.8 Dissolution Rate and Solubility of Forms I and II of Sulfathiazole

Temperature (°C)	Dissolution Rate		Solubility	
	Form I ($mg\ cm^{-2}\ sec^{-1}$)	Form II ($mg\ cm^{-2}\ sec^{-1}$)	Form I (g/1000 gm)	Form II (g/1000 gm)
59.1	0.185	0.239	31.5	40.7
48.8	0.102	0.145	19.8	28.1
39.4	0.0598	0.0913	14.0	21.4
29.6	0.0355	0.0597	9.93	16.7
24.1	0.0237	0.0413	8.15	14.2
20.4	0.0201	0.0371	7.10	13.1

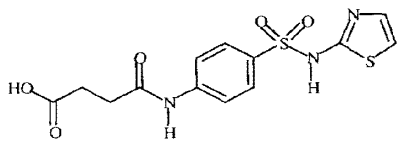
Milosovich, 1964.

The crystallographic data clearly established the existence of at least four polymorphs of sulfathiazole; however, at this point, it is worthwhile to review studies of the polymorphism of this drug using other techniques. As reported earlier in this section, Kuhnert-Brandstätter reported that sulfathiazole has four polymorphs based on hot stage microscopy. In the 1960's, three groups of workers [Milosovich (1964), Guillory (1967), and Higuchi *et al.* (1967)] reported only two polymorphs. DSC work by Shenouda (1970) also indicated the existence of only two polymorphs. Studies by Mesley (1971) using IR, DSC, and X-ray powder diffractometry showed the existance of three polymorphs. He suggested that most of the earlier workers had been dealing with mixtures of the three polymorphic forms. Burger and Dialer (1983) reinvestigated these findings and characterized four polymorphs by IR-spectroscopy, DSC, thermomicroscopy, solubility, and density.

To avoid prolonged confusion of this sort, studies of unfamiliar systems should involve separation of habits under a microscope and then crystallographic studies of each habit. X-ray powder diffraction patterns should be calculated from the single crystal X-ray data and compared with the experimentally observed XRPDs. This approach would make sure that mixtures of polymorphs are not involved.

The physical properties of sulfathiazole Forms I and II have been studied (Sunwoo and Eisen, 1971; Milosovich, 1964). These studies, which used a flow cell, measured the dissolution rate under conditions where Form II did not transform to Form I. The results in Table 10.8 show that Form II has a significantly higher dissolution rate and solubility than Form I. This is not consistent with the densities which predict that Form II should have a slower dissolution rate and be less soluble than Form I.

C. Succinylsulfathiazole



succinylsulfathiazole

In early studies of succinylsulfathiazole (Armour Research Foundation, 1949; Shefter and Higuchi, 1963) a large number of different crystal forms were found. The studies

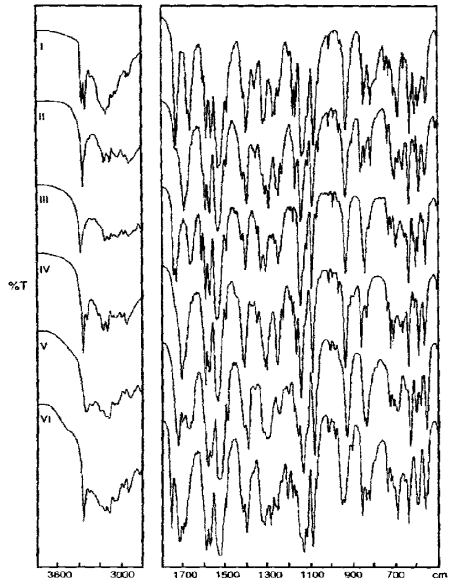


Figure 10.23 IR spectra (KBr pellets) of the unsolvated crystal forms of succinylsulfathiazole (Burger and Griesser, 1989).

by Burger and Griesser (1989; 1991) provide the most complete summary of the solid-state behavior of this compound. As summarized in Table 10.9, they found that succinylsulfathiazole crystallized in six anhydrous crystal forms, three polymorphic monohydrates, as well as an acetone solvate and an *n*-butanol solvate. These different crystal forms were prepared by a variety of methods involving crystallization from different solvents and by drying the different solvates. For example, Form IV was prepared by drying the acetone solvate at 150 °C. Form VI was prepared by dehydration of one of the monohydrates in vacuum at 100 °C. The three monohydrates are termed "polymorphic" because they contain the same chemical composition (compound and solvent) but exist in different crystal structures. The IR spectra of all eleven crystal forms were measured in KBr pellets. The polymorphs and solvates were also characterized by thermal microscopy and DSC. Figure 10.23 shows the IR spectra of the six unsolvated crystal forms and Figure 10.24 shows the DSC thermograms of these polymorphs. The IR spectra of the different crystal forms are different and indicate that these are different polymorphs. The DSC thermograms of Forms I through V show destinctive differences in melting points. The DSC thermogram of Form VI shows an incongruent melting process. However, IR appears to be better than DSC for distinguishing these forms. Figure 10.25 shows the X-ray powder diffraction patterns of the six crystal forms which are all different and confirm the IR results.

Table 10.9 Comparisor of Succinyl

Form	Stability (20 °C)	
I	Stable[a]	Suspen solv
II	< I	Evapor EtO
III	< II	Dehydr °C
IV	< III	Suspens EtO
V	< IV	Anneali 160
VI	< V	Dehydra
H_I	Stable	Suspens water
H_{II}	< H_I	Crystalli
H_{III}	< H_{II}	Suspens for 1

a in the absence of water. water at 20 °C. (Burger a

Figure 10.24 DSC therr Griesser, 1

Table 10.9 Comparison of the Physical Properties of the Polymorphic Anhydrates and Monohydrates of Succinylsulfathiazole

Form	Stability (20 °C)	Preparation	MP[b] (°C)	MP[c] (°C)	1st Peak in IR (cm^{-1})	Density ($g\ cm^{-3}$)	Solubility[d] Ratio to H_I
I	Stable[a]	Suspension of acetone solvate in EtOAC	204	205	3361	1.592	3.24
II	< I	Evaporation of absolute EtOH solution	195-199	195	3360	1.535	5.69
III	< II	Dehydration of H_I at 100 °C	189-194	188-191	3372	1.571	6.15
IV	< III	Suspension of V or VI in EtOAC	187-191	189	3338	1.518	9.26
V	< IV	Annealing of I at 160 °C	182-185	182-187	3330	1.488	~12.7
VI	< V	Dehydration of H_{II}	139-143	135-138	3350	1.463	—
H_I	Stable	Suspension of any form in water	123-125		3480 (OH) 3320 (NH)	1.527	1.00
H_{II}	< H_I	Crystallization from water	~110		3500 (OH) 3350 (NH)	1.520	1.81
H_{III}	< H_{II}	Suspension of III in water for 15 min	105		3450 (OH) 3335 (NH)		

a in the absence of water. *b* by thermomicroscopy. *c* by differential scanning calorimetry (DSC). *d* in water at 20 °C. (Burger and Griesser, 1991)

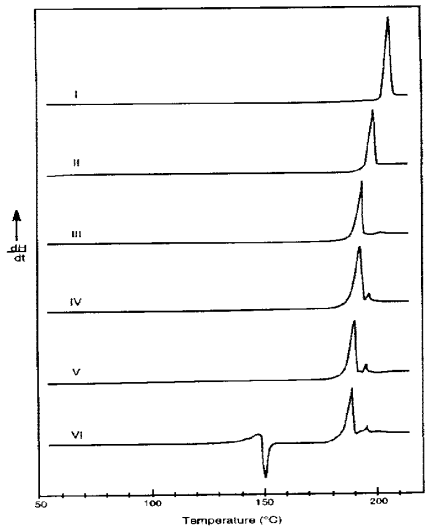


Figure 10.24 DSC thermograms of the unsolvated crystal forms of succinylsulfathiazole (Burger and Griesser, 1989).

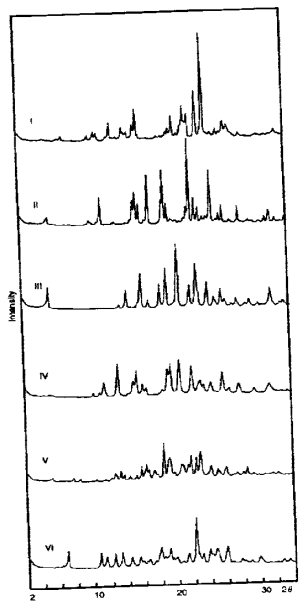


Figure 10.25 X-ray powder diffraction patterns of the six unsolvated crystal forms of succinylsulfathiazole (Burger and Griesser, 1989).

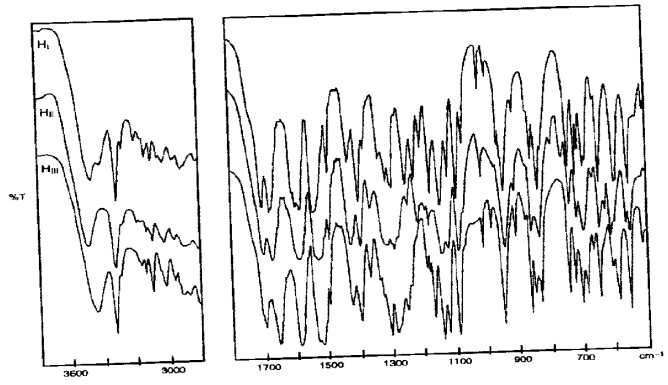


Figure 10.26 IR spectra of the polymorphic monohydrates of succinylsulfathiazole (Burger and Griesser, 1989).

Figure 10.26 shows
sulfathiazole. The IR sp
are different polymorph
tion patterns shown in F

The physical stabili
of succinylsulfathiazole
Figure 10.28. The mo
variety of methods used
crystal forms have diff
high humidity. The solu

Figure 10.27 X-ray powde
(Burger and

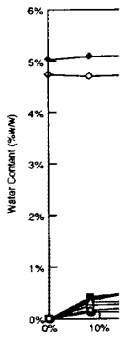


Figure 10.28 Water vapor
(Burger and

Figure 10.26 shows the IR spectra of the polymorphic monohydrates of succinylsulfathiazole. The IR spectra of these materials are also different establishing that these are different polymorphs. This conclusion is confirmed by the X-ray powder diffraction patterns shown in Figure 10.27.

The physical stability, water sorption, and solubility of the different crystal forms of succinylsulfathiazole have also been studied and are summarized in Table 10.9 and Figure 10.28. The most stable forms are Form I and hydrate H_I. In addition, the variety of methods used to prepare the different crystal forms are noted. The different crystal forms have differences in hygroscopicity and interconvert in the presence of high humidity. The solubilities of the different forms are also different. Most notable

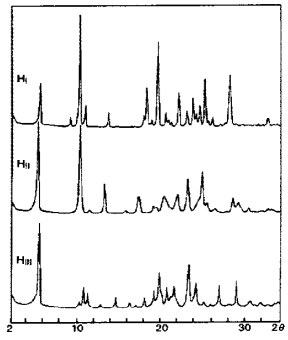


Figure 10.27 X-ray powder diffraction patterns of the three monohydrates of succinylsulfathiazole (Burger and Griesser, 1989).

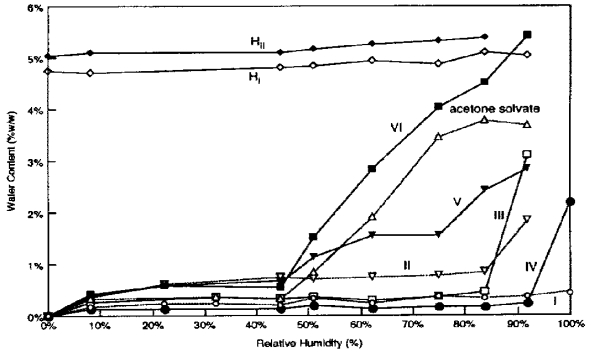


Figure 10.28 Water vapor sorption isotherms of the different crystal forms of succinylsulfathiazole (Burger and Griesser, 1991).

ns of succinylsulfa-

hiazole (Burger and

is that the differences in solubility among the anhydrate crystal forms is as large as a factor of 4 and that differences in solubility between anhydrate and hydrate crystal forms are as large as a factor of 12. This is one of many cases where anhydrate crystal forms have significantly higher solubilities than the hydrate.

Figure 10.28 shows the water vapor sorption isotherms for the different succinylsulfathiazole crystal forms. It is clear that some of the anhydrate forms absorb water relatively easily; furthermore, this data shows that the metastable forms are more hygroscopic.

Figure 10.29 shows the dissolution behavior of the different crystal forms of succinylsulfathiazole in buffer solution at pH 1.20 at 20 °C. It is clear that at equilibrium many of the anhydrates recrystallize and approach the solubility of the hydrates as might be expected. Figure 10.30 shows a van't Hoff plot for four of the crystal forms of succinylsulfathiazole. These curves do not cross in the temperature ranges studied and this indicates, in connection with the thermodynamic data, that all of the forms are monotropically related. Recall that monotropic forms retain the order of stability at all temperatures (see Section 5.2).

Figure 10.31 shows a scheme which illustrates the interconversion of the different crystal forms and methods to prepare each form. This figure illustrates how complicated interconversion of the different crystal forms can be. The van't Hoff plot clearly shows that the transformation of the more soluble form into the less soluble hydrate will occur at room temperature. This indicates the complications that can arise by relying on just one study and shows that several different approaches should be used to try to understand the interconversion of different crystal forms.

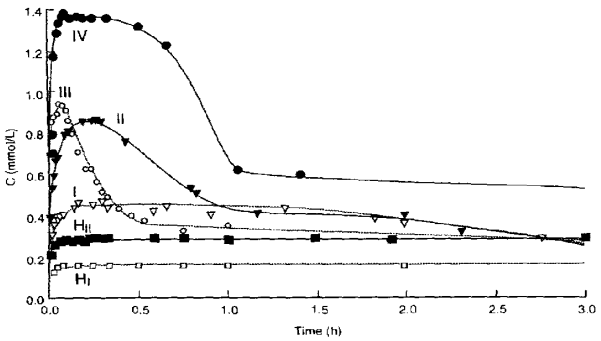


Figure 10.29 Dissolution behavior of the different crystal forms of succinylsulfathiazole in buffer solution, pH 1.3 at 20 °C (Burger and Griesser, 1991).

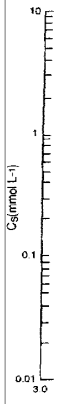


Figure 10.30 Van't Hoff pl
pH 1.3 (Burge

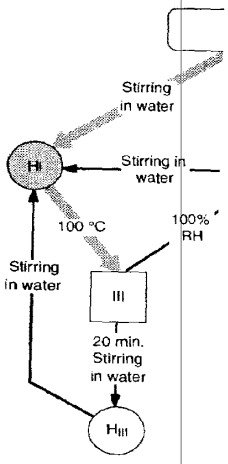


Figure 10.31 Diagram illu
produce the
mark paths w
most stable f

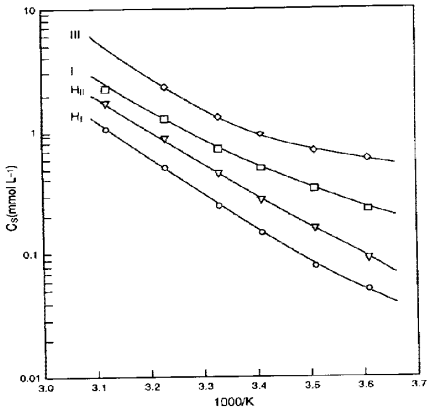


Figure 10.30 Van't Hoff plot of the solubility of four of the crystal forms of succinylsulfathiazole at pH 1.3 (Burger and Griesser, 1991).

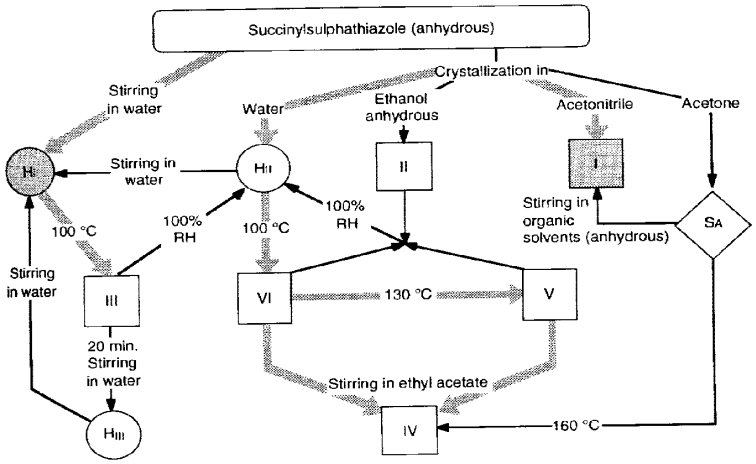
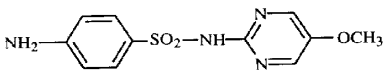


Figure 10.31 Diagram illustrating the most important transformation paths and production ways to produce the different crystal forms of succinylsulfathiazole. The thick, gray arrows mark paths whereby the different crystal forms can be produced in gram quantities. The most stable forms, Forms I and H_I, are shaded (Burger and Griesser, 1991).

D. SULFAMETER



sulfameter

Sulfameter (sulfamethoxydiazine) exists in at least six different forms (Moustafa *et al.*, 1971). Form I (see Figure 10.32 and Table 10.10) is obtained by crystallization from boiling water or by heating any other form to 150 °C. Form II is prepared by rapid cooling of a saturated ethanol solution. Form III (see Figure 10.33 and Table 10.10) is obtained from a number of solvents including methanol, isopropanol, and ethanol. Forms IV and V are probably solvates and are obtained from dioxane and chloroform, respectively. An amorphous form is also known.

These forms were characterized by their infrared spectra, which are all slightly different, particularly in the 800-875, 900-970, 1550-1600, and 3000-3500 cm^{-1} regions of the spectrum. The powder diffraction patterns of these forms are also significantly different.

The forms can be interconverted by heating or grinding. Heating converts all forms to Form I, while grinding or suspension in water converts all forms to Form III. This behavior is discussed in more detail in the interconversion section (see Section 13.2B).

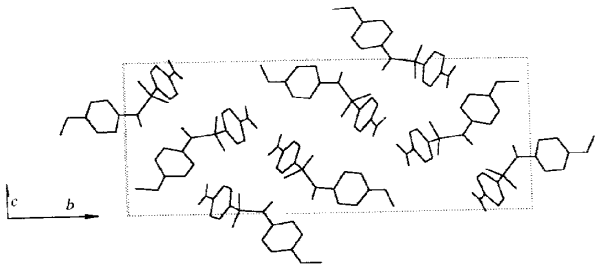


Figure 10.32 Crystal packing of sulfameter Form I (Giuseppetti *et al.*, 1977).

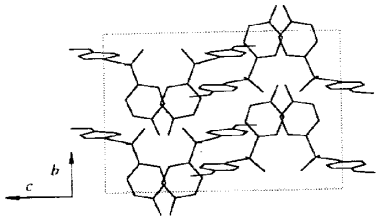


Figure 10.33 Crystal packing of sulfameter Form III (Giuseppetti *et al.*, 1977).

Table 10.10 Crystallographi

Parameter	
Space Group	
a (Å)	
b (Å)	
c (Å)	
β	1
Z	
ρ_{calc} (gm cm^{-3})	
V (Å^3)	

Giuseppetti *et al.*, 1977.

The dissolution rates
their relative bioavailabilit
ments are shown in Figu
dissolve most rapidly. F
Form II. It is also interest
amorphous form, sugges
surface area of Form II ma

Commercial preparati
mixtures of Forms I and I
ing. The significance of a
to be determined in separa

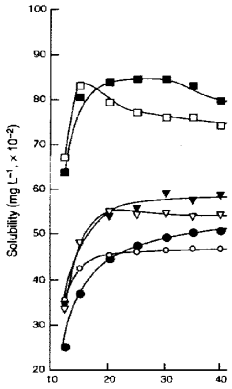


Figure 10.34 Dissolution rate

Table 10.10 Crystallographic Parameters for Sulfameter Forms I and III

Parameter	Form I	Form III
Space Group	$P2_1/c$	$C2/c$
a (Å)	8.358	13.370
b (Å)	26.833	11.735
c (Å)	11.964	15.928
β	111.36°	97.90°
Z	8	8
ρ_{calc} (gm cm^{-3})	1.490	1.504
V (Å^3)	2499	2475

Giuseppetti *et al.*, 1977.

The dissolution rates of these forms have been measured as a means of estimating their relative bioavailabilities (Moustafa *et al.*, 1971). The results of these measurements are shown in Figure 10.34. Obviously, Form II and the amorphous form dissolve most rapidly. Form III has the slowest dissolution rate, about half that of Form II. It is also interesting to note that Form II has a faster dissolution rate than the amorphous form, suggesting that the amorphous form may crystallize or that the surface area of Form II maybe much larger than that of the amorphous form.

Commercial preparations were also studied and, in general, contained Form I or mixtures of Forms I and III. These forms are the most stable and the slowest dissolving. The significance of any such differences with respect to bioavailability would have to be determined in separate experiments.

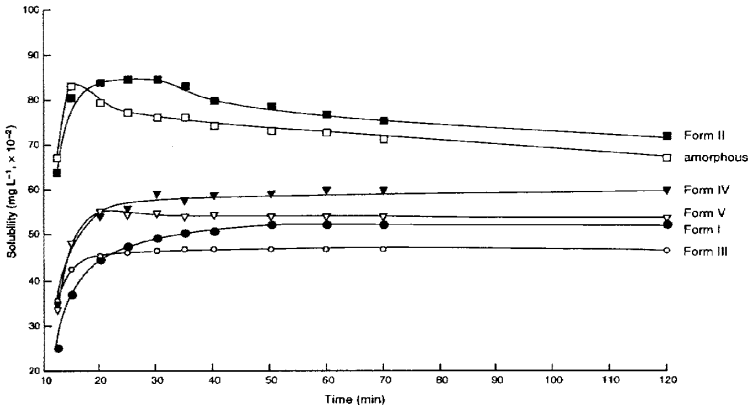
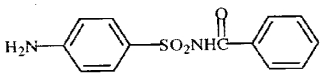


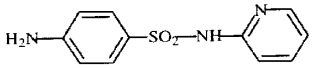
Figure 10.34 Dissolution rates of the different forms of sulfameter (Moustafa *et al.*, 1971).

E. Other Sulfonamides



sulfabenzamide

Sulfabenzamide. Sulfabenzamide exists in four polymorphs and three solvates (Yang and Guillory, 1972). Form III can be transformed to Form I by **trituration**, and Form IV can be transformed to Form III and then Form I by heating. Desolvation of two of the solvates yielded Form II (see Figure 10.35).



sulfapyridine

Sulfapyridine. Sulfapyridine (see Figures 10.35–10.39) exists in at least four polymorphs and one amorphous form (Yang and Guillory, 1972). The infrared spectra of two of these forms are identical, but their X-ray diffraction patterns are completely different. In addition, hot-stage experiments indicated that sulfapyridine crystallized in at least seven forms (Kuhnert-Brandstätter, 1971).

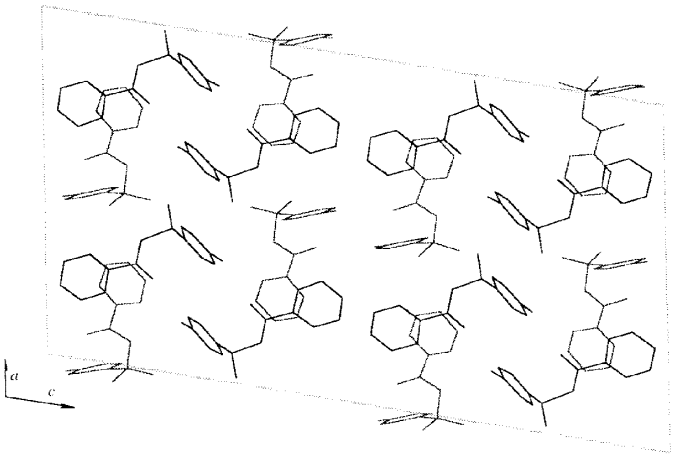


Figure 10.35 Crystal packing of sulfabenzamide Form II (Rambaud *et al.*, 1980).

Figure 10.36 Cryst

Figure 10.37 Cryst

Figure 10.38 Cryst

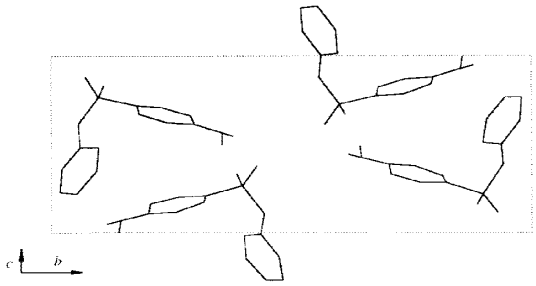


Figure 10.36 Crystal packing of sulfapyridine Form II (Bar and Bernstein, 1985).

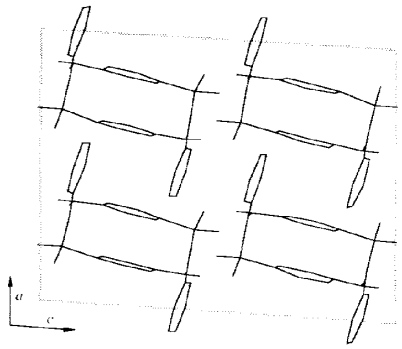


Figure 10.37 Crystal packing of sulfapyridine Form III (Basak *et al.*, 1984).

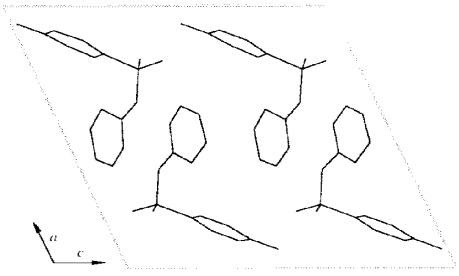


Figure 10.38 Crystal packing of sulfapyridine Form IV (Bernstein, 1988).

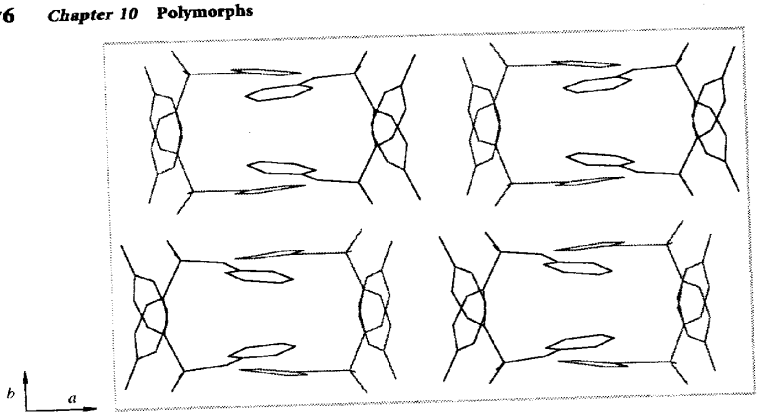


Figure 10.39 Crystal packing of sulfapyridine Form V (Bar and Bernstein, 1985).

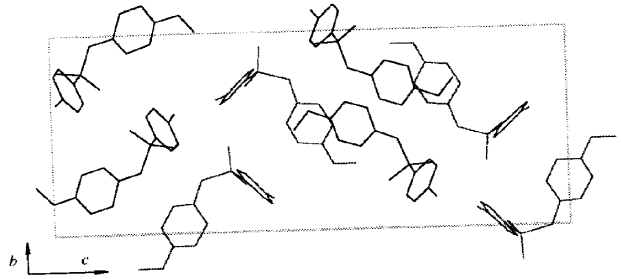
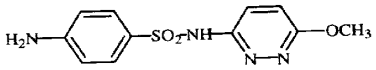
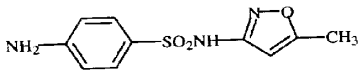


Figure 10.40 Crystal packing of sulfamethoxypyridiazine Form I (Basak *et al.*, 1987).



sulfamethoxypyridazine

Sulfamethoxypyridiazine. Sulfamethoxypyridiazine (see Figure 10.40) exists in at least three crystalline forms (Yang and Guillory, 1972). Form II can be transformed to Form I at 154 °C.



sulfamethoxazole

Sulfamethoxazole. Sulfamethoxazole (see Figures 10.41–10.42) exists in three polymorphs, and Form II can be converted to Form I at 164 °C (Yang and Guillory, 1972). These studies are in agreement with Kuhnert-Brandstätter (1971) who also

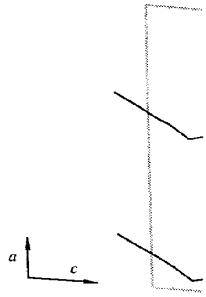


Figure 10.41 Crystal packing

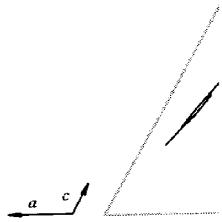
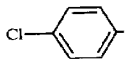


Figure 10.42 Crystal packing

showed there were three p
two forms of sulfamethox
10.41 and 10.42 show the
the conformations of the n



Chlorpropamide. Ch
morphs that have differe
obtained from aqueous et
or II at 110 °C. The infi

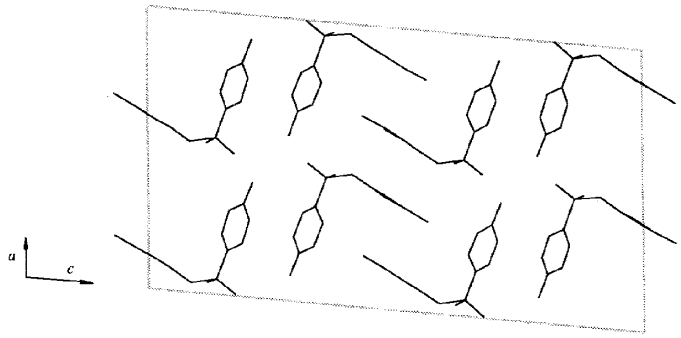


Figure 10.41 Crystal packing of sulfamethoxazole Form I (Bettinetti *et al.*, 1982).

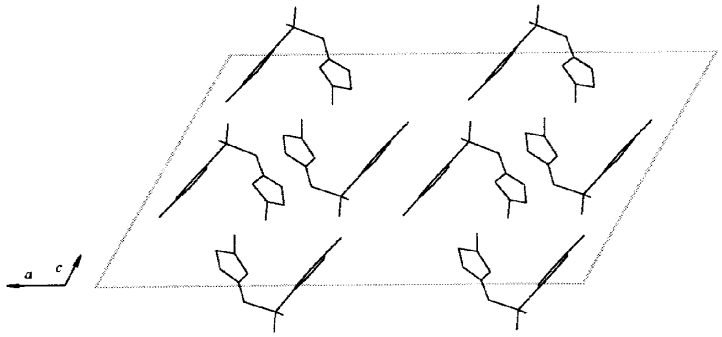
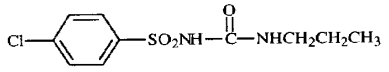


Figure 10.42 Crystal packing of sulfamethoxazole Form II (Bettinetti *et al.*, 1982).

showed there were three polymorphs of sulfamethoxazole. The crystal structures of the two forms of sulfamethoxazole were determined by Bettinetti *et al.* (1982). Figures 10.41 and 10.42 show the crystal packing in these two different forms. It appears that the conformations of the molecule in the two crystal forms are similar.



chlorpropamide

Chlorpropamide. Chlorpropamide (see Figure 10.43) exists in at least three polymorphs that have different diffraction patterns (Simmons *et al.*, 1973). Form I is obtained from aqueous ethanol, Form II from benzene, and Form III by heating Form I or II at 110 °C. The infrared spectra of all three forms are slightly different and the

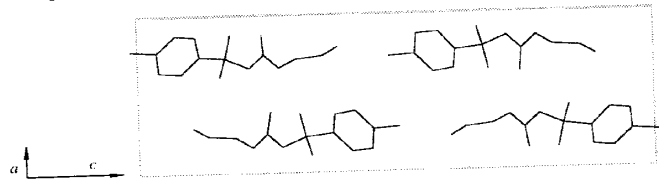
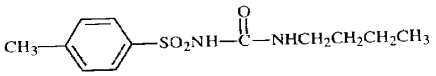


Figure 10.43 Crystal packing of chlorpropamide Form I (Koo *et al.*, 1980).

X-ray powder patterns of all three forms are significantly different, whereas the DSC thermograms obtained for the three forms are very similar.

The three forms of chlorpropamide have different dissolution rates. The dissolution rates of Forms I and III in water are identical, while Form II dissolves about half as fast. However, in beagle dogs, the serum levels following oral administration are identical for all three forms (Simmons *et al.*, 1973). Further single-crystal studies are necessary to completely characterize these forms and explain these results.



tolbutamide

Tolbutamide. Early studies (Simmons *et al.*, 1972) showed that tolbutamide crystallizes in two forms. Form I (see Figure 10.44) is obtained from benzene-hexane, and the crystals are prismatic with mp 127–128 °C. Form II is obtained from aqueous ethanol and the crystals are plates with mp 126–128 °C. Both the infrared spectra and the DTA thermograms of Forms I and II are slightly different. The DTA of Form II shows an endotherm at 113 °C that is not present in Form I. This endotherm apparently corresponds to the conversion of Form II to Form I. The dissolution rates of Forms I and II are the same in water at pH 5.5 and 7.3. The serum levels of these two forms are also identical. One explanation of this data is that, upon exposure to liquid, Form II is converted to Form I by a solution-mediated phase transformation.

More recent studies showed that tolbutamide exists in four crystal forms (Burger, 1975). In addition, aqueous suspensions of tolbutamide were found to thicken to an unpourable state upon occasional agitation. Analysis of the IR spectra and X-ray diffraction patterns confirmed that Form III had crystallized (Rowe and Anderson,

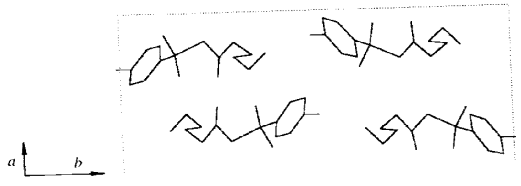


Figure 10.44 Crystal packing of tolbutamide Form I (Donaldson *et al.*, 1981; Nirmala and Gowda, 1981).

Figure 10.45 Van
trans

1984). This is su
thought to be the
shown in Figure
close. Because
suspensions; how
lower energy for
other solvents.

These data s
and that Form I is
was verified by
were placed in m
for several hours
the temperature v
grow throughout
room temperatur
dissolved. These
shown in Figure
thermal microsco

F. Conclusion

This section sho
polymorphism of
availability of a
number of ring-

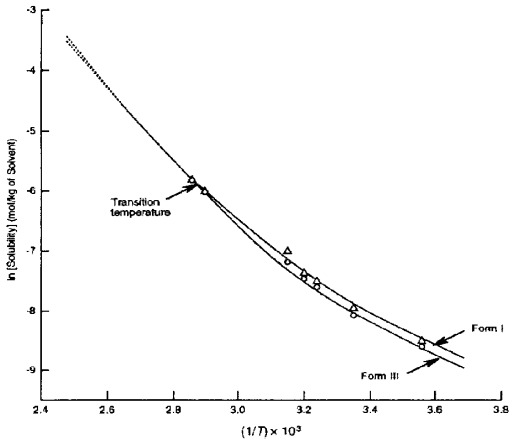


Figure 10.45 Van't Hoff plot of the solubilities of Forms I and III of tolbutamide showing the transition temperature (Rowe and Anderson, 1984).

1984). This is surprising since the suspensions were prepared with Form I which was thought to be the most stable polymorph. Solubility studies gave the van't Hoff plot shown in Figure 10.45. The aqueous solubilities of Form I and Form III are very close. Because of this, Form I may appear to be quite stable at low temperatures in suspensions; however, given sufficient time, Form I will transform to the Form III, the lower energy form. This interconversion was observed at room temperature in ten other solvents.

These data suggests that Form III is more stable than Form I at room temperature and that Form I is more stable than Form III at higher temperatures. This observation was verified by microscopy (Rowe and Anderson, 1984) in which Form III crystals were placed in mineral oil on a microscope hot stage. The sample was heated at 100 °C for several hours with periodic agitation by pressing and rotating the cover slip. When the temperature was reduced to 95 °C, prismatic crystals, typical of Form I, began to grow throughout the oil mixture and the Form III crystals dissolved. Upon cooling to room temperature, fine needles, typical of Form III, grew and the Form I crystals dissolved. These observations experimentally verify the result of the van't Hoff plot shown in Figure 10.45. These studies show the power of van't Hoff plots and also thermal microscopy in studying the interconversion of polymorphs.

F. Conclusion

This section shows the extent of polymorphism in the sulfonamides. The fact that polymorphism of these drugs is widespread yet unpredictable is probably due to (a) the availability of a variety of hydrogen-bonding schemes and (b) the occurrence of a number of ring-ring stacking modes. Further study of the polymorphism of these

compounds using single-crystal X-ray techniques should, no doubt, lead to a better general understanding of polymorphism.

10.5 STEROIDS

Steroids exhibit widespread polymorphism that may affect their bioavailability. A few examples of the polymorphism of steroids have been discussed in preceding sections.

Kuhnert-Brandstätter (1971) has studied the polymorphism of steroids using a Kofler hot stage, and the results of her studies are summarized in Table 10.11. This table clearly shows the extent of polymorphism in this important class of compounds. It should be noted that these studies are based mainly on hot-stage results. Other methods would be useful to verify the existence of these polymorphs and clarify the possible involvement of solvates.

Table 10.11 Melting Points of Polymorphic Steroids[a]

Compound	Forms I	II	III	IV	V
Allopregnane-3β,20α-diol	215–219	162–168			
Allopregnane-3,20-dione	202–206	198–203			
Androstane-3β,17β-diol	168–169	163–164	158–161	146–147	
Androstane-3,17-dione	132–134	128–130			
Androstanolone	182	168			
Δ^5-Androstene-3β,17α-diol	202–205	180–195			
Δ^5-Androstene-3β,17β-diol	181–185	177–180	155–158		
Δ^4-Androstene-3,17-dione	170–174	142–145			
Corticosterone	180–186	175–179	162–168	155–160	
Cortisone enanthate	138–140	135–137	129–132		
Dehydroepiandrosterone	149–153	139–141	137–140	130–136	
Dehydroepiandrosterone acetate	170–172	132–135	94–96	65–69	
Epiandrosterone	174–176	167–169			
α-Estradiol	225	223			
β-Estradiol	178	169			
Estradiol benzoate	188–195	177.5	176		
Estradiol dipropionate	107	97	82		
Estradiol 17-propionate	198–200	154–156			
Estrone	260–263	256	254		
Estrone methyl ether	172–174	123–126	88–92		
Etiocholane-3α-ol-17-one	150–152	141–143	133		
Etiocholane-17β-ol-3-one	141–143	103			
Fluorocortisone trimethylacetate	192–198	184–190			
9α-Fluorohydrocortisone acetate	225–233	208–212	205–208		
Hydrocortisone hemisuccinate	198–205	182–188	168–172		
Methandriol	205–208	202–205	196–198		
Methandriol dipropionate	83–86	74–75			
17α-Methandrostane-3β,17β-diol	213	205			

a Data from Kuhnert-Brandstätter (1971)

Table 10.11 (continued) Me

Compound
1-Methylandrostenolone acet
17α-Methylestradiol
6α-Methylprednisolone aceta
17-Norethisterone
Prednisolone
Prednisolone acetate
Progesterone
Testosterone
Testosterone isobutyrate
Testosterone nicotinate
Testosterone propionate

a Data from Kuhnert-Brandstät

A. ESTRONE

HO

As indicated in Table 10.1 of all three polymorphs ha of the estrone molecule is three forms is shown in molecules, but not obviou and stacks of estrone mol molecules. The crystal pa of 2.26 and 2.47 Å; the c

Table 10.12 Crystallographic

	Form I
Space group	$P2_12_12_1$
a (Å)	12.188
b (Å)	16.301
c (Å)	7.463
β	90.00°
Z	4
V (Å^3)	1481
Source	Sublimati

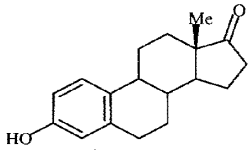
Busetta *et al.*, 1973

Table 10.11 (continued) Melting Points of Polymorphic Steroids[a]

Compound	Forms I	II	III	IV	V
1-Methylandrostenolone acetate	143	106			
17α-Methylestradiol	190–194	188			
6α-Methylprednisolone acetate	225–229	208–212	205–210		
17-Norethisterone	200–207	199			
Prednisolone	218–234	215			
Prednisolone acetate	232–241	225–228	217–220		
Progesterone	131	123	111	106	100
Testosterone	155	148	144	143	
Testosterone isobutyrate	131–133	88–90			
Testosterone nicotinate	194–196	185–188			
Testosterone propionate	122	74			

a Data from Kuhnert-Brandstätter (1971)

A. Estrone



estrone

As indicated in Table 10.12 estrone exists in three polymorphs. The crystal structures of all three polymorphs have been determined (Busetta *et al.*, 1973). The conformation of the estrone molecule is similar in all three polymorphs. The crystal packing of these three forms is shown in Figures 10.46–10.48. Form I contains layers of estrone molecules, but not obvious stacks of estrone molecules. Form III contains both layers and stacks of estrone molecules. Form II has a herringbone arrangement of estrone molecules. The crystal packing of Form I appears to be controlled by H···H contacts of 2.26 and 2.47 Å; the crystal packing of Form II appears to be controlled by C···C

Table 10.12 Crystallographic Parameters of Three Estrone Polymorphs

	Form I	Form II	Form III
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
a (Å)	12.188	10.043	9.271
b (Å)	16.301	18.424	22.285
c (Å)	7.463	7.787	7.610
β	90.00°	90.00°	111.45°
Z	4	4	4
V (Å^3)	1481	1440	1461
Source	Sublimation	Acetone	Sublimation

Busetta *et al.*, 1973

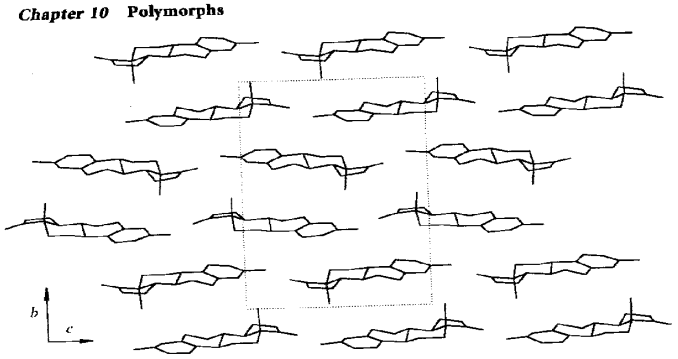


Figure 10.46 Crystal packing of estrone Form I (Busetta *et al.*, 1973).

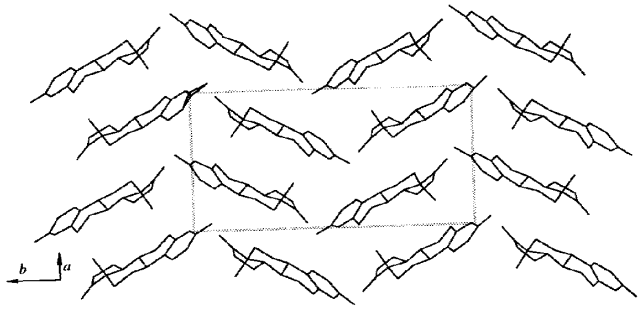


Figure 10.47 Crystal packing of estrone Form II (Busetta *et al.*, 1973).

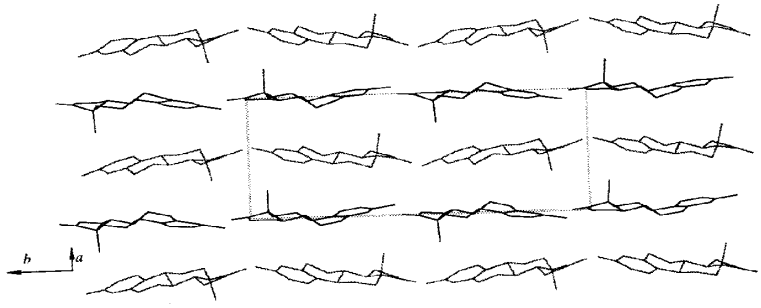


Figure 10.48 Crystal packing of estrone Form III (Busetta *et al.*, 1973).

contacts of 3.35
reported; howeve

B. PREDNISOLO

In our laboratory
Three crystal forn
parameters and c
10.13. The cryst
ture of Form III
prednisolone in t

Table 10.13 Cryst

Space Group
a (Å)
b (Å)
c (Å)
β
Z
ρ_{calc} (g cm^{-3})
V (Å^3)
R

Sutton, 1984

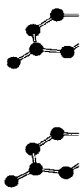
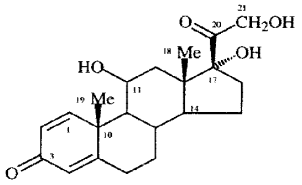


Figure 10.49 Stereo
(Sutto

contacts of 3.35 Å. No transformations or interconversions of these forms have been reported; however, it is likely that the densest form, Form II, is the most stable.

B. Prednisolone



prednisolone

In our laboratory we have investigated the polymorphs of prednisolone (Sutton, 1984). Three crystal forms were obtained by crystallization from various solvents. The cell parameters and other crystallographic data for these three forms are shown in Table 10.13. The crystal structures of Forms I and II were determined but the crystal structure of Form III could not be refined to an acceptable R value. The conformation of prednisolone in the two crystal forms (Forms I and II) is shown in Figure 10.49 and

Table 10.13 Crystallographic Data for the Polymorphs of Prednisolone

	Form I	Form II	Form III
Space Group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	6.350 (3)	11.808 (7)	24.56 (2)
b(Å)	12.985 (8)	6.009 (2)	24.77 (4)
c (Å)	10.971 (9)	25.643 (12)	6.415 (3)
β	91.24°	90.00°	90.00°
Z	2	4	8
ρ_{calc} (g cm^{-3})	1.32	1.32	1.29
V (Å^3)	904.4	1819.5	3903.5
R	0.672	0.672	> 0.10

Sutton, 1984

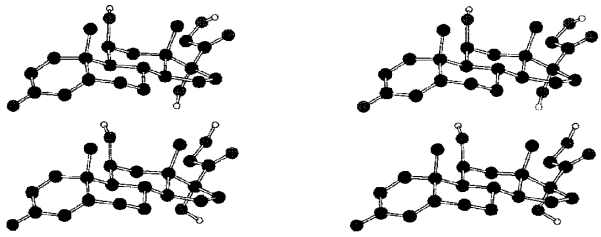


Figure 10.49 Stereoview of prednisolone Forms I (upper) and II (lower) conformations in the crystal (Sutton, 1984).

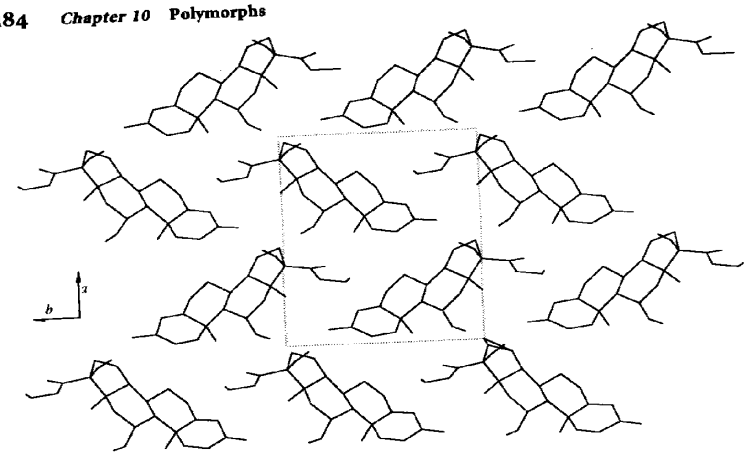


Figure 10.50 Crystal packing stereoview of prednisolone Form I (Sutton, 1984).

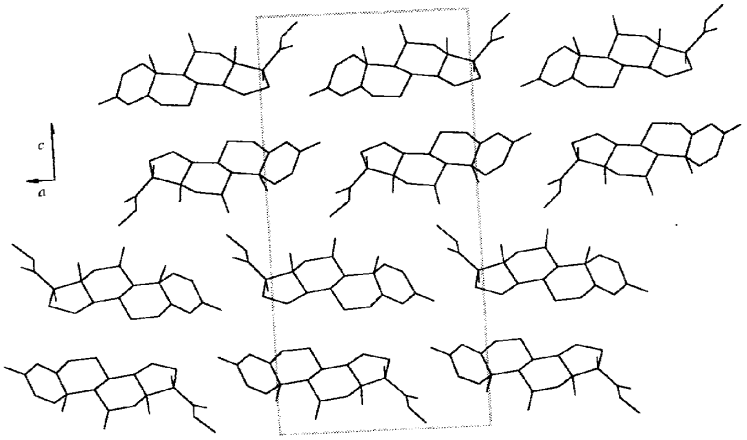


Figure 10.51 Crystal packing stereoview of prednisolone Form II (Sutton, 1984).

the crystal packing is shown in Figures 10.50–10.51.

The crystal packing shows that the arrangements of the prednisolone molecules in the unit cells of Forms I and II are similar but not identical. However, the solid-state NMR spectra of Forms I and II of prednisolone are different as illustrated by the spectra and the chemical shifts in Figure 10.52 and Table 10.14 (Saindon *et al.*, 1993).

Especially important for pu
the resonances assigned to
respectively.

The solid-state CP/M
(labeled amount of 5 mg)
10.53 and required long
comprises only about 5%
spectra shows that product
Further analysis showed th

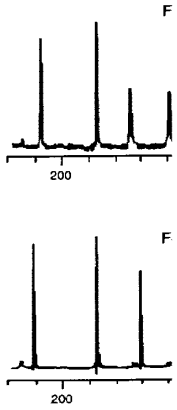


Figure 10.52 Solid-state CP/
(Saindon *et al.*,

Table 10.14 ^{13}C NMR Chem

Atom	Form I	Form II
C20	209.5	211.8
C3	188.1	187.g
C5	175.1	171.0
C13	159.8	157.3
C2	125.9	130.2
C4	121.8	123.8
C17	91.4	90.2
C11	69.9	70.4
C21	67.1	67.7
C9	55.4	54.8
C14	52.2	52.8

The assignment of this peak

Especially important for purposes of identification is the difference in chemical shifts of the resonances assigned to carbons C2 and C4 which occur between 120 and 140 ppm, respectively.

The solid-state CP/MAS ^{13}C NMR spectra of three generic prednisolone products (labeled amount of 5 mg) were also determined. These spectra are shown in Figure 10.53 and required long acquisition times since the active ingredient (prednisolone) comprises only about 5% of the approximately 100 mg tablets. Inspection of these spectra shows that products A and B contain Form I while product C contains Form II. Further analysis showed that all three products passed the USP dissolution test. Thus,

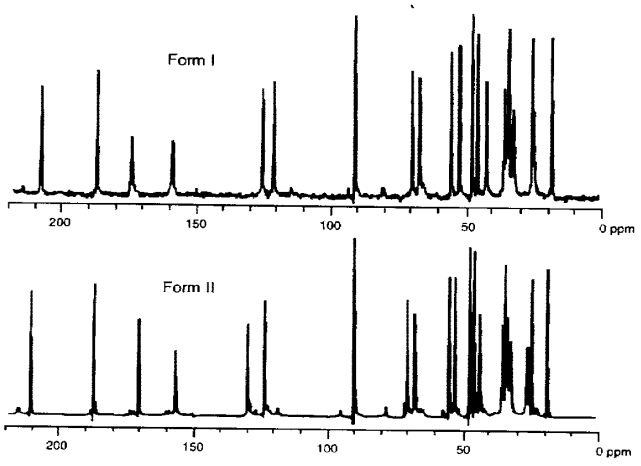


Figure 10.52 Solid-state CP/MAS ^{13}C NMR spectra of prednisolone Forms I (top) and II (bottom) (Saindon *et al.*, 1993).

Table 10.14 ^{13}C NMR Chemical Shifts of Prednisolone in the Solid-State and Solution

Atom	Form I	Form II	Solution	Atom	Form I	Form II	Solution
C20	209.5	211.8	211.5	C13	47.5	47.1	46.7
C3	188.1	187.g	185.1	C10	45.3	45.1	43.9
C5	175.1	171.0	170.5	C12	42.1	43.1	39.0
C13	159.8	157.3	156.8	C8[a]	35.3	34.7	34.1
C2	125.9	130.2	127.2	C16[a]	34.3	33.5	33.0
C4	121.8	123.8	121.7	C15[a]	33.5	32.7	32.7
C17	91.4	90.2	88.5	C6[a]	31.8	31.5	31.6
C11	69.9	70.4	68.6	C7[a]	24.6	25.4	31.2
C21	67.1	67.7	66.1	C18[a]	23.9	23.7	21.0
C9	55.4	54.8	55.5	C19[a]	17.3	18.1	17.0
C14	52.2	52.8	51.2				

[a] The assignment of this peak should be considered tentative (Saindon *et al.*, 1993)

molecules in
he solid-state
trated by the
et al., 1993).

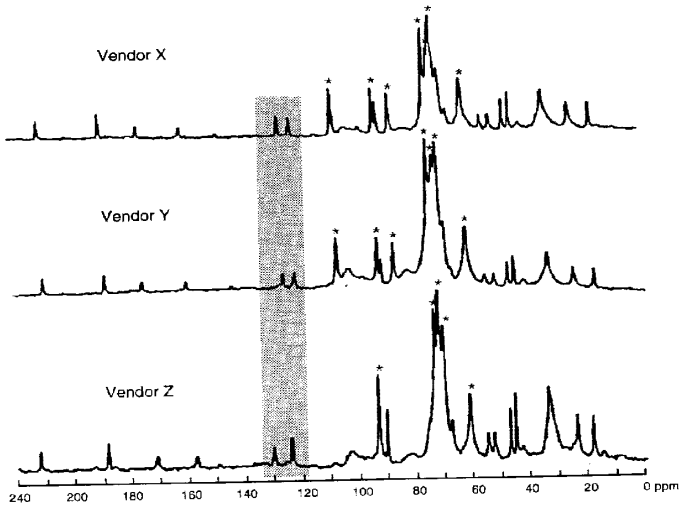
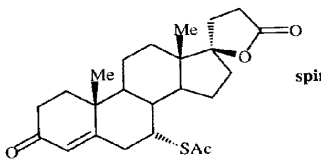


Figure 10.53 Solid-state CP/MAS ^{13}C NMR spectra of prednisolone tablets from three different vendors. The most evident differences are noted within the shaded region and the excipient signals are labeled with a star. (Byrn *et al.*, 1988).

these tablets represent a control problem because they contain different crystal forms but hopefully do not represent a serious clinical problem since they all meet the USP dissolution test.

C. Spironolactone



spironolactone

The polymorphism of spironolactone has been carefully studied using X-ray crystallography (Agafonov *et al.*, 1991). The data for the different forms are described in Table 10.15.

Spironolactone is of interest because it shows variable solubility and dissolution rate as well as pharmaceutical performance as an oral drug. Recently, a number of crystal forms of this compound have been discovered (see Table 10.15). As is the case for many steroids, both solvated and unsolvated crystal forms have been obtained. Figure 10.54 shows the TGA curves of the different crystal forms, clearly Forms III

Table 10.15 Spironolacto

Solvent	Method[a]
Acetone	1
Acetone	2
Dioxane	1
Dioxane	2
Chloroform	1
Chloroform	2
Acetonitrile	—[b]
Ethanol	—[b]
Ethyl acetate	—[b]
Methanol	—[b]

a Method 1—the sample is
0° C within a few hours; me
ture and the solvent allowe
the two methods of prepar
fraction pattern. (Agafono

through VI are solvates
crystal forms confirmin

Table 10.16 lists t
spironolactone, clearly
10.17 tabulates the pow
that Forms I through I
(Agafonov *et al.*, 1991
crystal forms of spiron
(Form I) is shown in Fi
Figure 10.57. The conf
it is clear that the crystal

Mass (mg)

Figure 10.54 TGA curves

Table 10.15 Spironolactone Single-Crystal Preparation Methods and Thermodynamic Data

Solvent	Method[a]	Form Obtained	T_{dec} (°C)	ΔH_{dec} (J/g)	T_f (°C)	ΔH_f (J/g)
Acetone	1	I	...	...	205 ± 1	48 ± 3
Acetone	2	II	...	...	210 ± 1	53 ± 4
Dioxane	1	Glass[c]	...	...	...	...
Dioxane	2	II	...	...	210 ± 1	53 ± 4
Chloroform	1	Glass[c]	...	...	...	...
Chloroform	2	II	...	...	210 ± 1	53 ± 4
Acetonitrile	—[b]	Solvate (2:1) (III)	137 ± 2	38 ± 2	210 ± 1	52 ± 4
Ethanol	—[b]	Solvate (2:1) (IV)	100 ± 2	28 ± 2	210 ± 1	54 ± 4
Ethyl acetate	—[b]	Solvate (4:1) (V)	102 ± 6	28 ± 1	210 ± 1	54 ± 4
Methanol	—[b]	Solvate (1:2) (VI)	25-126	50 ± 2	210 ± 1	52 ± 3

a Method 1—the sample is dissolved in the solvent at close to its boiling point and cooled to 0° C within a few hours; method 2—the sample is dissolved in the solvent at room temperature and the solvent allowed to evaporate slowly during several weeks. *b* For these solvents, the two methods of preparation give the same results. *c* Glass-like solid without X-ray diffraction pattern. (Agafonov *et al.*, 1991)

through VI are solvates. Figure 10.55 shows the DSC thermograms of the different crystal forms confirming that Forms III through VI contain solvent of crystallization.

Table 10.16 lists the crystallographic parameters of the different crystal forms of spironolactone, clearly showing that the different forms have distinct structures. Table 10.17 tabulates the powder patterns for Forms I through III. It is clear from this table that Forms I through III have different powder diffraction patterns. These workers (Agafonov *et al.*, 1991) were able to determine the crystal structures of three of the crystal forms of spironolactone and the contents of the unit cell for the needle form (Form I) is shown in Figure 10.56, the contents of the unit cell for Form II is shown in Figure 10.57. The conformation of the steroid is the same in all three crystal forms but it is clear that the crystal packing is different.

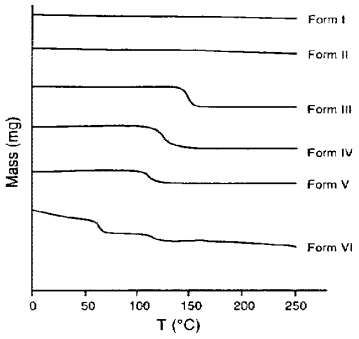


Figure 10.54 TGA curves of spironolactone crystal forms (Agafonov *et al.*, 1991).

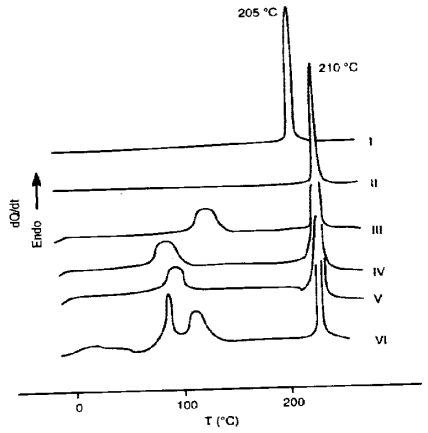


Figure 10.55 DSC thermograms of spironolactone crystal forms (Agafonov *et al.*, 1991).

Table 10.16 Crystallographic Data for the Crystal Forms of Spironolactone

Parameter	Form I	Form II	Form III	Form IV	Form V
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	9.979	10.584	11.857	10.14	10.15
b (Å)	35.573	18.996	19.655	36.21	36.22
c (Å)	6.225	11.005	11.346	6.28	6.29
β	90.00	90.00	118.13	90.00	90.00
Z	4	4	2	4	4
V (Å^3)	2209.8	2212.6	2318.7	2306	2315
Crystal System	Orthorhombic	Orthorhombic	Monoclinic	Orthorhombic	Orthorhombic
Morphology	Needle-like	Prisms	Trigonal prisms	Needle-like	Needle-like
Solvate	...	...	½ acetonitrile	½ ethanol	¼ ethyl acetate

Agafonov *et al.*, 1991.

Table 10.17 X-ray Powder Diffraction Data for the Different Crystal Forms of Spironolactone

Form I			Form II			Form III		
d_{hkl} (Å)	I^a	$h\ k\ l$	d_{hkl} (Å)	I^a	$h\ k\ l$	d_{hkl} (Å)	I^a	$h\ k\ l$
17.8	w	0 2 0	9.5	s	0 2 0	9.8	s	0 2 0
8.9	m	0 4 0	7.63	w	1 0 1	8.9	w	0 1 1
8.7	vs	1 2 0	7.00	m	1 2 0	8.8	w	1 1 1
7.63	s	1 3 0	5.43	s	1 3 0	6.99	w	1 2 1
6.64	m	1 4 0	5.29	s	0 1 2	5.55	s	1 3 0

a vs–very strong intensity, s–strong intensity, m–medium intensity, w–weak intensity, vw–very weak intensity (Agafonov *et al.*, 1991).

Table 10.17 (continued)

Form I		
d_{hkl} (Å)	I^a	$h\ k\ l$
6.13	w	0 1 1
5.93	vw	0 6 0
5.10	w	1 6 0
4.94	m	2 1 0
4.68	vs	0 5 1
4.599	s	2 3 0
4.528	s	1 7 0
4.351	m	2 4 0
3.870	m	2 0 1
3.699	m	1 9 0

a vs–very strong intensit
intensity (Agafonov *et a*

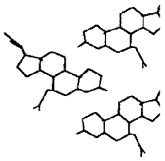


Figure 10.56 Contents o

Figure 10.57 Contents of

Table 10.17 (continued) X-ray Powder Diffraction Data for the Different Crystal Forms of Spironolactone

Form I			Form II			Form III		
d_{hkl} (Å)	I^a	*h k l*	d_{hkl} (Å)	I^a	*h k l*	d_{hkl} (Å)	I^a	*h k l*
6.13	w	0 1 1	5.10	m	2 1 0	5.48	s	0 3 1
5.93	vw	0 6 0	4.87	w	1 0 2	5.46	s	1 3 1
5.10	w	1 6 0	4.73	w	1 1 2	5.09	s	1 2 1
4.94	m	2 1 0	4.333	m	1 4 0	5.05	w	2 1 0
4.68	vs	0 5 1	4.263	w	2 1 2	4.97	m	2 0 –2
4.599	s	2 3 0	4.032	m	1 4 1	4.91	s	0 4 0, 1 2 2
4.528	s	1 7 0	3.815	w	2 0 2	4.456	m	0 2 2, 1 4 0
4.351	m	2 4 0	3.741	w	2 1 2	4.287	m	1 3 2
3.870	m	2 0 1	3.576	w	1 5 0	3.931	w	2 0 1
3.699	m	1 9 0	3.540	w	2 2 2	3.837	w	3 1 1, 3 0 2

a vs–very strong intensity, s–strong intensity, m–medium intensity, w–weak intensity, vw–very weak intensity (Agafonov *et al.*, 1991).

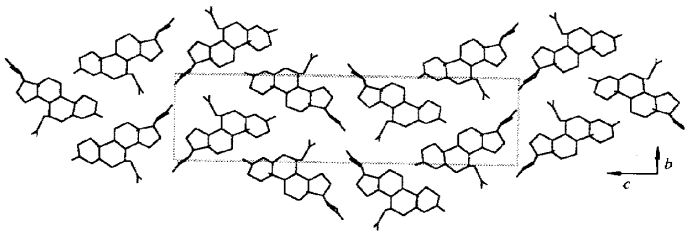


Figure 10.56 Contents of the unit cell of Form I of spironolactone (Dideberg *et al.*, 1972).

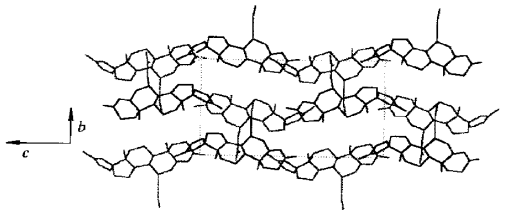
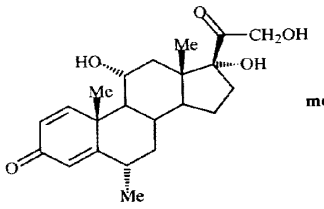


Figure 10.57 Contents of the unit cell of Form II of spironolactone (Agafonov *et al.*, 1989).

D. Methylprednisolone



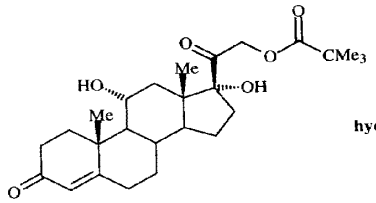
methylprednisolone

Methylprednisolone exists in two polymorphs. Form I can be prepared by recrystallization from acetone, and Form II by sublimation at 190 °C (Hamlin *et al.*, 1962). Dissolution rates of pellets of these two forms were studied under varying conditions of agitation. Under all conditions, except the most rapid agitation, Form II has a faster dissolution rate than Form I. *In vivo* tests of the rate of dissolution of Forms I and II using pellet implants in rats showed that Form II has a faster dissolution rate than Form I.

Studies of the intrinsic dissolution rates (see Chapter 6) of Forms I and II also showed that Form II has a faster dissolution rate than Form I. At increased stirring rates, Forms I and II had more similar dissolution rates. These studies also indicated that low agitation rates give data that correlate with the pellet-implant *in vivo* data, while higher agitation rates are required to give results that correlate with data from trials involving tablets dissolving in the stomach (Levy and Procknal, 1964).

Infrared spectroscopy showed that the surfaces of pellets of Form II revert to Form I in water, even after only a 2-minute exposure. This appears to be a water-mediated phase transformation of the type discussed by Haleblian and McCrone (1969). This observation explains some of the conflicting data obtained in measuring the dissolution rates of Form II in water (Higuchi *et al.*, 1969).

E. Hydrocortisone 21-*tert*-Butylacetate



hydrocortisone 21-*tert*-butylacetate

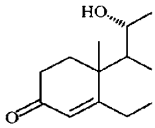
Biles (1963) reported that hydrocortisone 21-*tert*-butylacetate crystallizes in three forms. X-ray diffraction studies in our laboratory indicate that there are actually at least four different forms, and elemental analysis shows that two of these forms contain different amounts of ethanol. The results of these studies are shown in Table 10.18. Several other forms (from other solvents or from desolvation of a solvate by heating) are also known and have a melting point of 234–238 °C (Lin *et al.*, 1982).

Table 10.18 Crysta

Crystal Form
I
II
III
IV

a The exact melting
at this temperature r
melt resolidified as

During recry
III, often formed
new form, design
120 °C. Forms I
while Form III ch



hydrocortiso

All crystal fo
light. Form I
ultraviolet light ir
°C. The formatio
NMR chemical sh
by gas chromato
21-*tert*-butylaceta

Table 10.19 Desol
Butyl

Days
1
2
3
6
10
14
21

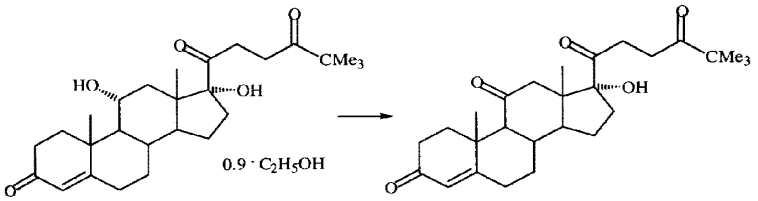
Lin *et al.*, 1982.

Table 10.18 Crystal Forms of Hydrocortisone 21-*tert*-Butylacetate

Crystal Form	Ethanol Content (mole ratio)	Oxidation in UV Light	Mp[a] (°C)
I	0.9 (variable)	Reaction	170-180
II	1.0	No Reaction	110-120[b]
III	0	No Reaction	123-126[c]
IV	0	No Reaction	234-238

a The exact melting temperature may vary from one crystal to another. *b* Opaque at this temperature range with final melting at 234–238 °C. *c* After melting, the melt resolidified as the temperature was increasing. (Lin *et al.*, 1982)

During recrystallization from ethanol, a mixture of crystal forms, Forms I, II, and III, often formed but a pure single form could be obtained under certain conditions. A new form, designated Form IV, was produced when Forms I, II, and III were heated at 120 °C. Forms I and II underwent desolvation and phase transformation to Form IV, while Form III changed from one phase to another.



hydrocortisone 21-*tert*-butylacetate **cortisone 21-*tert*-butylacetate**

All crystal forms, except for Form I, were stable upon irradiation with ultraviolet light. Form I was oxidized to cortisone 21-*tert*-butylacetate upon irradiation with ultraviolet light in air. A known weight of crystals was put in vials and irradiated at 30 °C. The formation of cortisone 21-*tert*-butylacetate was determined by the change in the NMR chemical shift of the C18 methyl signal, and the content of ethanol was measured by gas chromatography. The percent of desolvation and oxidation of hydrocortisone 21-*tert*-butylacetate to cortisone 21-*tert*-butylacetate is shown in Table 10.19. The loss

Table 10.19 Desolvation and Oxidation of Crystalline Hydrocortisone 21-*tert*-Butylacetate Form I (0.9 Ethanolate) upon Exposure to UV Light

Days	% Oxidation	Ethanol Lost
1	20.0	43.3%
2	38.9	75.6%
3	50.0	83.3%
6	52.9	88.9%
10	56.3	93.3%
14	66.7	95.6%
21	71.4	96.7%

Lin *et al.*, 1982.

of ethanol is faster than oxidation but does not completely precede oxidation. In addition, ethanol loss does not occur from crystals stored in the dark, indicating that oxidation is required for ethanol loss to begin. Further studies of this interesting reaction are in order. This behavior is different from that of dihydrophenylalanine hydrate, in which water loss almost completely preceded oxidation (Byrn and Lin, 1976).

F. Conclusion

The steroids exhibit a wide range of polymorphic and solvate behavior which appears to affect both the bioavailability and stability of these compounds. Of particular interest are the cases where one form is chemically reactive in the solid state while the others are stable.

10.6 Barbiturates

Barbiturates are another class of drugs which generally exhibit polymorphism. As in the discussions of the polymorphism of sulfonamides and steroids just presented, this section begins with Table 10.20 describing the results of hot-stage experiments on barbiturates (Kuhnert-Brandstätter, 1971).

Table 10.20 Melting Points of Polymorphs of Barbiturates[a]

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Allobarbital	173	~122									
5-Allyl-5-(2-Cyclopentenyl-1-yl)barbituric acid	148	126	124	115	—						
5-Allyl-5-phenyl-barbituric acid	159	133	130	129	128	126					
Amobarbital	157	151									
Aprobarbital	141	139	133	130	~116	~95					
Barbital	190	184	183	181	176	159					
Butallylonal	131	128	104								
Buthalitone	149	117	~95								
5-Crotyl-5-ethyl-barbituric acid	117	90									
Cyclobarbital	173	161									
Dipropylbarbital	148	146	126	120	~110	105	85				
Dormovit	171	146									
Ethallobarbital	160	149	137	129	117	108					
5-Ethyl-5-(1-piperidyl)-barbituric acid	217	210	204								
Heptabarbital	174	150	145	143	141	137	127	100			
Hexobarbital	146										

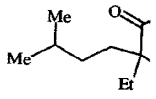
a Kuhnert-Brandstätter (1971).

Table 10.20 (continued) Melting Points

Compound	I
5-Methyl-5-phenyl-barbituric acid	226
Pentobarbital	129
Phenobarbital	176
Propallylonal	184
Secobutabarbital	166
Thialbarbital	146
Thiothyr	176
Vinbarbital	166

a Kuhnert-Brandstätter (1971).

A. Amobarbital



ven and Vizzini (1969) have det
phs of amobarbital (5-ethyl-5-iso
parameters shown in Table 10.21
The conformation of amobarbita
rystal packing is different (see F
double-ribbon arrangement; ho
ets, while in Form II an interl
density.

.21 Crystallographic Parameters fo

eter	Form I
oup	*C2/c*
	21.480
	11.590
	10.370
	97.07°
	8
	2562.0
-3)	1.171
it	Plates developed on
	154–156

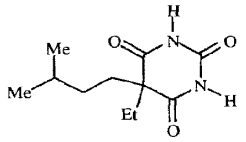
zini, 1969.

Table 10.20 (continued) Melting Points of Polymorphs of Barbiturates[a]

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
5-Methyl-5-phenyl-barbituric acid	226	226	200								
Pentobarbital	129	114	108								
Phenobarbital	176	174	167	163	160	157	153	141	133	126	112
Propallylonal	184	180	~179	~127	~123						
Secobutabarbital	166	—									
Thialbarbital	146	125									
Thiothyr	176	172									
Vinbarbital	166	129	106								

a Kuhnert-Brandstätter (1971).

A. Amobarbital



amobarbital

Craven and Vizzini (1969) have determined the crystal structures of the two polymorphs of amobarbital (5-ethyl-5-isopentylbarbituric acid). The two forms have the cell parameters shown in Table 10.21.

The conformation of amobarbital is virtually identical in the two polymorphs but the crystal packing is different (see Figures 10.58–10.59). Both forms show the so-called double-ribbon arrangement; however, in Form I there is no interaction between the sheets, while in Form II an interlocking structure is present resulting in a slightly higher density.

Table 10.21 Crystallographic Parameters for the Two Forms of Amobarbital

Parameter	Form I	Form II
Space group	$C2/c$	$P2_1/c$
a (Å)	21.480	10.281
b (Å)	11.590	22.061
c (Å)	10.370	11.679
β	97.07°	109.10°
Z	8	8
V (Å^3)	2562.0	2503.1
ρ_{calc} (g cm^{-3})	1.171	1.178
Crystal habit	Plates developed on 1 0 0	Needles elongated along b-axis
Mp (°C)	154–156	160–162

Craven and Vizzini, 1969.

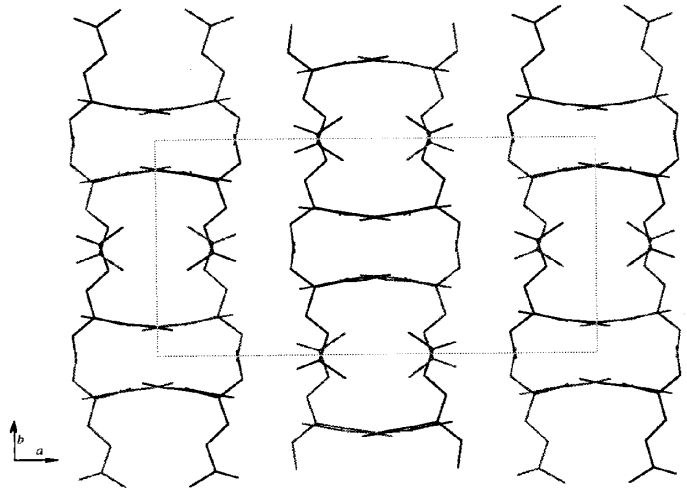


Figure 10.58 The crystal structure of Form I of amobarbital viewed down the *c* axis (Craven and Vizzini, 1969).

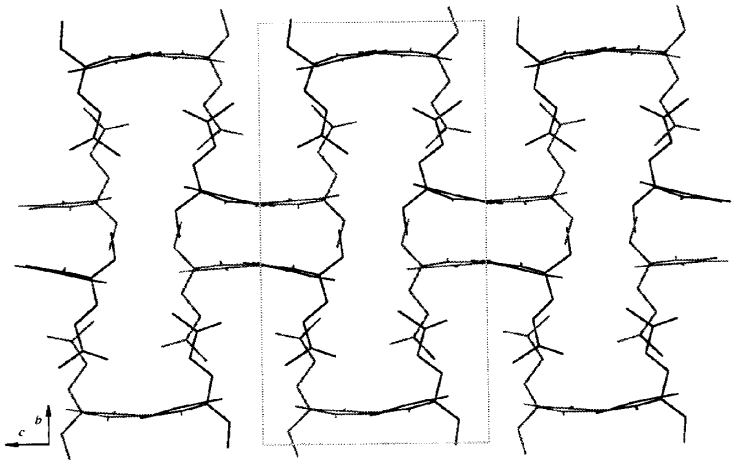


Figure 10.59 The crystal structure of Form II of amobarbital viewed down the *a* axis (Craven and Vizzini, 1969).

B PHENOBARBITAL

Phenobarbital (5-ethyl-5-pl
many as thirteen modificati
least four distinct anhydrou

The crystal structures
have been determined (W
phenobarbital, including the
two forms. The crystal pac
somewhat different; howev
hydrogen-bonded pyrimidi

Kopp *et al.* (1988) rep
of polymorphic phenobarbi
can easily lead to misunder
to identify the different cry
obtained if different heating
also influenced the DSC re
DSC methodology outlined

A study by Szabó-Révé
ers Avicel® PH 101 or Hew
(obtained by heating a comr
two commercial sources lab
phenobarbital. The dissolut
were different as shown in
and other similar observat
dissolution rates.

Table 10.22 Crystallographic I

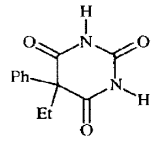
Parameter	Form I[a]
Space group	$P2_1/n$
a (Å)	6.800
b (Å)	47.174
c (Å)	10.695
α	90.00°
β	94.18°
γ	90.00°
Z	12
V (Å^3)	3421.7
ρ_{calc} (gm cm^{-3})	1.352

a Williams, 1973. *b* Williams,

c axis (Craven and

e *a* axis (Craven and

B PHENOBARBITAL



phenobarbital

Phenobarbital (5-ethyl-5-phenylbarbituric acid) has been reported to crystallize in as many as thirteen modifications. Single-crystal studies of these polymorphs revealed at least four distinct anhydrous forms and one hydrate (see Table 10.22).

The crystal structures of the hydrate (Form XIII) and of Forms I, II, III, and V have been determined (Williams, 1973; Williams, 1974). The conformations of phenobarbital, including the angle between the two rings, are slightly different in these two forms. The crystal packing of these two forms, shown in Figures 10.60–10.61, is somewhat different; however, both forms contain layers of phenyl rings and layers of hydrogen-bonded pyrimidine rings.

Kopp *et al.* (1988) reported a study of DSC and X-ray powder diffraction patterns of polymorphic phenobarbital. Their work demonstrates that using one technique alone can easily lead to misunderstandings. It was not possible to use the DSC thermograms to identify the different crystal forms of phenobarbital because different results were obtained if different heating rates were used. In addition, they found that particle size also influenced the DSC results. These results are consistent with the discussion of DSC methodology outlined in Chapter 5.

A study by Szabó-Révész *et al.* (1987) used direct compression with the dry binders Avicel® PH 101 or Heweten® 40 to evaluate manufactured tablets containing Form I (obtained by heating a commercial product near 160 °C for 3 h), Form II (obtained from two commercial sources labeled II_1 and II_2), or Form III (obtained by spray drying) of phenobarbital. The dissolution rates of the tablets containing the various crystal forms were different as shown in Figure 10.62 but by only a few percent. This observation and other similar observations suggest that different polymorphs may give similar dissolution rates.

Table 10.22 Crystallographic Parameters for the Crystal Forms of Phenobarbital.

Parameter	Form I[a]	Form II[a]	Form III[b]	Form V[a]	Form XIII (hydrate)[a]
Space group	$P2_1/n$	$P\bar{1}$	$P2_1/c$	$P2_1/c$	$Pbca$
a (Å)	6.800	6.784	9.534	12.66	7.157
b (Å)	47.174	23.537	11.855	6.75	30.879
c (Å)	10.695	10.741	10.794	27.69	10.87
α	90.00°	91.89°	90.00°	90.00°	90.00°
β	94.18°	94.43°	111.56°	106.9°	90.00°
γ	90.00°	89.03°	90.00°	90.00°	90.00°
Z	12	6	4	8	8
V (Å^3)	3421.7	1708.8	1134.6	2264.1	2402.3
ρ_{calc} (gm cm^{-3})	1.352	1.354	1.360	1.362	1.384

a Williams, 1973. *b* Williams, 1974.

The effect of additives on the crystallization of phenobarbital has also been investigated (Kato *et al.*, 1984). Kato and co-workers prepared two forms of phenobarbital by adding barbital or cyclobarbital to the crystallization. In these studies rather large quantities of additive (7.5% for barbital and 7% cyclobarbital) were required to achieve the effect.

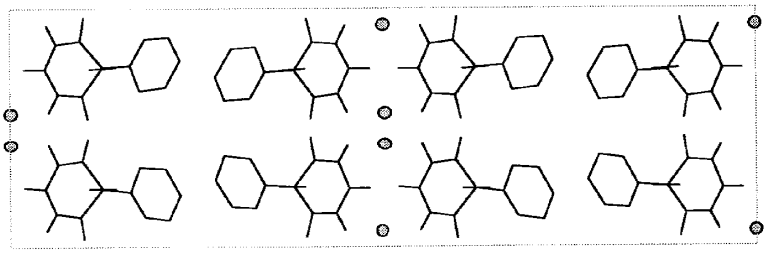


Figure 10.60 Crystal packing of phenobarbital Form XIII hydrate (◎ water molecule) viewed down the *z* axis. (Williams, 1973).

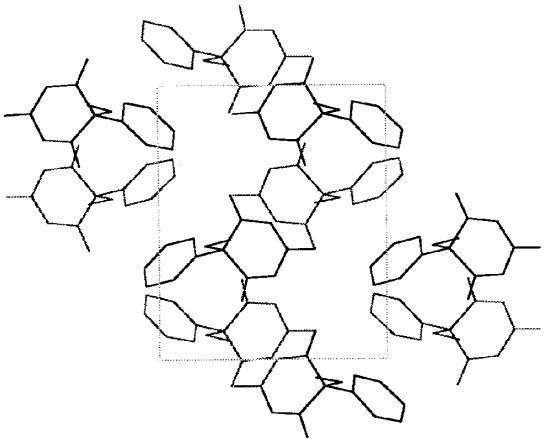


Figure 10.61 Crystal packing of phenobarbital Form III viewed down the *b* axis (Williams, 1974).

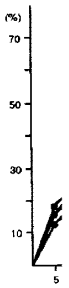
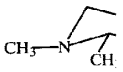


Figure 10.62 Dissolution rate of
pressure of 20 kN,
cial sources), and H

10.7 OTHER DRUGS

In this section the polymorph
this review is not exhaustive,
pharmaceuticals.

A. PROMEDOL ALCOHOL



DeCamp and Ahmed (1972
monoclinic and rhombohedr
methyl-4*e*-phenylpiperidin-4*e*
alcohol is the same in bot

Table 10.23 Crystallographic Pa

Parameter	Monoc
Space Group	
a (Å)	
b (Å)	
c (Å)	
β	
Z	
V (Å^3)	1
ρ_{calc} (gm-cm^{-3})	

a DeCamp and Ahmed, 1972a. *b* I

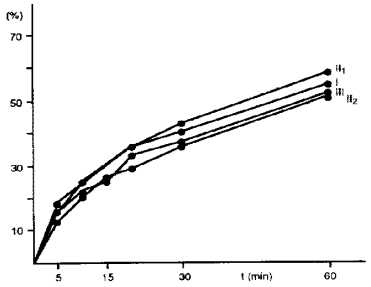
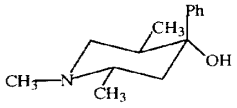


Figure 10.62 Dissolution rate of phenobarbital tablets prepared using the binder Heweten® 40, a pressure of 20 kN, and the four different crystal forms. Forms I, II (from two commercial sources), and III (Szabó-Réveśz *et al.*, 1987).

10.7 OTHER DRUGS

In this section the polymorphic properties of several other drugs are reviewed. While this review is not exhaustive, it illustrates several important studies of polymorphism in pharmaceuticals.

A. PROMEDOL ALCOHOL



(±)-β-promedol alcohol

DeCamp and Ahmed (1972a–b) have determined the crystal structure of both the monoclinic and rhombohedral forms of (±)-β-promedol alcohol, (±)-α-1,2*a*,5*e*-trimethyl-4*e*-phenylpiperidin-4*a*-ol, (see Table 10.23). The conformation of β-promedol alcohol is the same in both forms, but the crystal packing differs (see Figures

Table 10.23 Crystallographic Parameters for the Two Forms of (±)-β-Promedol Alcohol

Parameter	Monoclinic Form[a]	Rhombohedral Form[b]
Space Group	$P2_1/n$	$R\bar{3}$
a (Å)	13.298	29.754
b (Å)	7.721	29.754
c (Å)	12.776	7.713
β	90.09°	60.0°
Z	4	18
V (Å^3)	1311.8	5913.5
ρ_{calc} (gm·cm^{-3})	1.109	1.110

a DeCamp and Ahmed, 1972a. *b* DeCamp and Ahmed, 1972b

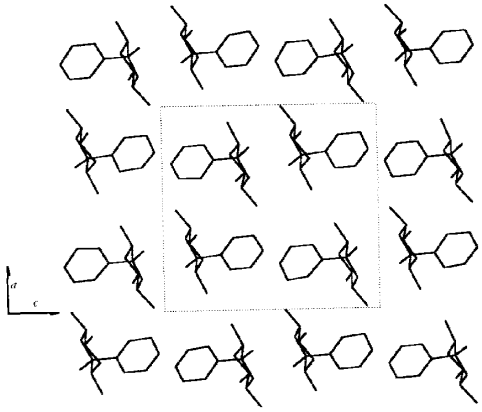


Figure 10.63 Crystal packing of (±)-β-promedol alcohol monoclinic form (DeCamp and Ahmed, 1972a).

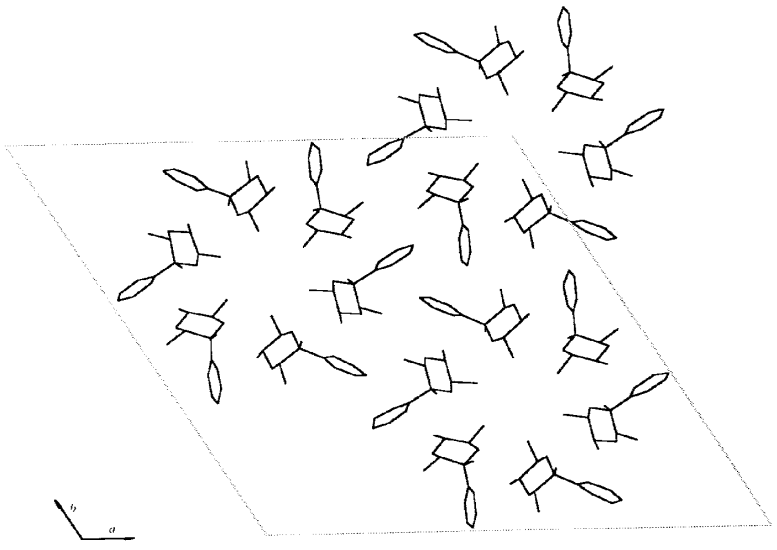


Figure 10.64 Crystal packing of (±)-β-promedol alcohol rhombohedral form (DeCamp and Ahmed, 1972b).

10.63–10.64). In the
same chirality to form
gen bonds; however,
Despite the differenc
have almost the sar
104.5–105 °C, where
difference in melting
since the OH···N dis
the densities indicate
DeCamp and Ahmed
rings of molecules of
molecules of the san
ordering results in a
monoclinic form. S
(1971).

B. ENALAPRIL MA

This example illustra
morphs. Enalapril
different solid-state
ethyl ester methyl a
respectively. The
forms as shown in F
of the two crystal fo
the DSC analysis, th
solution data, as sho
dissolution for the

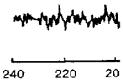
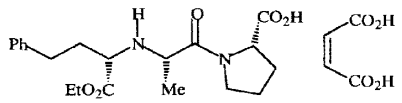


Figure 10.65 Solid-st

10.63–10.64). In the monoclinic form, OH···N hydrogen bonds link molecules of the same chirality to form chains. In the rhombohedral form, there are also OH···N hydrogen bonds; however, these link molecules of alternating chirality into hexameric rings. Despite the differences in crystal packing, the monoclinic and rhombohedral crystals have almost the same density. The melting point of the rhombohedral form is 104.5–105 °C, whereas the melting point of the monoclinic form is 90.5–91 °C. This difference in melting point is probably not related to differences in hydrogen bonding since the OH···N distances are approximately the same in the two forms. In addition, the densities indicate that the two forms have nearly equal packing energies. Thus, DeCamp and Ahmed (1972a) suggested that, since the rhombohedral form contains rings of molecules of alternating chirality while the monoclinic form contains stacks of molecules of the same chirality, the monoclinic form is more ordered. This increased ordering results in an entropy difference that results in a lower melting point for the monoclinic form. Similar arguments were also advanced by Krigbaum and Wildman (1971).

B. Enalapril Maleate



enalapril maleate

This example illustrates the need for using more than one method in looking for polymorphs. Enalapril maleate (Ip *et al.*, 1986) exists in two crystal forms which give different solid-state ^{13}C NMR spectra. (Figures 10.65 and 10.66). The signals of the ethyl ester methyl and maleate carbon signals are at 11–13 ppm and 137–138 ppm, respectively. The XRPD patterns also display a difference between the two crystal forms as shown in Figures 10.67 and 10.68. However, the FT-IR and Raman spectra of the two crystal forms are very similar. Under the experimental conditions used in the DSC analysis, the thermograms of both forms cannot be distinguished. Heat of solution data, as shown in Table 10.24, indicate that there are differences in the heats of dissolution for the two forms, although both crystal forms have virtually identical

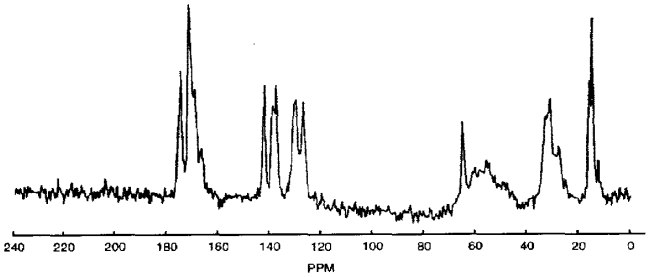


Figure 10.65 Solid-state ^{13}C NMR of enalapril maleate Form I (Ip *et al.*, 1986).

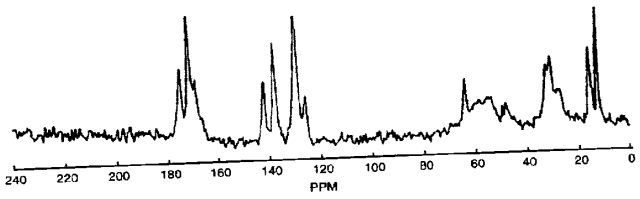


Figure 10.66 Solid-state ^{13}C NMR of enalapril maleate Form II (Ip *et al.*, 1986).

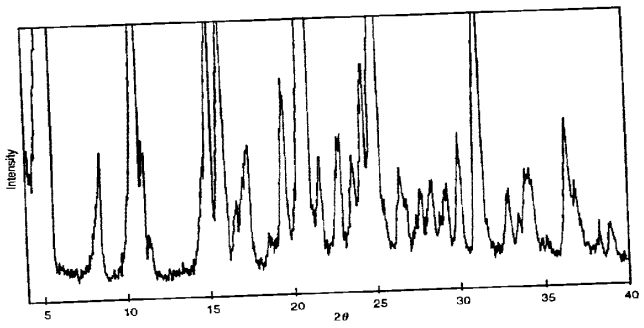


Figure 10.67 Powder X-ray diffraction pattern of enalapril maleate Form I (Ip *et al.*, 1986).

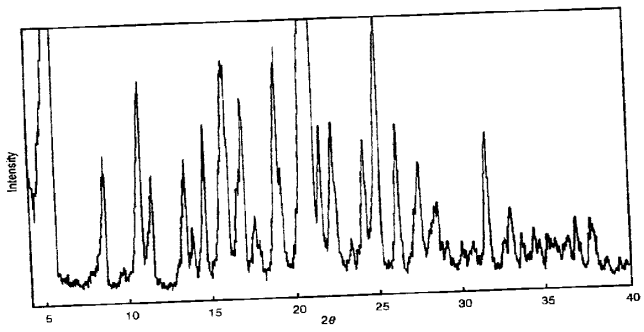


Figure 10.68 Powder X-ray diffraction pattern of enalapril maleate Form II (Ip *et al.*, 1986).

in vitro dissolution rates (s
number of methods on two
two crystal forms are very
properties.

Table 10.24 Heats of Solution

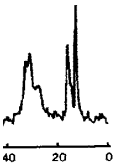
Solvent	Form I Δ (kJ/mo
Methanol	36.5(
	35.6
	35.9
	36.2
	36.4
Mean ± S.D.	36.33 ±
Acetone	59.4
	59.7
	59.1
	59.7
Mean ± S.D.	59.52 ±

Ip *et al.*, 1986.

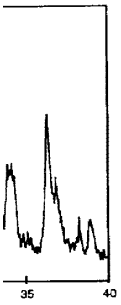
Table 10.25 Dissolution Dat

Enalapril Maleate Formulation	Crys
Capsules	
	I
	I
Tablets	

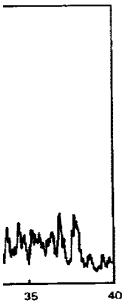
Ip *et al.*, 1986.



86).



p *et al.*, 1986).



(Ip *et al.*, 1986).

in vitro dissolution rates (see Table 10.25). In summary, this represents a study by a number of methods on two crystal forms of an important compound. It is clear that the two crystal forms are very similar in structure and have very similar pharmaceutical properties.

Table 10.24 Heats of Solution and Transition of Enalapril Maleate Polymorphs

Solvent	Form I ΔH_{soln} (kJ/mol)	Form II ΔH_{soln} (kJ/mol)	ΔH_{Trans} (kJ/mol)
Methanol	36.50	38.47	
	35.64	38.21	
	35.95	38.54	
	36.20	38.62	
	36.46		
Mean ± S.D.	36.33 ± 0.25	38.46 ± 0.11	2.05
Acetone	59.44	62.71	
	59.73	61.99	
	59.19	62.66	
	59.73	62.54	
Mean ± S.D.	59.52 ± 0.25	62.41 ± 0.29	2.89

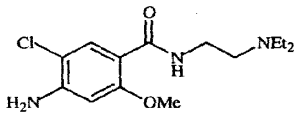
Ip *et al.*, 1986.

Table 10.25 Dissolution Data for Enalapril Maleate Capsules and Tablets

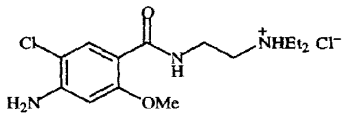
Enalapril Maleate Formulation	Crystal Form	Potency (mg)	Average Percent Dissolved at 30 min
Capsules	II	2.5	89
	I	2.5	100
	I and II	2.5	101
	I	2.5	96
	I and II	20	82
	I	20	99
	II	20	95
	I	20	92
Tablets	I	10	100
	II	10	99
	I	10	99
	I and II	10	98
	I	40	103
	I and II	40	102
	II	40	96

Ip *et al.*, 1986.

C. Metoclopramide and Metoclopramide Monohydrochloride



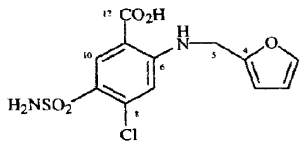
metoclopramide



metoclopramide monohydrochloride

Mitchell (1985) has studied the polymorphism of both metoclopramide and metoclopramide monohydrochloride. Each exists in two crystal forms and metoclopramide monohydrochloride also forms a monohydrate. Metoclopramide exists in two enantiotropic polymorphs with a transition temperature of 125 °C from Form I (stable at low temperature) to Form II (stable at high temperature) having a melting point of 147 °C. This process can also be reversed. Dehydration of metoclopramide monohydrochloride monohydrate, depending on the conditions, give rise to one of two anhydrous polymorphs; Form I (mp 187 °C) is formed from the melt under slow crystallization conditions, whereas, Form II (mp 155 °C) is formed from the melt under fast crystallization conditions. All of these crystal forms were detected by DSC, thermal microscopy, X-ray diffraction, and infrared spectroscopy.

D. Furosemide



furosemide

Doherty and York (1988) described the two crystal forms of furosemide readily detected by X-ray powder diffraction. In a more recent study, Matsuda and Tatsumi (1990) discovered three additional polymorphs as well as two solvates and an amorphous form. Interestingly, it was found that the forms produced could be related to the boiling point of the solvent. Thus, Form I was obtained from the lower boiling solvents used [acetone (bp 57 °C), methanol (bp 65 °C), ethanol (bp 79 °C), and methyl ethyl ketone (bp 80 °C)], Form II was obtained from the higher boiling solvents used [isobutyl alcohol (bp 108 °C), butanol (bp 118 °C), and pentanol (bp 138 °C)], and mixtures of both forms were obtained from solvents with intermediate boiling points used [isopropyl alcohol (bp 83 °C) and propanol (bp 97 °C)] by slow crystallization from a hot solution. To our knowledge this is the first such relationship which has been reported. In addition, they reported that the rate of solvent evaporation affected the crystal form obtained. Figure 10.69 shows the XRPDs of furosemide and Figure 10.70 shows the IR spectra of the different crystal forms.

Doherty and York (1988) also showed that Forms I and II had different solid-state NMR spectra as shown in Figure 10.71. Figure 10.72 shows the DSC and TG

thermograms of the six dif
all forms are unique and w

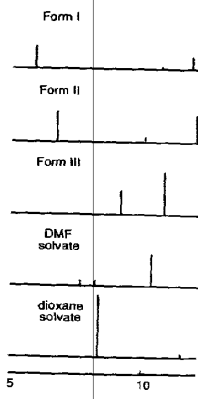


Figure 10.69 X-ray powder di
and Tatsumi, 19

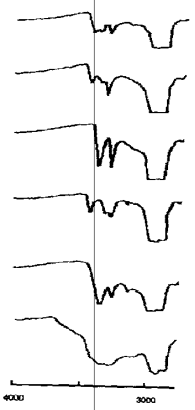


Figure 10.70 Infrared spectr
1990).

thermograms of the six different forms of furosemide. It is clear from these studies that all forms are unique and well characterized.

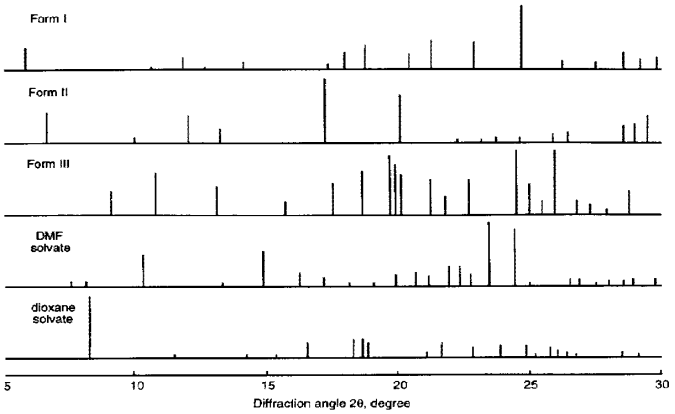


Figure 10.69 X-ray powder diffraction patterns of the different crystal forms of furosemide (Matsuda and Tatsumi, 1990).

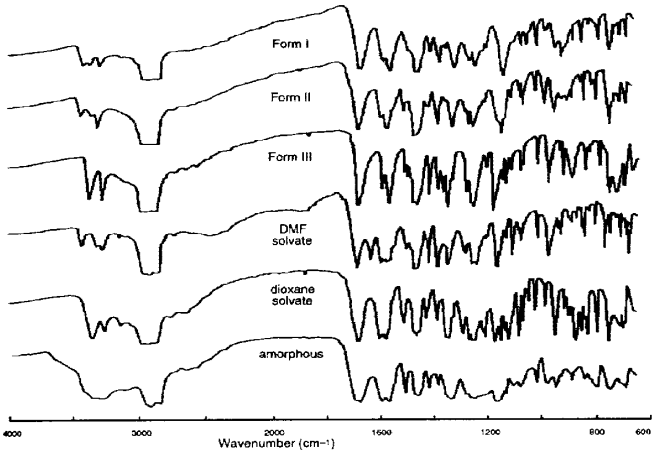


Figure 10.70 Infrared spectra of the different crystal forms of furosemide (Matsuda and Tatsumi, 1990).

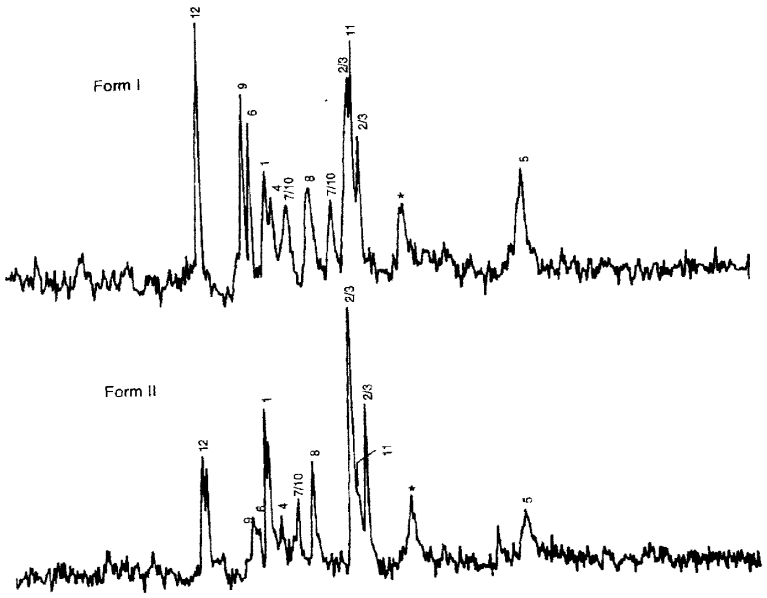


Figure 10.71 Solid-state ^{13}C CP/MAS NMR spectra for two furosemide forms at ambient temperature with peak assignments. The peaks marked with a star are due to the Delrin® rotor (Doherty and York, 1988).

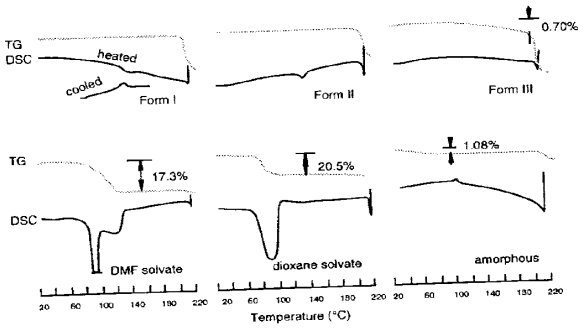


Figure 10.72 DSC and TG thermograms of the different crystal forms of furosemide (Matsuda and Tatsumi, 1990).

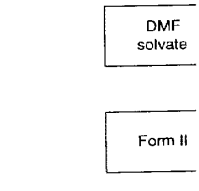


Figure 10.73 Interconvers
and Tatsum

Matsuda and Tats
which could be obtaine
studied the interconvers
rized in Figure 10.73.
most stable form, Form
Form I upon heating (s
Matsuda and Tats

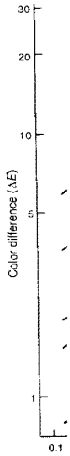


Figure 10.74 Double-log
forms unde

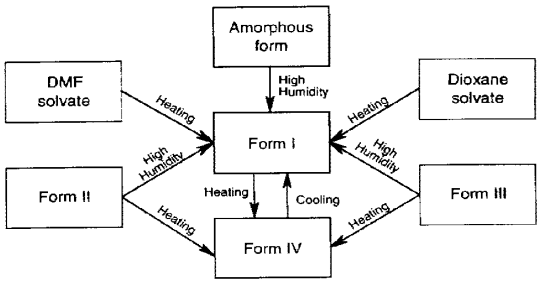


Figure 10.73 Interconversion scheme of furosemide crystal forms under various conditions. (Matsuda and Tatsumi, 1990).

Matsuda and Tatsumi (1990) found a high temperature crystal form (Form IV) which could be obtained by heating Forms I, II, or III to 180 °C. In addition, they studied the interconversion of the crystal forms and these interconversions are summarized in Figure 10.73. It is clear that all of the crystal forms can be converted into the most stable form, Form I, at room temperature. The solvated forms also converted to Form I upon heating (see Figure 10.73).

Matsuda and Tatsumi also studied the physical and chemical properties of the

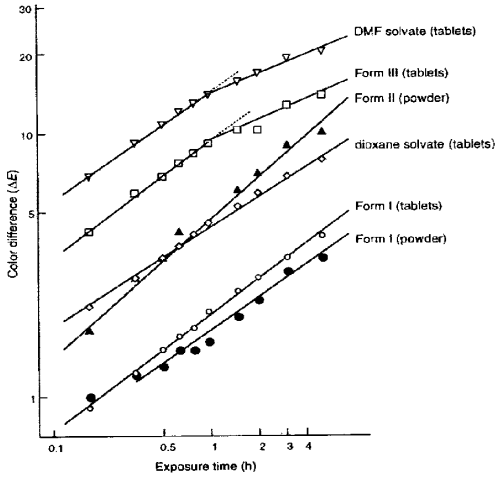


Figure 10.74 Double-logarithmic plots for the coloration process of different furosemide crystal forms under irradiation by a mercury vapor lamp (Matsuda and Tatsumi, 1990).

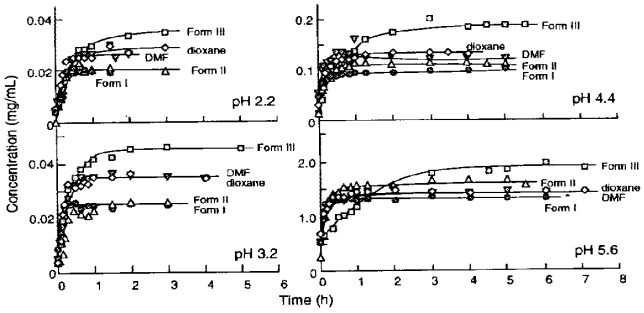
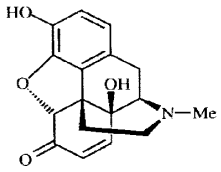


Figure 10.75 Dissolution profiles of the different crystal forms of furosemide in buffer solution at various pH values at 37° C (Matsuda and Tatsumi, 1990).

different crystal forms of furosemide. Figure 10.74 shows the studies on the photostability of the different crystal forms. It is apparent that the different crystal forms have a different amount of coloration initially but that the rate of change in coloration is about the same for all crystal forms. However, the relationship between coloration and degradation remains unknown.

Figure 10.75 shows the dissolution profiles of furosemide at different pH (2.2, 3.2, 4.4, and 5.6). It is apparent that Form II reaches the highest solubility at all pH's and that Form II and the DMF solvate are the least soluble. Judging by these profiles, some of the forms appear to interconvert in these experiments.

E. 14-Hydroxymorphinone—Color Dimorphism



14-hydroxymorphinone

The phenolic α,β-unsaturated ketone 14-hydroxymorphinone exists in two crystalline modifications (see Table 10.26), which are interconvertible by dissolution and recrystallization (Chiang *et al.*, 1978). Recrystallization from polar solvents (ethanol) yields yellow crystals, while crystallization from benzene gives colorless (white) crystals. Both forms are stable indefinitely in the solid state.

Infrared spectra show that the yellow form has a carbonyl absorption at 1685 cm^{-1}, while the colorless form has a carbonyl absorption at 1660 cm^{-1}. Since both forms have a carbonyl absorption, neither form contains an enol tautomer.

Crystallographic studies show that the conformation of 14-hydroxymorphinone in the two forms is similar; however, the yellow form contains an intermolecular OH···O

Table 10.26 Crystallogr

Parameter
Space group
a (Å)
b (Å)
c (Å)
Z
ρ_{calc} (g cm^{-3})
V (Å^3)

Chiang *et al.*, 1978.

hydrogen bond, while bond.

The color of the y hydrogen bond, since dihydroxyterephthalate is that there is a weak adjacent phenyl ring in tion between these two

Numerous other re that are not drugs. The *et al.*, 1978; Byrn *et a.* important compound f thebaine gave metathe sodium bicarbonate and NaOH or NH_3 and recr melting point, and both solution in benzene. U color and no investigati been reported.

M

F

F. Miscellaneous St

Kuhnert-Brandstätter an polymorphs of pharmace spectroscopy, and in son shown in Table 10.27. I of the different polymor

Table 10.26 Crystallographic Parameters for the Two Forms of 14-Hydroxymorphinone

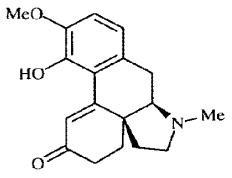
Parameter	Colorless Form	Yellow Form
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	12.918	13.150
b (Å)	14.074	13.508
c (Å)	8.035	7.837
Z	4	4
ρ_{calc} (g cm^{-3})	1.36	1.428
V (Å^3)	1460.8	1392.1

Chiang *et al.*, 1978.

hydrogen bond, while the white form contains an intramolecular OH···O hydrogen bond.

The color of the yellow form may, in part, result from the intermolecular OH···O hydrogen bond, since a similar effect was found for dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate (Byrn *et al.*, 1972; see Section 8.1). An alternative explanation is that there is a weak charge-transfer interaction between the C=O group and an adjacent phenyl ring in the yellow form, but not in the colorless form. A clear distinction between these two explanations is not possible.

Numerous other reports of color dimorphism have been published for compounds that are not drugs. These reports are briefly reviewed by (Desiraju *et al.*, 1977; Chiang *et al.*, 1978; Byrn *et al.*, 1972). Color dimorphism of at least one other biologically important compound has been reported (Small and Meitzner, 1933); reduction of thebaine gave metathebainone. Neutralization of a metathebainone solution with sodium bicarbonate and recrystallization gave yellow crystals, while neutralization with NaOH or NH_3 and recrystallization gave colorless crystals. Both crystals had the same melting point, and both gave a yellow solution in ethanol or water and a colorless solution in benzene. Unfortunately, no structural explanations of these differences in color and no investigation of differences in polymorphism of these compounds have been reported.



metathebainone

F. Miscellaneous Studies by Kuhnert-Brandstätter and Co-workers

Kuhnert-Brandstätter and co-workers have carried out an extensive study on the polymorphs of pharmaceuticals. Their studies generally use thermal microscopy, IR spectroscopy, and in some cases powder diffraction. The results of these studies are shown in Table 10.27. In many cases they were able to determine the relative stability of the different polymorphs and whether they were monotropic (one forms is most

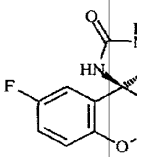
Table 10.27 Studies of Polymorphic Pharmaceuticals by Kuhnert-Brandstätter's Group

Pharmaceutical	No. of Forms	Thermodynamics*	Reference
Amiperone	2	II → I	Kuhnert-Brandstätter and Porsche, 1989b
Anilamate	3	III → II, II → I	Kuhnert-Brandstätter *et al.*, 1982c
Benactyzine HCl	2	II → I	Kuhnert-Brandstätter. and Wurian, 1982a
Bentiromide	3 + hydrates	II → I, ···	Kuhnert-Brandstätter and Porsche, 1989b
Bromopride	2	II → I	Kuhnert-Brandstätter *et al.*, 1982b
Brotizolam	4	IV → III, III → I, ···	Kuhnert-Brandstätter and Porsche, 1989b
Bumetanide	2	II → I	Kuhnert-Brandstätter *et al.*, 1982b
Bupicomide	3	Monotropic	Kuhnert-Brandstätter and Porsche, 1989a
Buspirone HCl	2	Monotropic	Kuhnert-Brandstätter and Porsche, 1989a
Clenbuterol HCl	2	II → I	Kuhnert-Brandstätter. and Wurian, 1982a
Dimethoxanate HCl	2	II → I	Kuhnert-Brandstätter *et al.*, 1982c
Diphenadione	2	II → I	Kuhnert-Brandstätter *et al.*, 1982c
Diphenidol HCl	3	III → II, III → I	Kuhnert-Brandstätter. and Wurian, 1982a
Dipyridamole	2	II → I	Kuhnert-Brandstätter. and Wurian, 1982a
Dobutamine HCl	4	···	Kuhnert-Brandstätter and Porsche, 1989b
Famotidine	2	II → I	Kuhnert-Brandstätter and Porsche, 1990
Fenbufen	3	III → II, III → I	Kuhnert-Brandstätter and Porsche, 1989b
Flucabril	2	II → I	Kuhnert-Brandstätter *et al.*, 1982b
Flupirtine Maleate	2	II → I	Kuhnert-Brandstätter and Porsche, 1990
Gallic Acid Ethyl Ester	3	III → II, III → I	Kuhnert-Brandstätter. and Wurian, 1982a
Halofenate	3	Monotropic	Kuhnert-Brandstätter and Völlenklee, 1986
Heptolamide	3	···	Kuhnert-Brandstätter and Porsche, 1989a
Iprindol HCl	3	III → II, ···	Kuhnert-Brandstätter *et al.*, 1982b
Levobunolol HCl	5		Kuhnert-Brandstätter and Porsche, 1989a
Lorcainide HCl	2	II → I	Kuhnert-Brandstätter and Völlenklee, 1986
Maprotiline HCl	3	III → II, II → I	Kuhnert-Brandstätter *et al.*, 1982c
Mexiletine HCl	3	III → I, II → I	Kuhnert-Brandstätter and Völlenklee, 1987
Minoxidil	3	III → II, II → I	Kuhnert-Brandstätter and Völlenklee, 1986
Mopidamol	4	IV → I, II → I, ···	Kuhnert-Brandstätter and Völlenklee, 1986
Nafoxidine HCl	3	III → I, II → I	Kuhnert-Brandstätter *et al.*, 1982c
Naftifine HCl	3	Monotropic	Kuhnert-Brandstätter and Porsche, 1989a
Oxypendyl 2HCl	4	III → I, II → I, ···	Kuhnert-Brandstätter and Völlenklee, 1987
Paxamate	2	II → I	Kuhnert-Brandstätter and Porsche, 1990
Penbutolol Sulfate	4	IV → III, III → II, ···	Kuhnert-Brandstätter and Völlenklee, 1987
Piretanide	4	II → I, ···	Kuhnert-Brandstätter and Porsche, 1989a
Pirprofene	2	Monotropic	Kuhnert-Brandstätter and Völlenklee, 1987
Propentofylline	4	Monotropic	Kuhnert-Brandstätter and Porsche, 1990
Renytoline HCl	3	III → II, II → I	Kuhnert-Brandstätter *et al.*, 1982b
Terconazole	2	Monotropic	Kuhnert-Brandstätter and Porsche, 1989b
Triclabendazole	2	Monotropic	Kuhnert-Brandstätter and Porsche, 1990

* Some forms undergo inhomogeneous melting rather than transformation.

stable at all temperatures) o
peratures). Specifically, Ku
this table as cases where the
the highest melting point.

G. (2*R*,4*S*)-6-FLUORO-2-M



This aldose reductase inhil
studied by DSC, X-ray pow
1988). Figure 10.76 shov
indicates that the β-form is
tent with the X-ray powder
sion of the β-form to the α
the α- and β-forms as wel
heating the β-form, indicati
α-form to the β-form appe

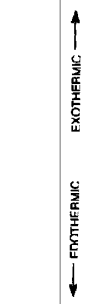
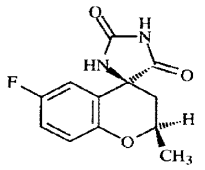


Figure 10.76 The DSC curv
dione (Ashizawa

stable at all temperatures) or enantiotropic (different forms are stable at different temperatures). Specifically, Kuhnert-Brandstätter defined enantiotropy for the purposes of this table as cases where the most stable form at room temperature is not the form with the highest melting point.

G. (2*R*,4*S*)-6-FLUORO-2-METHYLSPIRO[CHROMAN-4,4′-IMIDAZOLINE]-2′,5-DIONE



(2*R*,4*S*)-6-fluoro-2-methylspiro-[chroman-4,4′-imidazoline]-2′,5-dione

This aldose reductase inhibitor exists in two crystal forms, α and β, which were studied by DSC, X-ray powder diffraction, and infrared spectroscopy (Ashizawa *et al.*, 1988). Figure 10.76 shows the DSC behavior of the β–form. This thermogram indicates that the β–form is converted to the α–form at high temperature and is consistent with the X-ray powder diffraction and infrared spectra which showed interconversion of the β–form to the α-form. Figure 10.77 shows the X-ray powder patterns of the α– and β–forms as well as that of a 1:1 mixture and the product obtained upon heating the β–form, indicating it is being transformed into the α–form. Addition of the α–form to the β–form appears to provide nuclei which allow the conversion to occur

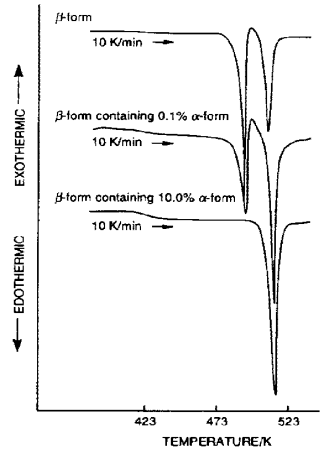


Figure 10.76 The DSC curve for (2*R*,4*S*)-6-fluoro-2-methylspiro[chroman-4,4′-imidazoline]-2′,5-dione (Ashizawa *et al.*, 1988).

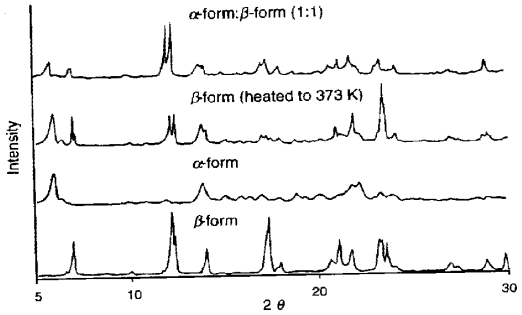
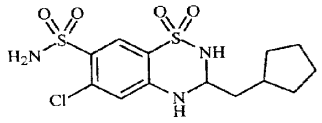


Figure 10.77 X-ray diffraction patterns of (2*R*,4*S*)-6-fluoro-2-methylspiro[chroman-4,4′-imidazoline]-2′,5-dione (Ashizawa *et al.*, 1988).

before melting of the β–form. This indicates the importance of nucleation in polymorphic interconversions.

The crystal structure of the β–form has been determined by single crystal X-ray methods (Ashizawa, 1989). They suggested that the crystal structure of the α–form is disordered and thus the structure could not be determined.

H. Cyclopenthiazide



cyclopenthiazide

The diuretic cyclopenthiazide exists in three polymorphic forms which are obtained by crystallization from ethanol:heptane:methanol (Form I), ethanol (Form II), and ethanol:water (Form III) (Gerber *et al.*, 1991).

These forms were characterized by DSC, thermomicroscopy, X-ray powder diffraction, scanning electron micrographs, IR, solid-state NMR, solution calorimetry, dissolution rates, and solubility determinations.

Figure 10.78 shows the DSC thermograms, Figure 10.79 shows the X-ray powder diffraction patterns, and Figure 10.80 shows the solid-state CP/MAS spectra. The DSC thermograms gave the following heats of fusion for the different polymorphs: Form I, 105.5 kJ/mol; Form II, 98.4 kJ/mol and Form III, 62.5 kJ/mol. The value for Form III is too low to be the ΔH_f and most likely represents a transformation process This was confirmed by thermomicroscopy in which Form III melted at 181 °C and recrystallized to Form I.

Figure 10.78 DSC the
238 °C;

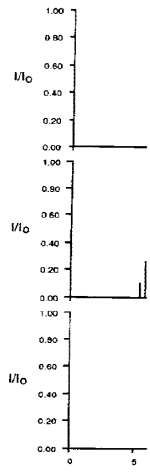


Figure 10.79 X-ray pov
1991).

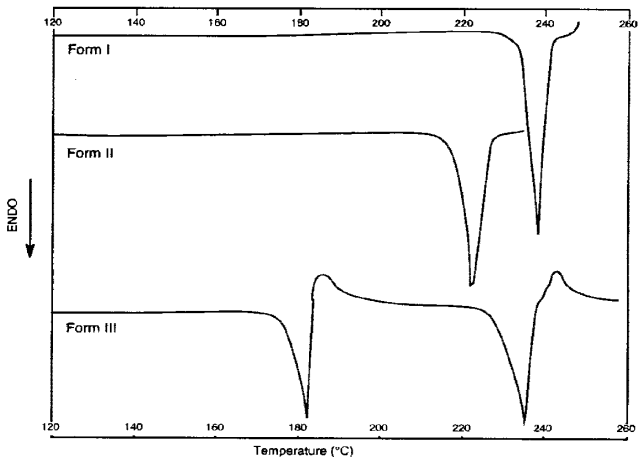


Figure 10.78 DSC thermograms of cyclopentathiazide polymorphs with melting points: Form I, 238 °C; Form II, 225 °C; and Form III, 181° and 235 °C (Gerber *et al.*, 1991).

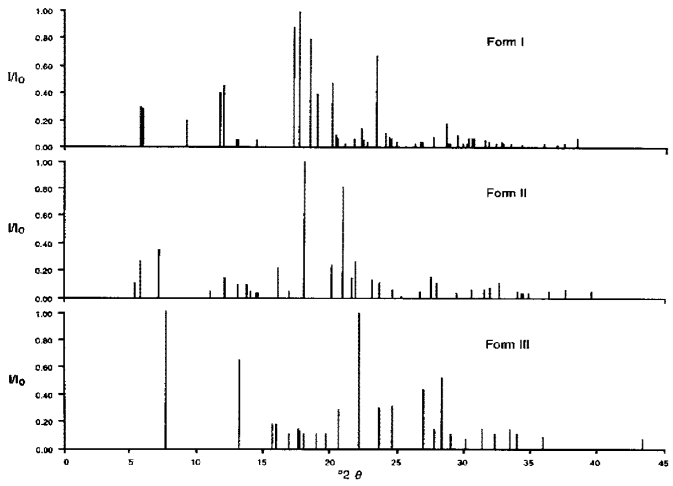


Figure 10.79 X-ray powder diffraction patterns of cyclopentathiazide polymorphs (Gerber *et al.*, 1991).

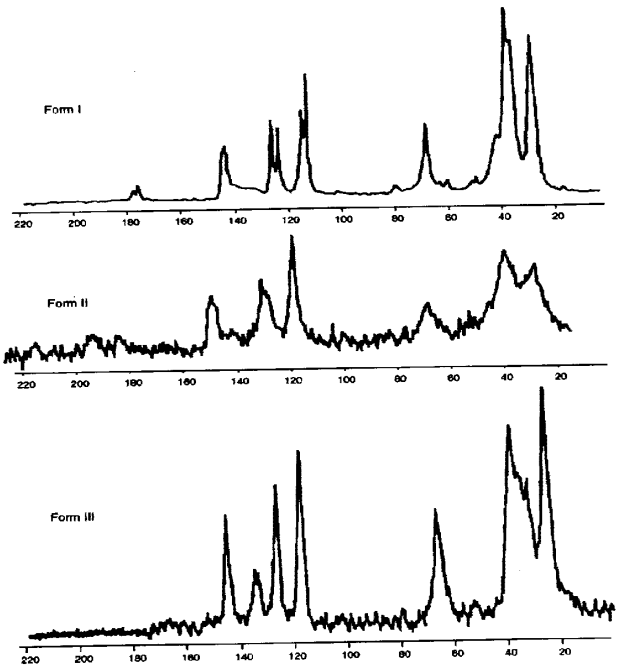


Figure 10.80 Solid-state ^{13}C NMR spectra of cyclopentathiazide polymorphs (Gerber *et al.*, 1991).

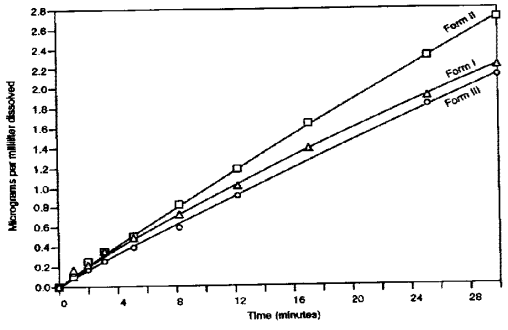
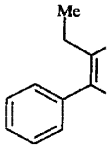


Figure 10.81 Intrinsic dissolution rates of cyclopentathiazide polymorphs (Gerber *et al.*, 1991).

It is evident from
of solution of the diff
Form I, 0.34 kJ/mol; F
these measurements ra
solution within experi
were measured and are
tion rates but Form II h
three forms were also
solubility was Form II
most stable polymorph

I. TAMOXIFEN CITR



Tamoxifen citrate is w
(1987) have reported th
Form B. Figure 10.82
morph of tamoxifen citr
of Form A; however, t
organized and less sta

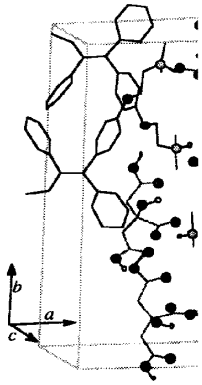
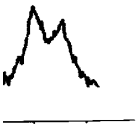
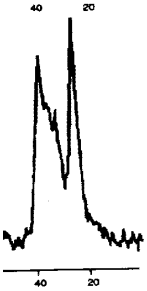
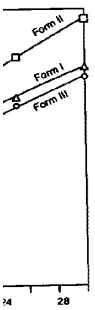


Figure 10.82 Stereoview o
Becker, 1987)





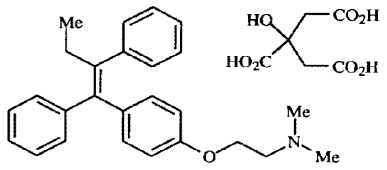
orphs (Gerber *et al.*, 1991).



hs (Gerber *et al.*, 1991).

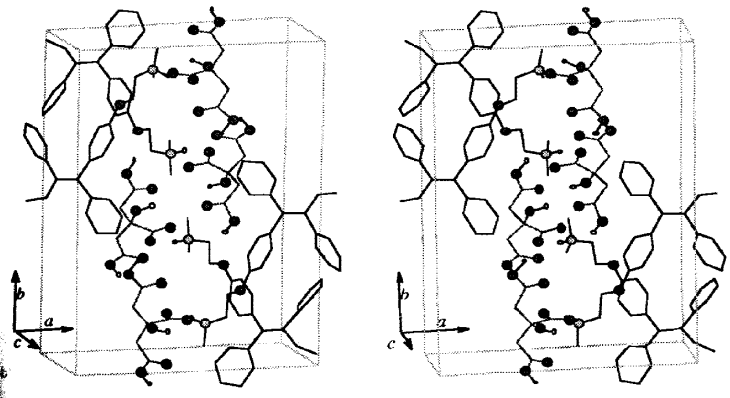
It is evident from all these data that these are truly different polymorphs. The heats of solution of the different polymorphs in 95% ethanol were also determined and are: Form I, 0.34 kJ/mol; Form II, 0.35 kJ/mol; and Form III, 0.86 kJ/mol. The errors in these measurements range 0.03–0.06 kJ/mol; thus Forms I and II have the same heat of solution within experimental error. The intrinsic dissolution rates of the three forms were measured and are shown in Figure 10.81. Forms I and III have similar dissolution rates but Form II has a significantly higher dissolution rate. The solubilities of the three forms were also determined in several solvents and in all cases the order of solubility was Form II > Form I > Form III. These data suggest that Form III is the most stable polymorph.

I. Tamoxifen Citrate



tamoxifen citrate

Tamoxifen citrate is well known as an antiestrogenic agent. Goldberg and Becker (1987) have reported the crystal structure of the more stable of two polymorphic forms, Form B. Figure 10.82 shows a stereoview of the crystal packing of the stable polymorph of tamoxifen citrate. Unfortunately they were not able to determine the structure of Form A; however, they point out that there are several indications that it is a less organized and less stable structure. For instance, they observed that at room



re 10.82 Stereoview of the crystal structure of Form B of tamoxifen citrate (Goldberg and Becker, 1987).

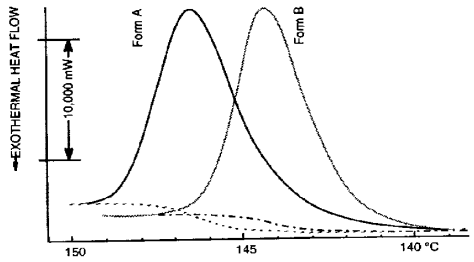


Figure 10.83 DSC thermograms of the two crystal forms of tamoxifen citrate (Goldberg and Becker, 1987).

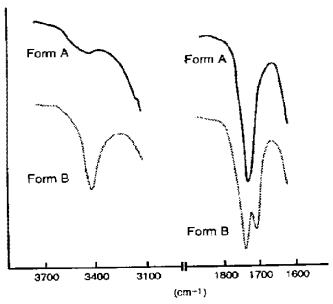


Figure 10.84 Infrared spectra of the two crystal forms of tamoxifen citrate: Form A, solid lines; Form B, dashed lines (Goldberg and Becker, 1987).

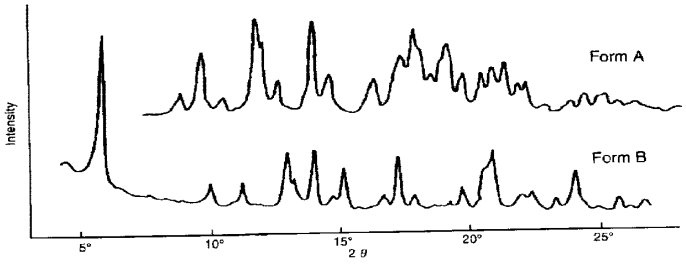


Figure 10.85 X-ray powder diffraction patterns of the two crystal forms of tamoxifen citrate (Goldberg and Becker, 1987).

temperature in an etl
They also reported
10.84), and the XRP

J. ANTIULCER AGE

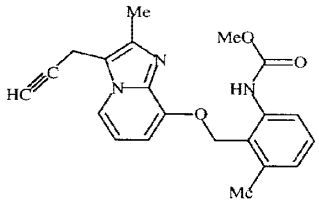
HC

Miyamac and co-worl
phism of an orally-ac
benzyloxy)-2-methyl-
in two crystal Forms A
crystal forms which
10.86–10.87). In ad
diffraction patterns an
IR spectra of the two c
complicated absorption
might be caused by dif

Figure 10.86 Stereoview of

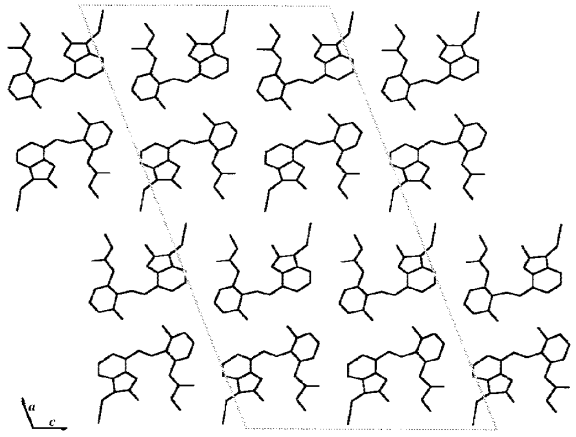
temperature in an ethanol suspension, Form A rearranges spontaneously to Form B. They also reported the DSC thermograms (Figure 10.83), the IR spectra (Figure 10.84), and the XRPDs (Figure 10.85) of the two polymorphs.

J. Antiulcer Agent FR101853



8-(2-methoxycarbonyl-amino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo[1,2-*a*]pyridine (FR101853)

Miyamae and co-workers (1990) have carried out an extensive study of the polymorphism of an orally-active antiulcer compound 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)imidazo[1,2-*a*]pyridine (FR101853) which exists in two crystal Forms A and B. Table 10.28 shows the crystallographic data for the two crystal forms which exhibit significantly different crystal packing (see Figures 10.86–10.87). In addition, the different crystal forms have different X-ray powder diffraction patterns and different DSC thermograms (Figure 10.88). Interestingly, the IR spectra of the two crystal forms are very similar (Figure 10.89) perhaps because the complicated absorptions of the molecule obscure any differences in infrared spectra that might be caused by different crystal packing.



re 10.86 Stereoview of the crystal packing of FR101853, Form A (Miyamae *et al.*, 1990).

Table 10.28 Crystal Data for the Two Crystal Forms of FR101853

Parameter	Form A	Form B
Space Group	$C2/c$	$P2_1/c$
a (Å)	42.936(14)	4.367(1)
b (Å)	4.356(1)	38.214(3)
c (Å)	21.536(6)	11.253(1)
β	109.92(4)°	95.47(2)°
Z	8	4
ρcalc (g cm^{-3})	1.275	1.292
V (Å^3)	3786.7(20)	1869.4(3)

Miyamae *et al.*, 1990.

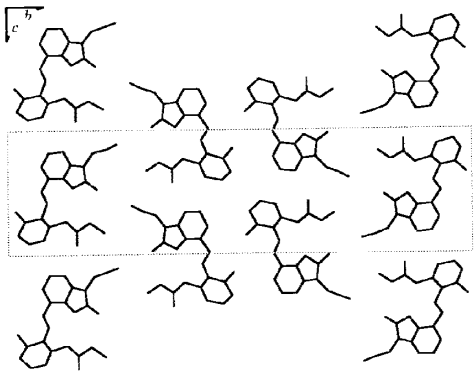


Figure 10.87 Stereoview of the crystal packing of FR101853, Form B (Miyamae *et al.*, 1990).

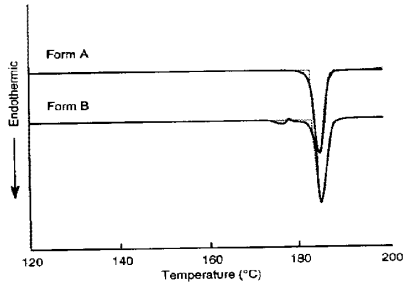


Figure 10.88 DSC thermograms of the different crystal forms of FR101853 (Miyamae *et al.*, 1990).

Form

Form

Figure 10.89 Infrared spect

10.8 CARBOHYDRAT

In this section, polymor substantial interest since ous carbohydrates exhib have been reported.

Mannitol exists in f isolated in the pure stat impurity. In addition, a The different compressib implications for their use tion patterns of the α- shows the X-ray powde apparent that material fr other preparations. The cial products were deter also carried out and it w tablets of different hardr but different amounts o related to the crystal form urements may be subject in the different crystal preparation and demonst excipients used in tablets

Several other carbol drate 4-methoxyphenyl- Each form has a distinct 161 °C (Shafizadeh an pyranoside also exists in can be converted to For acetamidotri-*O*-acetyl-β-

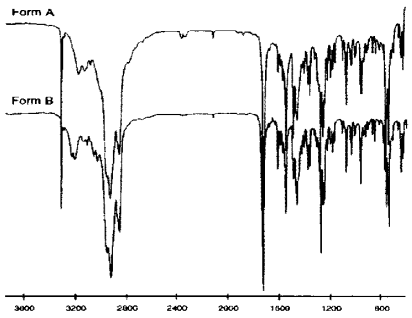


Figure 10.89 Infrared spectra of the different crystal forms of FR101853 (Miyamae *et al.*, 1990).

10.8 CARBOHYDRATES

In this section, polymorphism of carbohydrates is briefly discussed. This area is of substantial interest since carbohydrates are often used as excipients. Although numerous carbohydrates exhibit polymorphism, relatively few studies of these compounds have been reported.

Mannitol exists in four forms (Debord *et al.*, 1987). The α- and β-form have been isolated in the pure state, the δ-form has been isolated containing the α-form as an impurity. In addition, a fourth form was found but could not be characterized further. The different compressibilities and particle shapes of these forms could have important implications for their use as excipients. Figure 10.90 shows the X-ray powder diffraction patterns of the α- and β-forms as well as the "unknown" form. Figure 10.91 shows the X-ray powder patterns of different commercial products of mannitol. It is apparent that material from supplier 4 (S_4) contains a crystal form different from the other preparations. The water contents of the crystal forms and the different commercial products were determined after two months storage. Compression studies were also carried out and it was found that compression of the different samples produced tablets of different hardness. The different products and crystal forms took up small but different amounts of water, but the amount of water uptake did not seem to be related to the crystal form. The amounts of water uptake are so small that these measurements may be subject to variations from the amount of amorphous material present in the different crystal forms. Such studies have important implications for tablet preparation and demonstrate that it may be important to control the polymorphic form of excipients used in tablets.

Several other carbohydrates also exist in polymorphs. For example, the carbohydrate 4-methoxyphenyl-β-D-glucopyranoside exists in two forms (Forms I and II). Each form has a distinct powder pattern, and Form II can be converted to Form I at 161 °C (Shafizadeh and Susott, 1973). Phenyl-2-acetamidotri-*O*-acetyl-β-D-glucopyranoside also exists in two polymorphs that have different powder patterns. Form II can be converted to Form I at 185 °C (Shafizadeh and Susott, 1973). 4-Methoxy-2-acetamidotri-*O*-acetyl-β-D-glucopyranoside exists in four forms which have different

powder patterns (Shafizadeh and Susott, 1973). Form IV is converted to Form III at 158 °C, Form III can be converted to Form II at 177 °C, and Form II can be converted to the least stable form, Form I, at 183 °C. Form I melts at 192 °C.

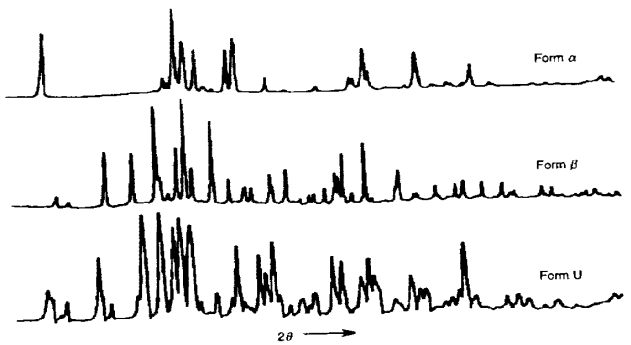


Figure 10.90 X-ray powder diffraction patterns of the α-, β-, and unknown forms of mannitol (Debord *et al.*, 1987).

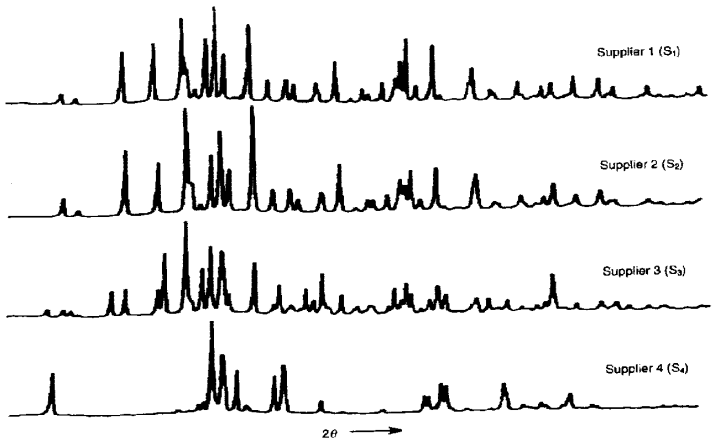


Figure 10.91 X-ray powder diffraction patterns of the commercial mannitol products S_1 through S_4 (Debord *et al.*, 1987).

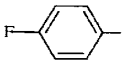
10.9 POLYMORPHS OF A

Antibiotics exhibit polym
In addition, cephalosporir
solvates as discussed in C

For the polyene antib
tallization have resulted in
differences are not due to p
mepartricin in methylene
which crystallized upon st
and between one-sixth an
tained by cooling an aceto

Studies of nystatin s
ter–methyl ethyl ketone so
ganisms, but half the solub
chloroform–methanol–amr
been proven by X-ray pov
that the differences in acti
solution rate. These solubi
ences.

A. CONFORMATIONAL PO



Azibi *et al.* (1983) describ
compound exists in two cr
10.92–10.93 and Table 10.
°C. The infrared spectra of

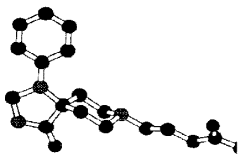


Figure 10.92 Stereoview of the
● N, ● O (Azit

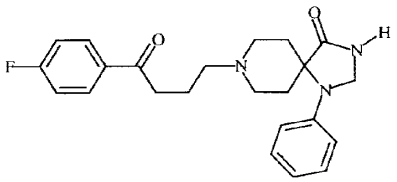
10.9 POLYMORPHS OF ANTIBIOTICS

Antibiotics exhibit polymorphism which could affect their stability and bioavailability. In addition, cephalosporin antibiotics crystallize in an extensive series of hydrates and solvates as discussed in Chapter 11.

For the polyene antibiotics, mepartricin and nystatin, different conditions of crystallization have resulted in products with different activity and acute toxicity. These differences are not due to particle size effects (Ghielmetti *et al.*, 1976). Evaporation of mepartricin in methylene chloride–methanol (9:1) at room temperature gave an oil which crystallized upon standing to form a solid which had one-fourth the oral activity and between one-sixth and one-tenth the LD_{50} (for mice) compared to the solid obtained by cooling an acetone–water–ether solution.

Studies of nystatin showed that crystals obtained by crystallization of a water–methyl ethyl ketone solution had approximately the same activity against microorganisms, but half the solubility and half to one-tenth the LD_{50} of crystals obtained from chloroform–methanol–ammonia. While the existence of nystatin polymorphs has not been proven by X-ray powder diffraction or other experimental techniques, it is likely that the differences in activity of the crystals are due to differences in solubility and solution rate. These solubility differences may, in turn, be due to polymorphic differences.

A. CONFORMATIONAL POLYMORPHISM OF SPIPERONE



spiperone

Azibi *et al.* (1983) described the conformational polymorphism of spiperone. This compound exists in two crystal forms (the structures and data are shown in Figures 10.92–10.93 and Table 10.29). Form I melted at 208.9 °C and Form II melted at 207 °C. The infrared spectra of the two crystal forms are different, and the crystal structure

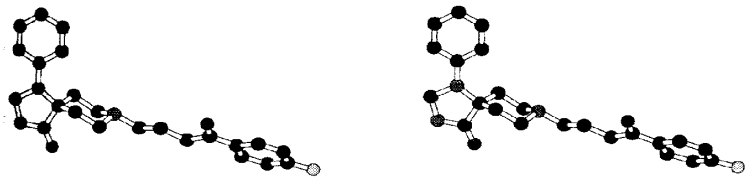


Figure 10.92 Stereoview of the molecular conformation of spiperone in Form I where: ● C, ○ F, ● N, ● O (Azibi *et al.*, 1983).

Figure 10.93 Stereoview of the molecular conformation of spiperone in Form II where: ● C, ◎ F, ● N, ● O (Koch and Germain, 1972).

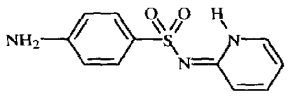
Table 10.29 Crystal Data of Spiperone Forms I and II

Parameter	Form I[a]	Form II[b]
Space Group	$P2_1/a$	$P2_1/c$
a (Å)	12.722	18.571
b (Å)	7.510	6.072
c (Å)	21.910	20.681
β	95.08°	118.69°
Z	4	4
V (Å^3)	2085.1	2045.7

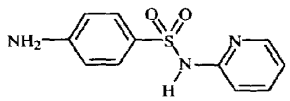
a Azibi *et al.*, 1983. *b* Koch and Germain, 1972.

showed that the conformation of the two forms are significantly different (see Figures 10.92–10.93). The authors analyzed the crystal packing and determined that hydrogen bonding was responsible for the polymorphism.

B. Sulfapyridine



"imide"



"amide"

Bar and Bernstein (1985) described the conformational polymorphism of 4-amino-*N*-2-pyridinylbenzenesulfonamide, sulfapyridine. The crystal structures of four forms of sulfapyridine were determined and are summarized in Table 10.30. The bond lengths and bond angles among the four structures are virtually identical, and are consistent with the imide structure. However, the conformations of the molecules are different in the different crystal structures, producing the phenomenon termed "conformational polymorphism." The conformations of the four different crystal forms are shown in Figure 10.94. It is clear that there is a different conformation about the —SO_2— bond in different molecules with some of the sulfapyridine rings pointing to the left in some forms and to the right in other forms.

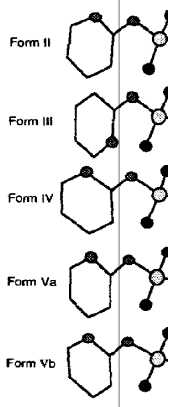


Figure 10.94 Stereoview of the
Bernstein, 1985; I

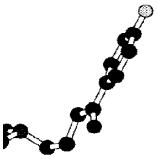
Table 10.30 Crystal Data for Su

Parameter	Form II[a]
Space group	$P2_1/c$
a (Å)	6.722
b (Å)	20.593
c (Å)	8.505
β	101.14°
Z	4
ρ_{calc} (g cm^{-3})	1.43
V (Å^3)	1155.1

a Bar and Bernstein, 1985. *b* Ba

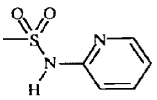
Bar and Bernstein (19
dine in the different crystal
tions showed that all four fc

Finally, the authors co
single crystal structures obt
the different crystal forms.
well with the published diff
of Form II and III did not
that there are additional cry
that a given powder patter
calculated pattern from a sii
either from observed single
nates using a program such



1 II where: ● C, ◎ F,

lifferent (see Figures
rmined that hydrogen



"amide"

ism of 4-amino-*N*-2-
res of four forms of
0. The bond lengths
al, and are consistent
ecules are different in
med "conformational
forms are shown in
ut the —SO_2— bond
ng to the left in some

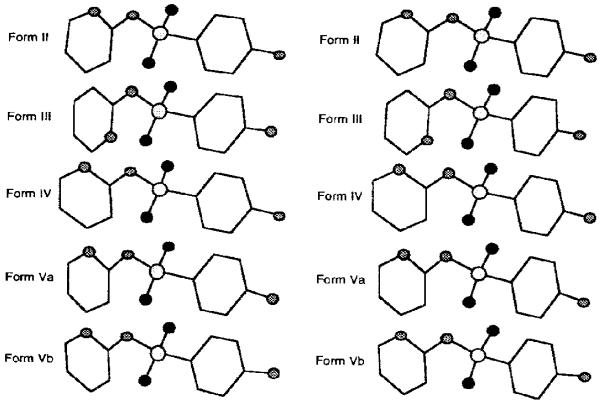


Figure 10.94 Stereoview of the molecular conformations in the four forms of sulfapyridine (Bar and Bernstein, 1985; Basak *et al.*, 1984; Bernstein, 1988).

Table 10.30 Crystal Data for Sulfapyridine

Parameter	Form II[a]	Form III[b]	Form IV[c]	Form V[a]
Space group	$P2_1/c$	$C2/c$	$P2_1/c$	$Pbca$
a (Å)	6.722	12.830	13.560	24.722
b (Å)	20.593	11.714	6.480	15.710
c (Å)	8.505	15.400	14.120	12.147
β	101.14°	94.12°	113.70	...
Z	4	8	4	16
ρ_{calc} (g cm^{-3})	1.43	1.44	1.46	1.41
V (Å^3)	1155.1	2308.5	1136.1	4717.7

a Bar and Bernstein, 1985. *b* Basak *et al.*, 1984. *c* Bernstein, 1988.

Bar and Bernstein (1985) also investigated the molecular energetics of sulfapyridine in the different crystal forms using extended Hückel calculations. These calculations showed that all four forms are within about 2.1 kJ/mol in energy.

Finally, the authors compared their data to research from other laboratories. The single crystal structures obtained allowed calculation of the X-ray powder patterns of the different crystal forms. The calculated X-ray powder pattern of Form I compared well with the published diffractogram. However, the calculated X-ray powder patterns of Form II and III did not agree with any previously reported patterns. This suggests that there are additional crystal forms. This study illustrates that the best way to prove that a given powder pattern is that of a pure polymorph is by comparing it with a calculated pattern from a single crystal structure. The powder pattern may be calculated either from observed single crystal diffraction intensity data or from the atomic coordinates using a program such as *Cerius*[2] (see Section 3.5).

10.10 POLYMORPHISM AND CHEMICAL STABILITY

Because polymorphs have different properties, including different melting points, densities, and crystal structures, it is not surprising that polymorphs have different chemical stabilities.

Perhaps the most striking effect of polymorphism on chemical reactivity is seen in the polymorphs of *trans*-2-ethoxycinnamic acid (see Figure 10.95). Irradiation of this compound in solution produces *trans*- to *cis*-isomerization, but no dimerization (Cohen and Green, 1973). Crystallization of this cinnamic acid yields three polymorphs, α, β, and γ. The α-form is obtained from ethyl acetate, ether, or acetone; the β-form is obtained from benzene or petroleum ether; and the γ-form is obtained from aqueous ethanol. Irradiation of the α-form gives the centrosymmetric dimer, irradiation of the β-form gives the mirror symmetric dimer, and irradiation of the γ-form produces no reaction. These reactions are summarized in Figure 10.95. Numerous examples of similar behavior have been found in other cinnamic acid derivatives and in anthracene dimerizations.

A number of pharmaceutical examples of different stabilities of polymorphs are also known. For example, methylprednisolone crystallizes in two forms. One form is stable while the other is reactive when exposed to heat, ultraviolet light, or high humidity (Munshi, 1973).

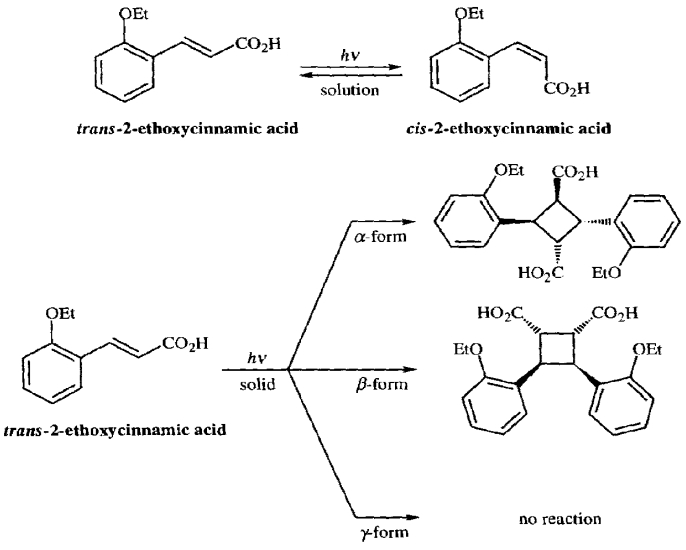


Figure 10.95 Summary of the reactivities of the α-, β-, and γ-crystalline forms of *trans*-2-ethoxycinnamic acid upon exposure to ultraviolet light (Cohen and Green, 1973).

In closely related stu
been reported. In our lab
polymorphs of hydrocort
ethanol in three crystalline
light, one of the solvates is
there are numerous cases
crystalline form. Macek (1
potassium penicillin G are
of the potassium salt can w
of the amorphous form re
have found similar differ
applied to sensitivity discs
detail in Chapter 12 (see Se
This discussion clearly
there is a need for careful c

10.11 POLYMORPHISM AN

The rate of absorption of a
dissolution rate is affected
the lowest solubility and, i
polymorphs will usually
ignored, significant dose-t
In a particular striking
taining various ratios of F
(*i.e.*, blood levels) (Aguia

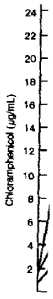


Figure 10.96 Comparison
suspensions
oral dose equi
creases, the pe
the next 25%
McCrone, 19

In closely related studies, different stabilities of polymorphs and solvates have been reported. In our laboratory, we have reinvestigated the behavior of the various polymorphs of hydrocortisone 21-*tert*-butylacetate. This steroid crystallizes from ethanol in three crystalline forms, one anhydrous and two solvates. When exposed to light, one of the solvates is reactive while the other two forms are stable. In addition, there are numerous cases where amorphous forms are much more reactive than the crystalline form. Macek (1965) has reported that the amorphous forms of sodium and potassium penicillin G are significantly less stable than the crystalline forms. Crystals of the potassium salt can withstand heating for several hours, while identical treatment of the amorphous form results in a significant loss of activity. Pfeiffer *et al.* (1976) have found similar differences between amorphous and crystalline cephalosporins applied to sensitivity discs. The reactivity of amorphous drugs is discussed in more detail in Chapter 12 (see Sections 12.1C–D).

This discussion clearly shows that in cases where chemical stability is a problem, there is a need for careful control of the polymorph or solvate.

10.11 Polymorphism and Bioavailability

The rate of absorption of a drug is sometimes dependent upon the dissolution rate. The dissolution rate is affected by the polymorph present, with the most stable form having the lowest solubility and, in most cases, the slowest dissolution rate. Other less stable polymorphs will usually have higher dissolution rates. Thus, if polymorphism is ignored, significant dose-to-dose variations can occur (Haleblian and McCrone, 1969).

In a particular striking example, a suspension of chloramphenicol palmitate containing various ratios of Form A and B showed significant variations in bioavailability (*i.e.*, blood levels) (Aguiar *et al.*, 1967). Figure 10.96 shows a comparison of mean

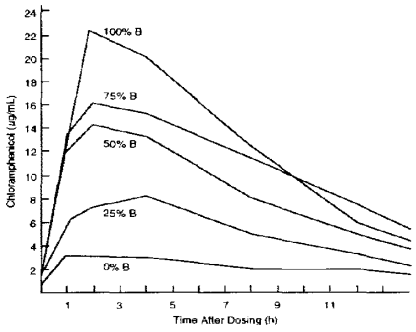


Figure 10.96 Comparison of the mean serum levels obtained with chloramphenicol palmitate suspensions containing varying ratios of the A and B polymorphs following a single oral dose equivalent to 1.5 gm of chloramphenicol palmitate. As the blood level increases, the percent of polymorph B increases. The lowest curve corresponds to 0% B, the next 25% B, the next 50% B, then 75% B, and the highest 100% B (Haleblian and McCrone, 1969).

blood serum levels of suspensions containing varying ratios of Form A and B. Clearly, the maximum blood levels are quite different, ranging from 3 to 22 μg/mL or by approximately a factor of seven. (Interestingly, a plot of peak blood levels versus percent Form B gave a straight line, as shown in Figure 10.97.) These data show that bioavailability is influenced by the type and concentration of the polymorph present. Obviously, if products are manufactured containing Form A, they will be largely inactive, while products containing Form B will show activity.

In another study, serum levels of the amorphous form and Form A of chloramphenicol palmitate have been compared in both children and Rhesus monkeys. Table 10.31 lists the results of these studies (Banerjee *et al.*, 1971) which show that the amorphous form has greater bioavailability than Form A.

Fluprednisolone crystallizes in three polymorphs and two solvates. These forms were pressed into pellets and implanted into rats, and their *in vivo* dissolution rates

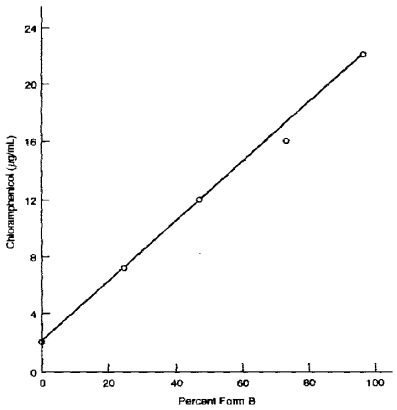


Figure 10.97 Plot of the peak chloramphenicol palmitate blood levels versus the percent of polymorph B (Haleblian and McCrone, 1969).

Table 10.31 Blood Levels (μg/100 mL) for Various Suspensions of Chloramphenicol Palmitate[a]

Suspension used	Hours after Feeding 2	4	6	8
		In Children		
Amorphous	102	60	42	26
Polymorph A	34	35	57	23
		In Rhesus Monkeys		
Amorphous	58	39	18	
Polymorph A	22	17	17	

a Banerjee *et al.*, 1971.

were measured (Hale
following order and
M^{-1}) > Form II (0.18
monohydrate (0.147 n
mately a factor of 1.6

The examples dis
matically affect the bic

10.12 POLYMORPHISM

Because polymorphs
choose the proper pol
22.10). In general, t
answers to the followi

1. What are t
2. Can pure,
3. Will the f

Furthermore, several r

1. How man
2. What is t
morphs?
3. Can the m

These basic quest
be determined by mic
DSC, IR, solid-state
(see Section 22.3).
solution phase transfo
in a drop of saturate
crystals of less stable
until only the most sta
of forms in successio
can also be used to pr
or decreased to the te
experiment repeated.

There are numer
tion of polymorphism
Tableting behavior d
(1972) showed that
causes **powder brid**
A, which is not plate

The behavior of
wrong polymorph of
occur producing a ch
is often undesirable a
syringeability of the

were measured (Haleblian and McCrone, 1969). The dissolution rates showed the following order and value: Form I (0.237 mg cm^{-2} M^{-1}) > Form III (0.209 mg cm^{-2} M^{-1}) > Form II (0.186 mg cm^{-2} M^{-1}) > β-monohydrate (0.162 mg cm^{-2} M^{-1}) > α-monohydrate (0.147 mg cm^{-2} M^{-1}). Thus, the variation in dissolution rate is approximately a factor of 1.6 when comparing Form I to the α-monohydrate.

The examples discussed in this section show that the polymorph present can dramatically affect the bioavailability of a drug.

10.12 Polymorphism and Its Pharmaceutical Application

Because polymorphs have different physical properties, it is often advantageous to choose the proper polymorph for the desired pharmaceutical application (see Section 22.10). In general, the pharmaceutical applications of polymorphism depends on the answers to the following questions:

1. What are the solubilities of each form?
2. Can pure, stable crystals of each form be prepared?
3. Will the form survive processing, micronizing, and tableting?

Furthermore, several more basic questions about polymorphs also need to be answered:

1. How many polymorphs exist?
2. What is the chemical and physical stability of each of these polymorphs?
3. Can the metastable states be stabilized?

These basic questions can be answered as follows: The number of polymorphs can be determined by microscopic examination and by subsequent analytical studies using DSC, IR, solid-state NMR, X-ray powder diffraction, and single-crystal X-ray studies (see Section 22.3). The physical stability of each form can be determined using the solution phase transformation method. This method involves placing two polymorphs in a drop of saturated solution under the microscope. Under these conditions, the crystals of less stable form will dissolve and crystals of the more stable form will grow until only the most stable form remains. Comparison of the relative stabilities of pairs of forms in succession gives the order of stability of the various forms. This method can also be used to prepare metastable forms. In this case, the temperature is increased or decreased to the temperature where the metastable form is most stable and then the experiment repeated.

There are numerous activities in the pharmaceutical industry that require consideration of polymorphism; these have been reviewed by Haleblian and McCrone (1969). Tableting behavior depends upon the polymorph present. For example, Simmons *et al.* (1972) showed that tolbutamide exists in Forms A and B. Form B is plate-like and causes **powder bridging** in the hopper and **capping** problems during tableting. Form A, which is not plate-like, showed no problems during tableting.

The behavior of suspensions also depends upon the polymorph present. If the wrong polymorph of a drug is used, a phase transformation to a more stable form may occur producing a change in crystal size and possibly caking. A change in particle size is often undesirable as it may cause serious caking problems, as well as changes in the syringeability of the suspension. In addition, the new polymorph may have altered

dissolution properties and, thus, bioavailability. Caking is a particularly serious problem since a caked suspension cannot be resuspended upon shaking. For example, oxyclozanide, upon standing in quiescent (undisturbed) suspensions, undergoes an increase in particle size (Pearson and Varney, 1969). This is due to a solvent-mediated phase transformation between two polymorphs. As discussed earlier, under these conditions, crystals of the more stable form grow and those of the less stable form dissolve. This produces cakes that cannot be resuspended by shaking.

REFERENCES

Agafonov, V., B. Legendre, and N. Rodier (1989) "A new crystalline modification of spironolactone" *Acta Crystallogr., Sect. C. Cryst. Struct. Commun.* **45** 1661–1663.

Agafonov, V., B. Legendre, N. Rodier, D. Wouessidjewe, and J.-M. Cense (1991) "Polymorphism of spironolactone" *J. Pharm. Sci.* **80** 181–185.

Aguiar, Arondo J., John Krc, Jr., Arlyn W. Kinkel, and Joseph C. Samyn (1967) "Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate" *J. Pharm. Sci.* **56** 847–853.

Alleaume, Marc, and Joseph Decap (1965) "Tridimensional refinement of β-sulfanilamide" *Acta Crystallogr.* **18** 731–736.

Alleaume, Marc, and Joseph Decap (1966) "Tridimensional refinement of γ-sulfanilamide" *Acta Crystallogr.* **19** 934–938.

Armour Research Foundation (1949) "Sulfasuxidine (*p*-2-thiazolylsulfamylsuccinanilic acid)" *Anal. Chem.* **21** 1293–1294.

Ashizawa, Kazuhide, Kiyohiko Uchikawa, Teiichi Hattori, Tadashi Sato and Yasuo Miyake (1988) "Polymorphic differences in α- and β-form crystals of 2*R*,4*S*,6-fluoro-2-methyl-spiro[chroman-4,4'-imidazoline]-2',5-dione (M79175) as determined by X-ray diffraction, infrared spectroscopy, and differential scanning calorimetry" *J. Pharm. Sci.* **77** 635–637.

Ashizawa, Kazuhide (1989) "Polymorphism and crystal structure of 2*R*,4*S*,6-fluoro-2-methyl-spiro[chroman-4,4'-imidazoline]-2',5-dione (M79175)" *J. Pharm. Sci.* **78** 256–260.

Azibi, M., M. Draguet-Brughmans, R. Bouche, B. Tinant, G. Germain, J. P. DeClercq, and M. Van Meerssche (1983) "Conformational study of two polymorphs of spiperone: possible consequences on the interpretation of pharmacological activity" *J. Pharm. Sci.* **72** 232–235.

Banerjee, Sachchidananda, Asok Bandyopadhyay, Ramesh Chandra Bhattacharjee, Arun Kumar Mukherjee, and Arup Kumar Halder (1971) "Serum levels of chloramphenicol in children, rhesus monkeys, and cats after administration of chloramphenicol palmitate suspension" *J. Pharm. Sci.* **60** 153–155.

Bar, I. and J. Bernstein (1985) "Conformational polymorphism. VI. The crystal and molecular structures of Form II, Form III, and Form V of 4-amino-*N*-2-pyridinylbenzenesulfonamide (sulfapyridine)" *J. Pharm. Sci.* **74** 255–263.

Basak, A. K., S. Chaudhuri, and S. K. Mazumdar (1984) "Structure of 4-amino-*N*-2-pyridylbenzenesulfonamide (sulfapyridine), $C_{11}H_{11}N_3O_2S$" *Acta Crystallogr., Sect. C, Cryst. Struct. Commun.* **C40** 1848–1851.

Basak, A. K., S. K. Mazumdar, and S. Chaudhuri (1987) "Structure of N'-(6-methoxy-3-pyridazinyl)sulfanilamide (sulfamethoxypyridazine)" *Acta Crystallogr., Sect. C, Cryst. Struct. Commun.* **C43** 735–738.

Bernstein, J. and A. T. Hagler (1978) "Conformational polymorphism. The influence of crystal structure on molecular conformation" *J. Am. Chem. Soc.* **100** 673–681.

Bernstein, Joel (1987) "Conformational polymorphism" in *Organic Solid State Chemistry*; G. R. Desiraju, Ed.; Studies in Organic Chemistry 32; Elsevier: Amsterdam; Chapter 13.

Bernstein, J. (1988) "Polymorph IV of 4-amino-*N*-2-pyridinylbenzenesulfonamide (sulfapyridine)" *Acta Crystallogr., Sect. C, Cryst. Struct. Commun.* **C44** 900–902.

Bettinetti, G. P., F. Giordano, and A. La Manna (1982) "Solid state molecular arrangements of sulfamethoxazole $C_{10}H_{11}N_3O_3S$: the crystal structure of two polymorphs" *Cryst. Strucut. Comm.* **11** 821–828.

Biles, John A. (1963) "So
hydrocortisone" *J. Phc*

Borchardt, Thomas B. (19
pharmaceutical precurs
due University: West L

Brown, Herbert C. and Sei
case of polymorphism

Burger, A. (1973) "The pol

Burger, A. (1973) "Solubil
pharmaceutical (sulfani

Burger, Artur (1975) "Poly

Burger, Artur and Regine I
zole" *Pharm. Acta Hel*

Burger, A. and U. J. Gries
poeia. IV. Identificat
Pharm. **57** 293–305.

Burger, Artur and Ulrich J
succinylsulfathiazole ci
poeia. VII" *Eur. J. Phc*

Busetta, Bernard, Christian
three polymorphous fo

Byrn, Stephen R., David Y.
white forms of dimeth
the yellow form to the

Byrn, Stephen R. and Chun
of hydrate crystals of
4004–4005.

Byrn, Stephen R., Brian Tc
Kozlowski (1988) "Re
morphs and solvates of

Chiang, Chian C., Wilson
Weiss (1978) "Color d
talline modifications"

Cohen, M. D. and Beman
490–497.

Craven, B. M. and E. A
isoamylbarbituric acid

Curtin, D. Y. and S. R.
hydrogen bonding. St
2,5-dihydroxyterephth

Curtin, David Y. and John
of 6-aroyloxyphenanth

Dabrowski, Janusz (1963)
pounds. I. Enamino I

Debord, B., C. Lefebvre,
"Study of different cry
Dev. Ind. Pharm. **13** 1

DeCamp, Wilson H. and F
and molecular structu
B., Struct. Sci. **B28** 1

DeCamp, Wilson H. and F
and molecular structu
Sect. B., Struct. Sci. 1

Desiraju, Gautam R., Iain
yellow to the red for
anisotropy of the reac

Biles, John A. (1963) "Some crystalline modifications of the *tert*-butylacetates of prednisolone and hydrocortisone" *J. Pharm. Sci.* **52**, 1066–1070.

Borchardt, Thomas B. (1997) "The derivatization, solid-state characterization, and crystallization of a pharmaceutical precursor that expresses color polymorphism in the solid state" Ph.D. Thesis, Purdue University: West Lafayette, IN 47907-1333.

Brown, Herbert C. and Sei Sujishi (1948) "Tri-1-naphthylboron as a highly hindered reference acid: a case of polymorphism ascribed to hindered rotation" *J. Am. Chem. Soc.* **70** 2793–2802.

Burger, A. (1973) "The polymorphs of sulfanilamide" *Sci. Pharm.* **41** 290–303.

Burger, A. (1973) "Solubility studies in the determination of thermodynamic data of a polymorphic pharmaceutical (sulfanilamide)" *Sci. Pharm.* **41** 303–314.

Burger, Artur (1975) "Polymorphism of oral antidiabetics. II. Tolbutamide" *Sci. Pharm.* **43** 161–168.

Burger, Artur and Regine D. Dialer (1983) "New research results on the polymorphism of sulfathiazole" *Pharm. Acta Helv.* **58** 72–78.

Burger, A. and U. J. Griesser (1989) "The polymorphic drug substances of the European Pharmacopoeia. IV. Identification and characterization of 11 crystal forms of succinylsulfathiazole" *Sci. Pharm.* **57** 293–305.

Burger, Artur and Ulrich J. Griesser (1991) "Physical stability, hygroscopicity and solubility of succinylsulfathiazole crystal forms. The polymorphic drug substances of the European Pharmacopoeia. VII" *Eur. J. Pharm. Biopharm.* **37** 118–124.

Busetta, Bernard, Christian Courseille, and Michel Hospital (1973) "Crystal and molecular structure of three polymorphous forms of estrone" *Acta Crystallogr., Sect. B., Struct. Sci.* **B29** 298–313.

Byrn, Stephen R., David Y. Curtin, and Iain C. Paul (1972) "X-ray crystal structures of the yellow and white forms of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate and a study of the conversion of the yellow form to the white form in the solid state" *J. Am. Chem. Soc.* **94** 890–898.

Byrn, Stephen R. and Chung-Tang Lin (1976) "The effect of crystal packing and defects on desolvation of hydrate crystals of caffeine and L-(–)-1,4-cyclohexadiene-1-alanine" *J. Am. Chem. Soc.* **98** 4004–4005.

Byrn, Stephen R., Brian Tobias, Donald Kessler, James Frye, Paul Sutton, Patricia Saindon, and John Kozlowski (1988) "Relationship between solid state NMR spectra and crystal structures of polymorphs and solvates of drugs" *Trans. Am. Crystallogr. Assoc.* **24** 41–54.

Chiang, Chian C., Wilson H. DeCamp, David Y. Curtin, Iain C. Paul, Sidney Shifrin, and Ulrich Weiss (1978) "Color dimorphism of 14-hydroxymorphinone. X-ray analysis of two different crystalline modifications" *J. Am. Chem. Soc.* **100** 6195–6201.

Cohen, M. D. and Bernard S. Green (1973) "Organic chemistry in the solid state" *Chem. Brit.* **9** 490–497.

Craven, B. M. and E. A. Vizzini (1969) "Crystal structures of two polymorphs of 5-ethyl-5-isoamylbarbituric acid (amobarbital)" *Acta Crystallogr., Sect. B., Struct. Sci.* **B25** 1993–2009.

Curtin, D. Y. and S. R. Byrn (1969) "Stereoisomerism at the oxygen-carbon single bond due to hydrogen bonding. Structures of the yellow and white crystalline forms of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate" *J. Am. Chem. Soc.* **91** 1865–1866.

Curtin, David Y. and John H. Englemann (1972) "Intramolecular oxygen-nitrogen benzoyl migration of 6-aroyloxyphenanthridines" *J. Org. Chem.* **37** 3439–3443.

Dabrowski, Janusz (1963) "Infrared spectra and structure of substituted unsaturated carbonyl compounds. I. Enamino ketones with primary amino group" *Spectrochim. Acta* **19** 475–496.

Debord, B., C. Lefebvre, A. M. Guyot-Hermann, J. Hubert, R. Bouché, and J. C. Guyot (1987) "Study of different crystalline forms of mannitol: comparative behavior under compression" *Drug Dev. Ind. Pharm.* **13** 1533–1546.

DeCamp, Wilson H. and F. R. Ahmed (1972a) "Structural studies of synthetic analgesics. II. Crystal and molecular structure of the monoclinic form of (±)-β-promedol alcohol" *Acta Crystallogr., Sect. B., Struct. Sci.* **B28** 1796–1800.

DeCamp, Wilson H. and F. R. Ahmed (1972b) "Structural studies of synthetic analgetics. III. Crystal and molecular structure of the rhombohedral form of (±)-β-promedol alcohol" *Acta Crystallogr., Sect. B., Struct. Sci.* **B28** 3484–3489.

Desiraju, Gautam R., Iain C. Paul, and David Y. Curtin (1977) "Conversion in the solid state of the yellow to the red form of 2-(4'-methoxyphenyl)-1,4-benzoquinone. X-ray crystal structures and anisotropy of the rearrangement" *J. Am. Chem. Soc.* **99** 1594–1601.

Dideberg, O., and L. Dupont (1972) "Crystal and molecular structure of spironolactone, 7α-acetylthio-3-oxo-17α-4-pregnene-21,17β-carbolactone" *Acta Crystallogr., Sect. B., Struct. Sci.* **28** 3014–3022.

Doherty, Chris and Peter York (1988) "Frusemide crystal forms; solid state and physiochemical analyses" *Int. J. Pharm.* **47** 141–155.

Donaldson, J. D., J. R. Leary, S. D. Ross, M. J. K. Thomas, and C. H. Smith (1981) "The structure of the orthorhombic form of tolbutamide (1-*n*-butyl-3-*p*-toluenesulphonylurea)" *Acta Crystallogr., Sect. B., Struct. Sci.* **B37** 2245–2248.

Dudek, Gerald O. and Gert P. Volpp (1963) "Nuclear magnetic resonance studies of keto-enol equilibria. V. Isomerization in aliphatic Schiff bases" *J. Am. Chem. Soc.* **85** 2697–1702.

Dunitz, Jack D. and Joel Bernstein (1995) "Disappearing polymorphs" *Acc. Chem. Res.* **28** 193–200.

Eistert, Bernd, Friedrich Weygand, and Ernst Csendes (1952) "Polymorphism of the chalcones" *Chem. Ber.* **85** 164–168.

Fletton, Richard A., Robert W. Lancaster, Robin K. Harris, Alan M. Kenwright, Kenneth J. Packer, David N. Waters, and Alan Yeadon (1986) "A comparative spectroscopic investigation of two polymorphs of 4'-methyl-2'-nitroacetanilide using solid-state infrared and high-resolution solid-state nuclear magnetic resonance spectroscopy" *J. Chem. Soc., Perkin Trans. 2* **1986** 1705–1709.

Gerber, J. J., J. G. vander Watt, and A. P. Lötter (1991) "Physical characterization of solid forms of cyclopenthiazide" *Int. J. Pharm.* **73** 137–145.

Ghielmetti, G., T. Bruzzese, C. Bianchi, and F. Recusani (1976) "Relationship between acute toxicity in mice and polymorphic forms of polyene antibiotics" *J. Pharm. Sci.* **65** 905–907.

Giuseppetti, G., C. Tadini, G. P. Bettinetti, and F. Giordano (1977) "2-Sulfanilamido-5-methoxypyrimidine, $C_{11}H_{12}N_4O_3S$" *Cryst. Struct. Commun.* **6** 263–274.

Goldberg, Israel and Yigal Becker (1987) "Polymorphs of tamoxifen citrate: detailed structural characterization of the stable form" *J. Pharm. Sci.* **76** 259–264.

Gougoutas, J. Zanos and L. Lessinger (1974) "Solid state chemistry of organic polyvalent iodine compounds. III. The crystal structures of 3-oxo-3*H*-2,1-benzoxiodol-1-yl *m*-chlorobenzoate (two polymorphs) and its isostructural derivative, 3-oxo-3*H*-2,1-benzoxiodol-1-yl benzoate" *J. Solid State Chem.* **9** 155–164.

Griesser, Ulrich J. and Xiaorong He (1998) Personal communication; Purdue University: West Lafayette, IN 47907-1336.

Guillory, J. Keith (1967) "Heats of transition of methylprednisolone and sulfathiazole by a differential thermal analysis method" *J. Pharm. Sci.* **56** 72–76.

Haleblian, John and Walter McCrone (1969) "Pharmaceutical applications of polymorphism" *J. Pharm. Sci.* **58** 911-929.

Hamlin, W. E., E. Nelson, B. E. Ballard, and J. G. Wagner (1962) "Loss of sensitivity in distinguishing real differences in dissolution rates due to increasing intensity of agitation" *J. Pharm. Sci.* **51** 432–435.

Herbstein, F. H. and G. M. J. Schmidt (1955) "The crystal and molecular structures of heterocyclic compounds. I. The analysis of the crystal structure of α-phenazine" *Acta Crystallogr.* **8** 399–405.

Higuchi, W. I., P. D. Bernardo, and S. C. Mehta (1967) "Polymorphism and drug availability. II. Dissolution rate behavior of the polymorphic forms of sulfathiazole and methylprednisolone" *J. Pharm. Sci.* **56** 200–207.

Higuchi, W. I., W. E. Hamlin, S. C. Mehta (1969) "Infrared attenuated total reflectance (ATR) method for observing the water-mediated surface phase reversion of methylprednisolone II to I during dissolution" *J. Pharm. Sci.* **58** 1145–1146.

Ip, Dominic P., Gerald S. Brenner, James M. Stevenson, Siegfried Lindenbaum, Alan W. Douglas, S. David Klein, and James A. McCauley (1986) "High resolution spectroscopic evidence and solution calorimetry studies on the polymorphs of enalapril maleate" *Int. J. Pharm.* **28** 183–191.

Kato, Yuriko, Yumi Okamoto, Sayoko Nagasawa, and Ichiko Ishihara (1984) "New polymorphic forms of phenobarbital" *Chem. Pharm. Bull.* **32** 4170–4174.

Koch, Michael H. J. and Gabriel Germain (1972) "Crystal and molecular structure of 4-[1-(4-hydroxy-4-*p*-fluorophenyl)piperidinyl]-4'-fluorobutyrophenone and its hydrochloride" *Acta Crystallogr., Sect. B., Struct. Sci.* **B28** 121–125.

Koo, Chung Hoe, Sung Il Cho, and Young Hee Yeon (1980) "The crystal and molecular structure of chlorpropamide" *Arch. Pharmacal. Res.* **3** 37–49.

Kopp, Sabine, Christian I
misinterpretations of I
Pharm. Technol. **34** 21

Krigbaum, W. R. and G.
Crystallogr., Sect. B

Kruger, G. J. and G. Gafner
Crystallogr., Sect. B

Kruger, G. J. and G. Gafner
Struct. Sci. **B27** 326–3

Kuhnert-Brandstätter, M. (1
New York, NY.

Kuhnert-Brandstätter, M. an
tions on enantiotropic p

Kuhnert-Brandstätter, M., I.
investigations on enanti

Kuhnert-Brandstätter, M., I.
investigations on enanti

Kuhnert-Brandstätter, M. an
Halofenate, lorcainide h
71–82.

Kuhnert-Brandstätter, M. an
Mexiletine hydrochlorid
Pharm. **55** 13–25.

Kuhnert-Brandstätter, M. an
Bupicomide, buspirone
chloride and piretanide"

Kuhnert-Brandstätter, M. an
Amiperone, bentiromide
Pharm. **57** 81–96.

Kuhnert-Brandstätter, M. an
Famotidine, flupirtine m
Pharm. **58** 55–67.

Levy, Gerhard and Josephine
polymorphs" *J. Pharm.*

Lin, Chung-Tang, Phillipe F
"Solid-state photooxidati
Chem. **47** 2978–2981.

Macek, Thomas J. (1965) "Th
forms for new pharmaceu

Matsuda, Yoshihisa and Ets
modifications" *Int. J. Pha*

Mesley, R. J. (1971) "The pol

Milosovich, George (1964) "
53 484–487.

Mitchell, A. G. (1985) "Pol
Pharm. Pharmacol. **37** 6(

Miyamae, Akira, Shigetaka K
(1990) "X-ray crystallogra
6-methylbenzyloxy)-2-me

Molecular Simulations, Inc. (

Moustafa, M. A., A. R. Ebia
crystal forms" *J. Pharm. I*

Munshi, Mayank V. (1973)
Thesis, University of Mic

Nirmala, K. A., and D. S. Sak
logr., Sect. B., Struct. Sci

Kopp, Sabine, Christian Beyer, Engelbert [illegible] (1988) "Potential misinterpretations of DEA/DSC [illegible] benobarbital" *Acta Pharm. Technol.* **34** 213–217.

Krigbaum, W. R. and [illegible] "[illegible]-nitrotoluene" *Acta Crystallogr., Sect. B: Struct. Sci.* **B27** [illegible].

Kruger, G. J. and G. Gafner (1971) "[illegible]sulfathiazole" *Acta Crystallogr., Sect. B: Struct. Sci.* **B27** [illegible].

Kruger, G. J. and G. Gafner (1971) [illegible] *Crystallogr., Sect. B: Struct. Sci.* **B27** 326–[illegible].

Kuhnert-Brandstätter, M. [illegible] Pergamon Press, New York, NY.

Kuhnert-Brandstätter, M. and [illegible] "[illegible] optical investigations on enantiotropic [illegible]".

Kuhnert-Brandstätter, M. [illegible] "[illegible] spectroscopical investigations on enantiotropic [illegible] **60** [illegible].

Kuhnert-Brandstätter, M. [illegible] "[illegible] spectroscopical investigations on enantiotropic [illegible] **60** [illegible].

Kuhnert-Brandstätter, M. and [illegible] "[illegible] of Drugs. II. Halofenate, lorcainide [illegible] *Pharm.* **54** 71–82.

Kuhnert-Brandstätter, M. [illegible] "[illegible] of Drugs. III. Mexiletine hydrochloride [illegible] suprofene" *Sci. Pharm.* **55** 13–35.

Kuhnert-Brandstätter, M. and [illegible] "[illegible] of Drugs. VI. Bupicomide, bisphosphonate [illegible] nafiverine hydrochloride and piretanide" [illegible] 88.

Kuhnert-Brandstätter, M. [illegible] "[illegible] of Drugs. V. Amiperone, benzoctamine [illegible] fenbufen" *Sci. Pharm.* **57** 81–96.

Kuhnert-Brandstätter, M. [illegible] "[illegible] of Drugs. VII. Famotidine, flupirtine [illegible] fludrocortisone" *Sci. Pharm.* **58** 55–67.

Levy, Gerhard and Joseph[illegible] "[illegible]-hydroxyprednisolone polymorphs" *J. Pharm.* [illegible] 83–[illegible].

Lin, Chung-Tang, Phillip [illegible] "Solid-state photooxidation [illegible] Byron (1982) "[illegible]-acetate" *J. Org. Chem.* **47** 2978–2981.

Macek, Thomas J. (1965) "The [illegible] forms for new pharmaceuticals" [illegible].

Matsuda, Yoshihisa and [illegible] "[illegible] of Spirosemide modifications" *Int. J. [illegible]* **60** [illegible].

Mesley, R. J. (1971) "De[illegible]" [illegible] 604.

Milosovich, George (1963) [illegible] *Pharm.* [illegible] **53** 484–487.

Mitchell, A. G. (1985) "[illegible] of Isopramide" *Pharm. Pharmacol.* **37** [illegible].

Miyamae, Akira, Shigetaka Kuda, [illegible] Yoshi Moriguchi (1990) "X-ray crystallographic [illegible] carbonylamino-6-methylbenzyloxy)-2-methyl [illegible] **79** 189–[illegible].

Molecular Simulations, Inc. [illegible].

Moustafa, M. A., A. R. [illegible] "[illegible]methoxybenzoic acid crystal forms" *J. Pharm. Pharm.* [illegible] 23–[illegible].

Munshi, Mayank, V. (1987) [illegible] Thesis, University of Michigan [illegible].

Nirmala, K. A., and D. S. [illegible] *Crystallogr., Sect. B: Struct.* [illegible].

O'Conner, B. H. and E. N. Maslen (1965) "The crystal structure of α-sulfanilamide" *Acta Crystallogr.* **18** 363–366.

Pearson, J. T. and G. Varney (1969) "Crystal growth studies involving phase transitions in aqueous drug suspensions" *J. Pharm. Pharmacol., Suppl.* **21** 60S–96S.

Perrin, M. and P. Michel (1973a) "Polymorphism of *p*-chlorophenol. I. Crystal structure and morphology of the stable form" *Acta Crystallogr., Sect. B., Struct. Sci.* **B29** 253–258.

Perrin, M. and P. Michel (1973b) "Polymorphism of *p*-chlorophenol. I. Crystal structure of the metastable form (β-form) at low temperature" *Acta Crystallogr., Sect. B., Struct. Sci.* **B29** 258–263.

Pfeiffer, Ralph R., Gary L. Engel, and Dennis Coleman (1976) "Stable antibiotic sensitivity disks" *Antimicrob. Agents Chemother.* **9** 848–851.

Phillips, D. C. (1956) "The crystallography of acridine. II. The structure of acridine III" *Acta Crystallogr.* **9** 237–250.

Phillips, D. C., F. R. Ahmed, and W. H. Barnes (1960) "The crystallography of acridine. III. The structure of acridine II" *Acta Crystallogr.* **13** 365–377.

Rambaud, J., R. Roques, S. Alberola, and F. Sabon (1980) "Crystallographic structure of 3-(4-aminobenzenesulfonamido)-5-methylisoxazole" *Bull. Soc. Chim. Fr.* **1980** 56–60.

Richardson, Mary Frances, Quing-Chuan Yang, Elisabeth Novotny-Bregger, and Jack D. Dunitz (1990) "Conformational polymorphism of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate. II. Structural, thermodynamic, kinetic and mechanistic aspects of phase transformations among the three crystal forms" *Acta Crystallogr., Sect. B* **B46**, 653–660.

Robertson, J. Monteath and J. G. White (1947) "The crystal structure of the orthorhombic modification of 1,2,5,6-dibenzathracene. A quantitative X-ray investigation" *J. Chem. Soc.* **1947** 1001–1010.

Robertson, J. Monteath and J. G. White (1956) "The crystal structure of the monoclinic modification of 1,2,5,6-dibenzanthracene. A quantitative X-ray investigation" *J. Chem. Soc.* **1956** 925–931.

Rowe, Englebert L. and Bradley D. Anderson (1984) "Thermodynamic studies of tolbutamide polymorphs" *J. Pharm. Sci.* **73** 1673–1675.

Saindon, Patricia J., Nina S. Cauchon, Paul A. Sutton, C.-j. Chang, Garnet E. Peck, and Stephen R. Byrn (1993) "Solid-state nuclear magnetic resonance (NMR) spectra of pharmaceutical dosage forms" *Pharm. Res.* **10** 197–203.

Schulenberg, John W. (1968) "Isolation of crystalline keto-enol tautomers. Conversion into indoles and oxindoles" *J. Am. Chem. Soc.* **90** 7008–7014.

Shafizadeh, Fred and Ronald A. Susott (1973) "Crystalline transitions of carbohydrates" *J. Org. Chem.* **38** 3710–3715.

Shefter, Eli and Takeru Higuchi (1963) "Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals" *J. Pharm. Sci.* **52** 781–791.

Shenouda, Latif S. (1970) "Various species of sulfathiazole Form I" *J. Pharm. Sci.* **59** 785–787.

Shieh, Tiee-Leou, Chung-Tang Lin, Ann T. McKenzie, and Stephen R. Byrn (1983) "Relationship between the solid-state and solution conformations of β-(benzylamino)crotonate" *J. Org. Chem.* **48** 3103–3105.

Simmons, D. L., R. J. Ranz, N. D. Gyanchandani, and P. Picotte (1972) "Polymorphism in pharmaceuticals. II. Tolbutamide" *Can. J. Pharm. Sci.* **7** 121–123.

Simmons, D. L., R. J. Ranz, and N. D. Gyanchandani (1973) "Polymorphism in pharmaceuticals. III. Chlorpropamide" *Can. J. Pharm. Sci.* **8** 125–127.

Small, Lyndon F. and Erich Meitzner (1933) "Metathebainone" *J. Am. Chem. Soc.* **55** 4602–4610.

Smith, Jay, Ernesto MacNamara, Daniel Raftery, Thomas Borchardt, and Stephen Byrn (1998) "Application of two-dimensional ^{13}C solid-state NMR to the study of conformational polymorphism" *J. Am. Chem. Soc.* **120** 11710–11713.

Stephenson, G. A., T. B. Borchardt, S. R. Byrn, J. Bowyer, C. A. Bunnell, S. V. Snorek, and L. Yu (1995) "Conformational and color polymorphism of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile" *J. Pharm. Sci.* **84** 1385–1386.

Sunwoo, Chimin and Henry Eisen (1971) "Solubility parameter of selected sulfonamides" *J. Pharm. Sci.* **60** 238–244.

Sutton, Paul Allen (1984) "Crystal packing effects on the photochemical oxidation and solid state carbon-13 NMR chemical shifts of several anti-inflammatory steroids" Ph.D. Thesis, Purdue University, West Lafayette, IN 47907-1330.

Szabó-Réveśz, Piroská, I
Kala, and U. Wenzel
IV. The influence o
phenobarbitone tablet

Weintraub, H. J. R. and
solution by empirical
tions" *Int. J. Quantu*

Williams, P. P. (1973)
phenylbarbituric acid

Williams, P. P. (1974) "I
Acta Crystallogr., Se

Yang, Shiu Shiang and J.
26–40.

Yang, Qing-Chuan, Mary
phism of dimethyl 3,6
parameters between
312–323.

Yu, Lian (1998) Personal

Szabó-Révész, Piroská, Klárá Pintye-Hódi, Máriá Miseta, B. Selmeczi, G. Kedvessy, J. Traue, H. Kala, and U. Wenzel (1987) "Investigations about polymorphism of drugs in powders and tablets. IV. The influence of the polymorphism of drugs on the physical properties and drug release of phenobarbitone tablets" *Pharmazie* **42** 179–181.

Weintraub, H. J. R. and A. J. Hopfinger (1975) "*CAMSEQ* [conformational analysis of molecules in solution by empirical and quantum mechanical techniques] software system in drug design calculations" *Int. J. Quantum Chem., Quantum Biol. Symp.* **1975** 203–208.

Williams, P. P. (1973) "Polymorphism of phenobarbitone: the crystal structure of 5-ethyl-5-phenylbarbituric acid monohydrate" *Acta Crystallogr., Sect. B., Struct. Sci.* **B29** 1572–1579.

Williams, P. P. (1974) "Polymorphism of phenobarbitone. II. Crystal structure of modification III" *Acta Crystallogr., Sect. B., Struct. Sci.* **B30** 12–17.

Yang, Shiu Shiang and J. Keith Guillory (1972) "Polymorphism in sulfonamides" *J. Pharm. Sci.* **61** 26–40.

Yang, Qing-Chuan, Mary Frances Richardson, and Jack D. Dunitz (1989) "Conformational polymorphism of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate. I. Structures and atomic displacement parameters between 100 and 350 K for three crystal forms" *Acta Crystallogr., Sect. B* **B45** 312–323.

Yu, Lian (1998) Personal communication; Eli Lilly and Company: Indianapolis, IN 46285–0001.

Analysis of Organic Polymorphs
A Review

Terence L. Threlfall,
Chemistry Department, University of York, Heslington, York, UK YO1 5DD

Summary of Contents

Keywords: *Polymorphism; phase transitions; amorphous materials; solvates; microscopy; thermal analysis; infrared spectroscopy; Raman spectroscopy; solid-state nuclear magnetic resonance spectroscopy; X-ray diffraction*

Introduction and Definition of Polymorphism

Polymorphism[1–7] in the chemical sense of the word* is a phenomenon of the solid state, associated with the structure of the solid. It has proved difficult to define precisely although the basic concept is readily understood. The definitions which have been offered vary in breadth but the implication of all of them is that polymorphs involve different packings of the same molecules in the solid.[4] The question of how similar the same molecules must be and of how dissimilar the different packing arrangements must be in order to qualify as polymorphs is more than a matter of semantics but goes to the root of our understanding of the organic molecular solid state.

McCrone has defined a polymorph as 'a solid crystalline phase of a given compound resulting from the possibility of at least two crystalline arrangements of the molecules of that compound in the solid state' and has listed those types of solid phenomena which are excluded from this definition.[1] Later writers who have accepted this definition have tended to substitute their own list of exclusions,[5] if they have addressed the matter at all. Buerger's tentative definition[3] 'ideally, two polymorphs are different forms of the same chemical compound which have distinctive properties' is broader and appears not to accept the need for separate phases and to include amorphous forms. The nature of the amorphous state[8,9] will be discussed later.

Polytypism[10] is one-dimensional polymorphism, referring to different stacking of the same layers. It is most familiar in inorganic systems, particularly silicon carbide, but has been recognized in organic crystals, both as ordered[11–13] and as disordered stacking.[14] There is no special term for two-dimensional polymorphism, although some liquid crystal systems display it. Liquid crystals are notorious for their ability to exist in different phases both in the mesomorphic and in the solid state[15–17] and this has led to the suggestion that the term polymorphism should apply to liquids as well as solids,[18] but it is only the solid dimensions of liquid crystals which can adopt distinct packing arrangements. Liquid-crystal polymorphism will not be dealt with specifically in this review except where it is related to the polymorphism of solids. The long standing question[19] of whether allotropy and polymorphism are distinct[20] is not an issue in the case of organic compounds. Inorganic polymorphs have been excluded because the extended structures of which most inorganic crystals are composed raise concepts not discussed here.[21,22] Protein polymorphism usually refers to minor molecular sequence changes[23,24] rather than to packing, but different crystal packing of protein molecules is also known.[25] Polymorphism of thin films[26,27] and polymers, both of biological[28,29] and of synthetic[30] origin, although of the same nature as the concept of polymorphism considered here, will not be discussed.

There is a profusion of words in the English language for the phenomena discussed in this review, yet not enough because of the overlapping usage. 'Polymorph' (dimorph, trimorph) 'form' and 'modification' are all used to describe polymorphic phases, but 'form' and 'modification' are also used in reference to crystal habit. 'Polymorph' and 'form' have been used to describe solvates, whilst 'pseudopolymorph' doubles for both solvates and for those solids which are otherwise not considered true polymorphic forms. The term 'pseudopolymorphic solvate' applied to crystals losing solvent molecules without change of crystalline form offers yet another source of confusion in terminology. Genetic polymorphism which is now the major use of the term is often described as 'polymorphisms' but this is occasionally seen also in chemical senses. In view of the almost universal use of 'polymorphic' as the appropriate adjective, the word 'polymorphous' seems superfluous despite dictionary support. There is an urgent need for consistent usages so as to be able to delineate the phenomena under consideration.

There is no clear choice as to the best method of designating polymorphs. Arbitrary systems are to be discouraged, but numbering based either on order of melting point or of room temperature stability have been recommended. Both are susceptible to change as a result of later identification of new polymorphic forms. Numbering based on order of discovery is unchangeable, but requires a knowledge of the history of the compound. The addition of the crystal class, as has been suggested for minerals[31] is not very practicable, since crystallographic classes are rarely determined from optical microscopic or X-ray powder diffraction studies for organic compounds. The assignment of a space group is even less realistic.

* An on-line search of Chemical Abstracts will reveal more than 47000 entries under 'polymorphism'. Over 90% of these relate to genetic polymorphism, which at least in its origins can claim the true etymology of the word. Some selectivity between biological and chemical uses can be achieved, but there is no certain searching strategy. Searching under 'phase transition' and related concepts will generate a further 44000 entries, most of which refer to inorganic systems, and cannot be easily disentangled. Nevertheless, these represent only a proportion of the papers containing information on polymorphs and polymorphism. Hence it is not possible to state how many publications relate to those aspects of polymorphism described here.

In any case the distribution of organic molecules amongst crystal classes and space groups is extremely limited, as is discussed later.[32,33] The addition of a melting or upper transition point to a Roman numeral is probably the best compromise,[1] although care must be taken to distinguish the melting point of the polymorph and that of the transformed product.

Significance of Polymorphism

The continuing investigation of polymorphism by the Innsbruck school (Kofler, Kuhnert–Brandstätter, Burger) over more than half a century has shown that around one-third of organic substances show crystalline polymorphism under normal pressure conditions.[34,35] A further third are capable of forming hydrates and other solvates.

Much of the literature on the polymorphism of organic compounds relates to pharmaceutical products.[1,36–40] The incentive for this interest in polymorphism began with the need to satisfy regulatory authorities in various countries as to the bioavailability of formulations of new chemical entities.[36,37] Of the several contributory factors to the bioavailability of finished products, the inherent solubility and rate of dissolution of the drug substance itself are of major importance. The solubility is dependent on the polymorphic state, as different polymorphs have different energies and therefore different solubilities.[40] It has been pointed out, particularly by Burger,[36] that the difference in solubility between polymorphs is likely to result in significant bioavailability differences, in practice, only in exceptional cases. Although some may think that this represents an extreme view, the consequences of polymorphism on bioavailability are commonly overstated. Chloramphenicol palmitate, over which the original concerns were voiced,[41] is unique in that the solubility is related to the rate of enzymic attack on the solid.[42] This and novobiocin,[43] which involves consideration of the amorphous state, are among the handful of examples of marketed products showing major bioavailability differences as a result of polymorphism.

As formulations have become more sophisticated and as the tolerances on products have become tighter, the need to identify polymorphic behaviour at an early stage of development has become important in the pharmaceutical industry as a means of ensuring reliable and robust processes[44] and conformity with good manufacturing practice. The aim is to avoid, *inter alia*, tabletting problems and subsequent tablet failure,[45,46] crystal growth in suspensions[47,48] and resultant caking, precipitation from solutions and problems with suppositories,[49] as well as chemical production problems such as filtrability[1] and to ensure analytical reproducibility. By extension such considerations relate to the control of quality in manufacture and product reliability in any industry by ensuring that the processes are well understood and under control so that unpleasant surprises do not occur.[50] This point is most dramatically illustrated in the explosives industry, where the wrong polymorph can have greatly increased sensitivity to detonation.[51,52] Pigment colour and solubility are polymorph dependent,[53–59] as are photographic and photolithographic sensitizers.[60] The performance of industrial products, particularly those based on natural fats and waxes[61,62] and derived soaps,[63] and on petroleum products[64,65] is in many cases related to polymorphic composition and degree of crystallinity. The same is true of the processing, acceptability and deterioration of foods and confectionery containing fats,[66,67] sugars,[68–72] polysaccharides[73] and other constituents.[74–75] A comprehensive summary of the solid-state properties of lipids has recently appeared.[76]

It is also worth establishing the polymorphic behaviour of a compound for the sake of good order in documentation so that reference works, for example, pharmacopoeias, do not contain conflicting data[34,77] such as a spectrum of one polymorph, but the melting point of another.

A major incentive to the study of polymorphism in the pharmaceutical industry during development has become strikingly apparent recently in the use of subsidiary patents on desirable polymorphic forms[78] to prolong the patent life of major products. Much recent pharmaceutical patent litigation has concerned polymorphs and particular interest has been taken in Glaxo's patent on the polymorph of ranitidine[79] (Zantac) which if held valid will extend the patent protection from 1995 to 2002 in many countries.[80] For a compound with annual sales of over 2 400 million pounds sterling,[81] the financial incentives to investigate polymorphs are obvious.

Finally, the very existence of polymorphism tells us something about the solid-state. Investigation of polymorphic systems, especially those with a large number of forms can help in understanding solid-state and molecular behaviour and intermolecular interactions[82] and the relationship between crystal structure, crystal growth and crystal habit[83] and their influence on bulk properties. Apart from knowledge for its own sake, this is of clear application in the development of organic electronic[84,85] and other specialty products[86–88] and in understanding the function of biological membranes.[89]

Distinction From Related Phenomena

At one time polymorphism was regarded only as different arrangements of rigid molecules in the solid state.[90,91*] A clear dichotomy existed between this and arrangements of molecules in different forms, such as could be imagined would occur with isomeric, tautomeric, zwitterionic and chiral structures and later with different conformers.[92] The early crystallographic studies on rigid aromatic molecules tended to reinforce the distinction. This simple division could only be maintained whilst details of the rich variety of solid-state structures were inaccessible. The early examples of dynamic isomerism and tautomerism were few[93,94] and the proposition that they could not be part of polymorphism was copied by reviewers until even the examples were forgotten.[95] A quoted example of a tautomeric solid-state structure, that of 3,5-dichloro-2,6-dihydroxy dimethyl terephthalic acid was shown in 1972 not to be tautomeric, but to involve conformational change with hydrogen bonding differences.[96] One would have expected examples of tautomerically related solid structures to be exceedingly numerous, since the molecular energetic requirements can easily be fulfilled as is shown by the widespread occurrence of tautomerism in solution.[97] Tautomeric polymorphism is surprisingly rare, but a well investigated example is now known, that of 2-amino-3-hydroxy-6-phenylazopyridine.[98]

There are a few papers in the literature either where tautomeric polymorphism is invoked[99–105] or where examination of the IR spectra is suggestive of forms whose difference resides in transfer of hydrogen between one part of the molecule and another.[106] The instances of 1,3-cyclohexadienone and squaric acid (3,4-dihydroxy-3-cyclobutene-1,2-dione are more difficult to place unambiguously in the category of tautomeric polymorphism. Proton transfer between donor and acceptor oxygen sites results in little change in over-all structure.[107]

Both tautomeric equilibrium and the neutral $\longleftrightarrow$ zwitterionic equilibrium formally involve such an intramolecular hydrogen transfer. The nominal difference is that a charge separation is produced in zwitterions which cannot be extinguished intramolecularly by a double-bond rearrangement cascade. The difference may be even smaller in practice because charge stabilization of zwitterions can occur intermolecularly, for example, in solution through solvation, whilst tautomeric structures can retain a substantial part of their charge as shown by dipole moment and IR spectroscopic studies.[108,109] Anthra-

* Earlier literature can be accessed *via* references 1, 2 and 10.

nilic acid exists as two metastable forms containing only uncharged molecules and a form stable at room temperature, half the molecules of which have been shown from crystallographic studies to be zwitterionic and half uncharged.[110] A related phenomenon is the changing of allegiance of hydrogen-bonded hydrogens between electron donor atoms, which is a prolific source of polymorphism.[111] The role of hydrogen-bonding networks in determining crystal structure has been discussed extensively.[112] Conformational differences between molecules of different structures have been admitted, perhaps reluctantly, and distinguished by the title conformational polymorphism.[113] The original examples form one extremity where molecules in distinctive conformations pack similarly,[92] but it is now obvious from the plethora of crystal structures, as could always have been deduced from elementary considerations of energy minimization, that any change of packing will cause geometrical change in molecules and conversely that any change in geometry will invite different packing of the molecules.[82] The extent will depend on the rigidity of the molecules. Although some floppy ring systems maintain their shape in different forms[114,115] even nominally rigid structures such as the ring systems of steroids[116] can show substantially different conformations in different polymorphs. Heteroaromatic[117–121]* and benzoquinone[122] planes are frequently bent and even benzene rings[123] may be. Thus it seems pragmatic to accept conformational polymorphism as a normal sub-set of polymorphism and the term will only be used here when it is necessary to distinguish cases of substantial conformational change.

The distinction between polymorphism and chirality is made in most accounts of polymorphism; yet it has recently been pointed out that if conformational polymorphism is accepted, then racemates and conglomerates of rapidly interconverting chiral systems are in fact polymorphs.[5] Such systems are generally ones with an easy conformational change where the trivial distinguishing feature from other conformational polymorphism is that the result of such a change is a reflection of an asymmetrical structure across a mirror plane. Although this seems difficult to accept, the dextrorotatory and laevorotatory forms of such systems are then equally polymorphs.[124] The narrow line of demarkation between polymorphism, conformational polymorphism and chirality first seems to have been recognized by Eistert *et al.*.[125] Examples of rapidly interchanging enantiomers in solution capable of independent existence in the solid state are known[126,127] but uncommon.

A further extension of the concept of conformational polymorphism is to be found where there is rapid interconversion between isomers.[128] As in the chiral examples, one molecular species or the other becomes exclusively incorporated in the crystal because the mechanism of crystal growth acts as such an exquisitely discriminatory process.[129]

Since a hydrate and an anhydrous form are constitutionally distinct, they cannot bear a strictly polymorphic relationship on the basis of any definition. However, the observation of material of different melting point or other properties during recrystallization may be due (apart from chemical reaction with solvent or decomposition) to solvation or polymorphism and the methods of examination are similar in either case. Hence the term 'pseudopolymorphism' has become common[130] particularly in the pharmaceutical industry. The term seems unnecessary and could lead to confusion[131] with its use to describe all other phenomena related to polymorphism[1] and so will not be used here. It must be emphasized, however, that the distinction between solvates and polymorphs is not as clear-cut as might be imagined, either conceptually or practically.

* In the case of phenothiazines[121] the point of interest is not that the ring system is bent, but that the heteroatoms are out of the plane of the aromatic rings and in the opposite sense to expectation.

The traditional narrow view of polymorphism, rigidly excluding chirality and isomerism, has caused considerable difficulty[128] to the investigators of the systems described above and it is suggested that the way to avoid these problems is to adopt the gloss originally proposed by McCrone and co-workers[1,37] on his definition of polymorphism, namely that the criterion is that the component molecules must have the same structure in solution irrespective of the polymorph from which they were derived; but, as has been suggested by Dunitz,[5] without excluding tautomerism, isomerism or conformers *per se*. Thus, rapidly interconverting species would be accepted, whilst slowly interconverting species would be excluded, as was surely within the original contemplation. Despite appearances, this proposal is likely to multiply examples of polymorphism very little and it avoids what otherwise must be artificial situations of accepting phases as polymorphs based on impeccable polymorph behaviour until their crystal structure reveals excluded molecular forms.[98,110,132] If, as asserted, the transition between polymorph I and polymorph II of 1,3-cyclohexadiene occurs by transfer of hydrogen from one oxygen to another, then this is nominally an example of tautomeric polymorphism.[107] If, on the other hand, the same change occurs or can be made to occur by a movement of the whole molecule then it is an example of regular polymorphism. The boundaries between the various alternative solid structural concepts are too subtle and too vague to be used to define polymorphism.

Although the requirement of the same structure in solution has been canvassed above, one-component phase diagrams are constructed on the basis of equilibrium with vapour, rather than liquid. It is just in the instance of conformational, configurational or hydrogen mobility that molecular differences between vapour,[133,134] melt, solution[126,135] and solid are found. The mobilities are inevitably of different magnitudes in different states. We shall be increasingly obliged to decide where to draw the boundaries of polymorphism as more comparative studies involving polymorphs and molecular structure in different states are undertaken.

One negative consequence of accepting the wider view of polymorphism should be noted, namely that the thermodynamic relationships discussed later are likely to be less certain for the wider polymorphic family.[90]

Stability of Polymorphs

Polymorphs, or strictly dimorphs where only two forms are under consideration, may be in an enantiotropic or monotropic relationship.[19,136] An enantiotropic relationship implies that each form has a range of temperature over which it is stable with respect to the other and a transition point at which the forms are equistable and in principle interconvertible.[137] Above that temperature the thermodynamic tendency is to the formation exclusively of the form stable at the higher temperature. Below the transition temperature the low- temperature form is the only stable one with respect to the other, although there is usually a greater tendency for the high temperature form to become frozen-in than for a low- temperature form to persist beyond its stability range.[8] Forms outside their range of stability are described here as metastable[138]. In the case of a monotropic relationship one form is metastable with respect to another at all temperatures. There is no observable transition point, although the thermodynamic description implies a theoretical transition point above the melting point which is therefore unattainable.[139] The use of the terms enantiotropic or monotropic in reference to a phase, as opposed to a transition, is ambiguous and likely to lead to confusion, since a polymorph can have a monotropic relationship to a second polymorph, but be enantiotropic in relation to a third polymorph. Flufenamic acid provides such an example.[140] The distinction between thermodynamic and kinetic transition points also needs to be drawn.[141]

Polymorphs only exist in the solid state: melting or dissolution destroys any distinctions. It is therefore important in examining polymorphs analytically not to submit them to conditions under which they melt, dissolve or are rendered more likely to interconvert. Heating and grinding[142–144] are obviously potentially hazardous operations in this context, but often cannot be avoided. The presence of solvent, even one in which the substance appears insoluble, will speed up the interconversion.[145] Trace moisture, acid or alkali on vessels can be similarly effective in interconverting polymorphs or in catalysing competing and confusing phenomena such as ring-opening reactions, for example, in 3,5-dihydroxy-3-methylvaleric acid derivatives,[146], or group transfer reactions.[147]

It might be supposed that a transition during grinding would always be from less stable polymorph to the polymorph more stable at that temperature, but in our experience, as well as from the literature,[145] this is not always true, presumably because the transformation takes place at a local temperature generated by the grinding and the unstable form becomes frozen-in by rapid cooling outside the immediate area of grinding.[148] This can only occur in cases in which the transition temperature does not lie too far above ambient. There may be alternative explanations, namely interconversion *via* amorphization or that a less stable polymorph may become the more stable one when in the form of small crystallites, as a result of surface effects. The latter phenomenon has been observed and investigated theoretically in the case of phthalocyanine pigments.[149] The possibility of growing unstable forms in microdrop conditions has been known for some time,[34] but recently the value of emulsions for this purpose has been suggested.[150] Although it would be desirable to have more compelling evidence than that obtained by differential scanning calorimetry (DSC) alone to establish the relationship between forms grown in this way, it does appear that new forms can be produced as well as metastable ones which are otherwise only accessible *via* the melt. The product of a polymorphic transition can also depend on particle size.[151,152]

Mnyukh and Petropavlov, in extensive studies of the transformation of individual crystals, observed that strict orientation of axes between mother and daughter phases was not preserved upon transformation.[153] They have concluded that only reconstructive transitions, *i.e.*, those involving the growth of new crystals in place of the old, take place for organic compounds. Even rapid transitions, described as atypical, were observed to follow the same patterns. No displacive (martensitic, co-operative) mechanism involving concerted structural change is therefore possible for organic compounds in Mnyukh's scheme. Whilst it would now appear that the reconstructive mechanism is the usual one, there are many examples involving preservation of axial orientation at phase transitions[4] some of which appear to be topotactic rather than only epitaxial.[154–157].

Irrespective of the mechanism and the rate of conversion at the point of transition, the stability in practice of a metastable polymorph at room temperature varies enormously,[158] from examples where the transformation is so rapid that the only evidence of the transient existence of a polymorph is its pseudomorphic outline,[1] to those which can be kept indefinitely and indeed refuse to transform in the absence of heat, high humidity or solvents.[152] The majority of systems are in fact quite robust to handling. It may therefore be thought that some of the present work presents over-concern with the possibility of transforming polymorphs during analytical examination. However, the modifications of some compounds show extraordinary sensitivity to handling in so many different ways. For example, with octakisphenylthionaphthalene, pressure on a cover-slip causes the yellow form to change to red;[159] with ethylenediamine hydrochloride, mere contact with KBr is stated to cause transformation;[160] with D,l-pantolactone 2,4-dihydroxy-3,3-dimethylbutyric acid γ-lactone, absorption of IR radiation in the spectrometer is sufficient for transformation;[161] and with meprobamate, high humidity may rapidly transform an otherwise indefinitely stable polymorph.[162] The problem is that this sensitivity may not be apparent until after the measurements have been made and then only if the analyst is alert, so that it is not possible to be too careful at the outset. Three of the commonest methods, IR spectroscopy, X-ray powder diffraction and differential scanning microscopy are unreliable for comparison of identity unless the sample is examined as a fine powder, but grinding can mislead into belief of identity if it induces transformation. This is why optical microscopy is so valuable for the initial examination. On the other hand, where transformation is sluggish, solubility determinations will be of more value than instrumental measurements for establishing the stability relationships.[34]

The existence of enantiotropically related polymorphs is indicative of the fact that the relative stabilities and therefore the Gibbs energies of the forms are very similar.[163,164] For this reason the empirical forecasting of polymorphism of a given compound is unlikely to be reliable.[88,165] Despite this, groups of compounds such as sulfonamides, barbiturates and steroids are known to be extraordinarily susceptible to polymorph formation.[39] Around 70% of these are now known to be polymorphic. Other examples include theophylline derivatives,[35] coumarins,[87] alkanes,[64,65] fatty acids and their derivatives[61,62] molecules which form liquid crystals,[15–17] and molecules which pack badly.[166] With the advent of molecular modelling techniques for crystal growth prediction, interest has been generated in the computer prediction of polymorphism.[87] The task is difficult because of the lacunae in our understanding of polymorph structure.

Methods for the Examination of Polymorphs

Polymorphs can be sought deliberately by cooling or quenching of melts, by condensation of vapour, or by crystallization under different conditions, although they are often encountered by chance. In the process of crystallization from solution, the expected effect of crystallization temperature may be overshadowed by other factors, particularly deliberate or adventitious seeds.[167] The importance of crystallization control during process development and the attitudes when unexpected polymorphic forms are encountered has been described by Bavin:[42] 'the process of crystallization is taken for granted by most chemists and it takes a reaction vessel clogged with an unstirrable mass to provoke serious thought'.

All the solid-state properties of the different polymorphic modifications of a compound will be different, but often only marginally so, to the point of instrumental indistinguishability. For this reason, it is important to look at potentially polymorphic systems by a variety of techniques to avoid erroneous conclusions. Failure to recognize a polymorph is the more obvious situation but it is also possible to identify polymorphs where none exist, if reliance is placed on too few techniques.[168] Substances with multiple forms can require substantial effort for their complete elucidation, especially when previous studies have characterized the forms inadequately.[142,148,151,169,170]

The techniques which have been available for a long time for the examination of polymorphs include those listed in Table 1. Which are the commonest methods depends to some extent on the area of interest, but in industrial practice, microscopy, IR spectroscopy, DSC, X-ray powder diffraction, solubility and density measurements have been the most widely used techniques. Within the past decade several new techniques and instrumental accessories have become widely available. These ease the manipulation of polymorphs and so lessen the danger of interconversion, or enable new properties to be investigated and allow measurements to be made which would have formerly

been impossible on the specimen under examination because of its size or microcrystallinity, for example. These developments are listed in Table 2. In general, the application of these newer techniques to polymorphism has not been adequately reviewed. Much of this article will therefore be devoted to a description of these methods in relation to examples taken from the literature on polymorphism. Some attention will also be devoted to aspects of the traditional techniques which have been given surprisingly little coverage in the reviews. Apart fom the techniques discussed below, there have of course been many other methods applied to particular aspects of polymorphism and solid–solid phase transitions. Examples include scanning tunnelling microscopy,[64] electron diffraction,[53] atomic force microscopy,[171] crystal etching,[172] electron microscopy[64,173] and thermobarometric measurements.[174]

The analytical strategy in approaching a polymorphism study will be dictated by the availability of instrumentation, time and material. At the beginning of a study, the fact that minimal quantities of a compound are required by IR spectroscopy, DSC and, particularly microscopy can be a significant consideration. Since thousands of compounds are put into pre-development in the pharmaceutical industry for each successful marketed product[175*] the cost of extensive investigation of polymorphism also needs to be borne in mind.

Microscopy

Although a theme of this review is that no one technique should be used in isolation, hot-stage microscopy has been often so used and remains the outstanding method for the examination and generation of polymorphs.[1] In the hands of experts, surprisingly comprehensive accounts of polymeric behaviour have been generated from microscopy alone,[37,39,140,176] but it is a technique which requires experience for rapid study and the drawing of confident conclusions. A preliminary examination under a binocular microscope will enable the overall characteristics of the sample to be ascertained. Temperature cycling and melt and solvent recrystallization experiments with a polarizing microscope equipped with a hot-stage[177–179] will allow the identification of transition points, the distinguishing of monotropic and enantiotropic relationships, estimation of the tendency of melts and individual phases to supercool, the generation of stable and unstable polymorphs and the recording of their optical properties.[140,180,181] The identification of solvates and the observation of sublimates and of any tendency to decompose are added information.[175] This can be carried out with minute amounts of material. The field has been excellently and comprehensively reviewed in the past,[1,37–39,178,179] and for that reason only the developments since then will be considered in detail here. The basic hot-stage methods have changed little in the intervening years, although there have been considerable improvements in the design of microscopes in terms of greater stability, versatility, ease of use and optical excellence. The availability of phase[182,183] and differential interference contrast (Nomarski) methods[184] and of interference microscopy has enabled precise refractive indices to be more readily determined.[185]

Several designs of hot-stage have been developed and are commercially available. Unfortunately, convenience is often sacrificed to temperature precision and many are unsatisfactory in maintaining temperature control whilst allowing for the manipulation of the specimen since the housings restrict access to the specimen. In fact in some designs, access cannot be gained at all whilst the stage is in position on the microscope. Recourse to a more open design, such as the Kofler stage, a graduated hot-stage[186–188] or a purpose-built heated microscope slide[189] will be necessary for such a requirement. The simplest rotating needle stages[177,185] are similarly more useful in practice than four-axis or five-axis Federov stages, because of the open access.

Although the determination of refractive indices and optic axis angles on birefringent specimens is time-consuming,[190] these optical measurements are critically distinctive of phases[140] especially when variation methods can be justified,[177,191,192] and such measurements ought to be more widely considered when doubt remains as to whether different specimens represent different phases. Such doubt is of more frequent occurrence than is ever suggested in the literature. This is owing, at least partly, to our inadequate understanding of the molecular solid state, and the relationship of that state to its properties. X-ray crystallographic studies have shown that hot-stage microscopic investigations have tended to overestimate the number of polymorphs,[193] presumably because crystal habits have been judged as modifications and because samples of different melting or transition points have been assumed necessarily to represent distinct forms. In fairness to the early investigators it is by no means clear how samples of the same polymorph, for example, can have the same unit cell yet melt 19 °C apart where purity considerations can be excluded.[146] Crystal strain which has been invoked in other,[179] less extreme cases, seems to be a rationalization rather than an explanation.

A major advance in microscopy for the analyst confronted with potential polymorphism has been the availability of video recording.[5] A change in a specimen or perhaps only in a few crystals of the specimen under examination is often only noticed after it has occurred. The ability to replay the video and re-observe the changes, perhaps in slow motion and to compare the timing of the changes in different crystals of the specimen can be exceedingly useful in making judgements of whether sample

Table 1 Techniques which have been available for many years for the examination of polymorphs

Hot-stage microscopy

Thermal methods—
- DTA
- DSC
- Thermogravimetric analysis

Solution calorimetry
Infrared spectroscopy
Solubility measurements

Density measurements—
- Flotation
- Pyknometry
- Dilatometry

X-ray powder diffraction
X-ray single-crystal diffraction

Table 2 Techniques of particular value for the examination of polymorphs which have become readily or more widely available within the past decade

Solid-state NMR
Diffuse-reflectance IR spectroscopy
Near-IR spectroscopy
Raman spectroscopy
Area detectors on diffractometers

Combined techniques including—
- Hot-stage IR spectroscopy
- IR microscopy
- Video recording on the microscope

* According to Lumley and Walker[172] '5000–10000 candidate substances have to be synthesized and screened for every one new medicine that reaches the market'.

homogeneity is in question, in determining transition temperatures or temperature ranges, in recording events in systems displaying irreproducible, erratic behaviour and in sorting out sequential but nearly concurrent events that sometimes occur. For example, a melting followed by resolidification of the low-temperature form will often accompany the transition without melting,[194], individual crystals or crystal domains within the field of view behaving independently.[110,122] A particularly valuable use is in distinguishing the movement of boundaries between domains or phases[178,195] and so distinguishing polymorphic changes from related behaviour such as crystal strain effects.[179]

A more elaborate arrangement has been described[196] in which a differential scanning calorimeter and a hot-stage microscope are linked through video recording. Commercial hot-stages with associated thermal sensors are also available which enable the optical changes and the associated changes in thermal properties to be examined simultaneously. There is a compromise[197] between optical and thermal excellence, versatility and convenience so that it is best regarded as a supplement for a microscope plus a calorimeter rather than a substitute. Close transitions or meltings are better resolved by microscopy than by DSC.[198] There are transitions which are seen by microscopy and not by DSC[106,199] and *vice versa*. The different behaviour of ethyl morpholine $HCl \cdot 2H_2O$ under the microscope and in DSC is particularly striking.[200] Thermomicrophotometry has been recommended and shown to be effective in detecting phase transitions that were not detected either by microscopy or DSC.[201]

A triple system of DSC–microscopy–microphotometry has also been described.[202] The combination of microscopes with other instruments is discussed in the following sections.

Infrared Spectroscopy

The first intimation of polymorphism not previously noticed as a melting point discrepancy or sought deliberately by hot-stage microscopy is often from inconsistencies in solid-state IR spectra. Infrared spectroscopy has had, of course, enormous exposure in the literature through books,[203] reviews[204] and papers but there are surprisingly few descriptions of the precautions to be taken when recording or interpreting the IR spectra of polymorphs. For example, in the case of non-matching spectra, a wide variety of causes might be suspected, including mis-labelling of a homologue,[205*] sample purity, crystal size,[206,207] crystal habit and orientation,[208,209] instability to comminution,[210] formation or partial decomposition of a salt,[211] solubility in the mulling medium, hydration,[212] dehydration[213] or other solvent loss under vacuum, level of impurities in the mulling or disk medium and instrumental variables[214] including the inadequate elimination of background peaks. The latter can be more of a problem with the Fourier transform instruments now in almost universal use, because of the high (often unnecessarily high) resolution which can be achieved in routine use. Experience of the expected levels of instrument and sample reproducibility is the best prophylactic against the discovery of non-existent polymorphs or the disregard of actual polymorphs.

The choice of routine sample presentation methods now includes mulls[215–217], disks[215–219], diffuse reflection[220,221] and attenuated total reflection (ATR).[222,223] All present hazards particularly for amorphous forms and for crystals of limited stability. The running of solution spectra is, of course, excluded for distinguishing between polymorphs, but can be used to check the molecular identity and purity of the specimens and so distinguish polymorphism from solvation, isomerism and other phenomena. The key factor in determining the sample procedure is simply the stability of the polymorph to the chosen conditions. Disks or mulls are usually most appropriate for routine use, but diffuse reflectance spectra are particularly suited for preliminary examination because the preparation technique will minimize polymorphic interconversion in most cases. For particularly sensitive compounds, the choice between ATR, photoacoustic spectroscopy or microspectroscopy will probably be determined by the availability of the appropriate accessories. Interconversion depends on the nature of the compound as well as the vigour of the preparatory stages of the examination. It is desirable to establish the sensitivity of the forms to grinding at an early stage of the investigation, but it is rarely indicated in the literature that this is ever considered.

In general the preparation of a mull is less likely to produce polymorphic changes than that of a disk,[224,225] presumably because the heat of grinding is carried away more efficiently by a liquid than by a solid. However, Nujol itself can cause polymorphic change.[128,143] There is also the belief that the pressure itself during disk formation can bring about polymorphic transitions.[226,227] KCl and KI have been recommended in place of KBr for various reasons,[206,211] but KBr is now most commonly used. It is softer than KCl[228] and so safer for this reason. On the other hand, it is less neutral and so can cause salt formation. Ethylenediamine dihydrochloride is so sensitive to KBr that merely placing a Nujol mull in contact with a KBr disk causes transformation, as previously noted, although a KCl disk is inert in the same circumstances.[160] Different alkali halides have different refractive indices.[204,228] Although not often a problem with organic materials, mismatch of refractive index of medium and sample can cause distorted spectra due to the Christiansen filter effect,[229] which in extreme cases also produces an apparent band shift to lower frequencies. Sometimes, with strong bands, substantial shifts in the opposite direction result[204] a phenomenon which has never been satisfactorily explained. This reinforces the importance of always comparing spectra run under the same conditions.

The use of a grinding or dispersion promoter such as acetone for disk making is excluded, as polymorphic changes are catalysed by solvents.[145] This raises the caveat that non-polar polymorphic systems should not be examined as paraffin mulls.[128,143] In an extreme case, there is the possibility of observing the solution spectrum of the compound being mulled. The further problem with mulls is that they are less quantitatively reproducible and parts of the spectra are obscured owing to the bands of the mulling agent which makes comparison of spectral identity or differences more difficult.[230] For this reason, the use of alternative mulling agents such as hexachlorobutadiene or Fluorolube[98] may be attractive if only the high-frequency region of the spectrum is of interest. This is only likely to be the case for hydrogen-bonded molecules. The most pronounced band shifts are, however, often to be found below 800 cm^{-1} and into the far IR (FIR) region.[231,232]

In the diffuse reflectance (DRIFTS)[233,234] technique the substance to be examined is dispersed in a matrix of a powdered alkali halide and placed in a sample cup in the diffuse reflectance accessory. The sample is illuminated by a wide cone of radiation and the reflected radiation collected over a wide angle. The effects of multiple scatter and multiple reflection within the sample over a wide range of permutations of angles of incidence and reflection tend to reduce orientation effects accompanying insufficient grinding of needle or platey crystals. The observed spectrum results primarily from the transmission of radiation through crystals rather than from reflection from individual faces. Acceptable spectra of polymorphs can generally be obtained by this technique, with much gentler grinding than either for disks or for mulls. For this reason it is to be regarded as the presentation method of choice[146,226,234] for the initial examination of the IR spectra of polymorphs. KCl has

* The fact that a homologue and a polymorph can produce similar degrees of difference was first noted by Jones as quoted by Rosenkrantz and Zablow.[205]

been recommended as the best diluent.[226] For quantitative work, it may be necessary to grind the sample thoroughly, but this may be avoidable for an initial examination. Care must be taken to ensure reproducible dispersion and packing of the sample in the sample cup.[235–237] The use of diffuse reflection is now becoming more commonly reported for the examination of polymorphic systems and the reader is referred to the literature[226,234] for details of the preparation of samples.

In ATR spectroscopy, also called frustrated total reflection or internal reflection spectroscopy, the evanescant wave that penetrates the low refractive index medium under total internal reflectance conditions at a high refractive index/low refractive index boundary is minutely absorbed. This is because the depth of penetration is only of the order of magnitude of the wavelength of the radiation or less. In practice IR radiation is directed through a thallium bromide iodide crystal which represents the high refractive index medium against which the sample is pressed. ATR spectroscopy is widely used for the examination of materials which present problems when examined by other methods. It is particularly valuable for samples which are strongly absorbing or which must be examined *in situ* or at least neat. ATR would thus appear at first sight to be the ideal way of obtaining the IR spectra of polymorphs[238-240] which is possibly why it has been preferred by some of the pharmacopoeias and authorities, for example, in Australia. In principle neither grinding nor any preparation other than possibly sprinkling the sample on to transparent sticky tape is required. However, ATR spectra are particularly susceptible to packing and crystal orientation problems. This, combined with the difficulty in obtaining sufficiently strong and acceptably reproducible spectra, without finely grinding the sample and pressing it to the face of the ATR crystal, makes the technique less attractive and it is rarely used in polymorphism studies. The potential presence of a dispersion component superimposed on the absorption component can also make the comparison of subtle differences less certain.[241] Nevertheless, if a sample proves susceptible to grinding, as in the case of phenylbutazone[239] or sulfathiazole,[242] ATR spectroscopy may be a valuable resort.

Sulfathiazole is one of the few substances in the literature for which spectra run as KBr disks,[243] Nujol mulls[169] and ATR[242] are displayed. The differences in scale make comparisons difficult. Therefore, in Fig. 1 a set of spectra of sulfathiazole polymorph III is displayed, to highlight typical differences. These are mostly in the background and in intensity variation; the position of bands, except those associated with hydrogen bonding, remain at the same wavelengths. Diffuse reflectance spectra of sulfathiazole forms are illustrated in Fig. 2 to give an idea of typical spectral differences between polymorphs. Comparison with spectra in the literature[169,242,243] reveal differences due, apart from the variation in sample presentation technique, to the possibility of interconversion during preparation for spectral examination and to the difficulty in producing pure polymorphs or even reproducible specimens. The spectra of III and IV show only minute differences. This is a consequence of the inherent similarity of the crystal structures and is reflected in the ease of conversion of IV to III. The largest spectral differences between polymorphs I and III are in the NH stretching region, reflecting the substantially different hydrogen bonding networks. Despite the curious appearance of the spectrum of polymorph II above 1700 cm^{-1}, all the features are genuine, but have become exaggerated because of the crystallinity of the sample. This illustrates the dilemma in examining polymorphs. Grinding would improve the appearance of the spectrum but at the risk of promoting a transition. The IR spectra of polymorph III shown[243] or implied[169] in the two most carefully conducted studies in the literature are those of an approximately (1 + 1) mixture of polymorphs III and IV, as are some samples of the commercial material. By near IR difference measurements (see below) the specimen of polymorph III used here was estimated to contain 8% of IV and the specimen of IV to contain 9% of III. The polymorphs of sulfathiazole must be

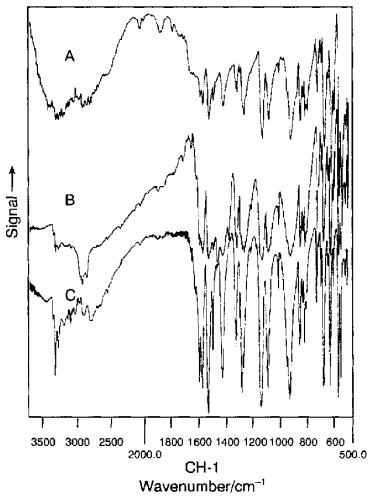


Fig. 1 The IR spectrum of polymorph III of sulfathiazole A, by attenuated total reflection; B, as a Nujol mull; and C, as a KBr disk, for comparison with the diffuse reflection spectrum, Fig. 2. Polymorph III is believed to be stable to grinding, hence any differences are due to orientation effects or to the optical differences inherent in the sample presentations. The intensity differences along the wavelength scale are due to the change in depth of radiation penetration.

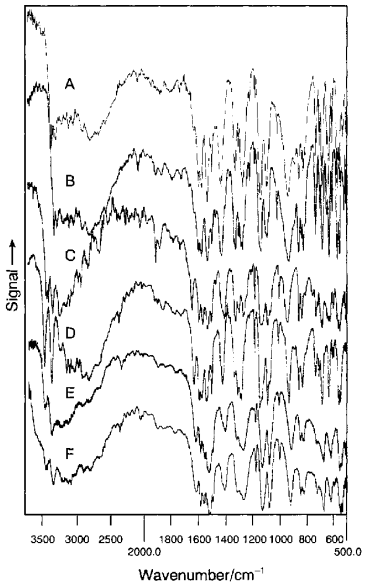


Fig. 2 Diffuse IR spectra of forms of sulfathiazole, admixed into a KBr matrix using minimal grinding. A, polymorph IV prepared inadvertantly; B, polymorph III, commercial sample; C, polymorph II by boiling an aqueous saturated solution to dryness; D, polymorph I by heating polymorph III above 175 °C; E, melt; and F, amorphous form produced by quenching the melt in liquid nitrogen. The spectrum of the melt (in a KBr matrix) is shown for comparison with the amorphous form.

regarded as amongst the most difficult to make and keep as pure specimens, as the number of papers on this topic reflect.[243]

Photoacoustic spectroscopy (PAS) relies on the detection of the acoustic signals generated by the absorption of modulated radiation[244,245] and is therefore not subject to the blacking out effect that occurs when IR spectra of too strongly absorbing samples are recorded by any other technique. Hence spectra can be obtained from neat samples and as such it might be expected to have been more widely explored for polymorphic systems.[246] Control of particle size is, however, important in ensuring reproducibility.[247] PAS has been used to obtain IR spectra of 2*R*,4*S*-6-fluoro-2-methylspiro(chroman-4,4′-imidazoline)-2′,5-dione because the forms were too sensitive to grind.[248] Comparisons of DRIFTS and PAS have been made.[249–251]. There is a difference in the over-all intensity relationship with wavelength between these techniques and transmission methods related to the variation of depth of penetration with wavelength and this needs to be taken into account in comparing spectra obtained by the different methods.

Spectra at low temperatures are more highly resolved and so more characteristic than those at room temperature, owing to suppression of the thermal motion. Low temperature spectra have been recommended for the examination of antibiotics.[252] The relative ease of obtaining spectra at −196 °C has been stressed and the technique has been applied to polymorphic steroids to achieve greater resolution and distinguishability.[116]

The absorption of polarized radiation is dependent on molecular orientation and therefore potentially of value in examining packing modes of molecules,[253] but appears to have been little explored for enhancing the distinguishability of polymorphs. The transformation of polymorphs of fatty acids has, however, been recently investigated. Monoclinic phases of fatty acids pack in layers with oblique orientation of the hydrocarbon chains within a layer. An orthorhombic polytypic phase of both the B and the E forms is known, in which alternate layers have the contrary orientation.[254] Polarized IR spectroscopic studies have been used in establishing the relationship between the orientation of crystal axes in crystals undergoing transformation.[255]

Recording the IR spectra on thin films made by rapid cooling of melts between salt plates or pressed KBr disks is a valuable way of investigating polymorphic propensities.[256,257] Ostwald's principle[257] predicts that the form involving the least loss of Gibbs energy, that is, the modification least stable at low temperatures will be first formed on cooling and if it can be trapped by rapid cooling, it may be possible to follow a whole series of polymorphic changes with time and temperature by IR spectroscopic examination of the film. This can be achieved by warming the centre of the disk with a hot rod,[258] although it is more elegantly carried out on a hot-stage. This technique of making thin films can only be used for substances stable at moderate melting temperatures because of the possibility of fracture of the salt plates from thermal shock.[230]

Commercial heated stages for IR spectrometers have been available for some time, but have not always had sufficient temperature control or insulation to enable differential scanning calorimetric or hot-stage microscopic observations, for example, to be matched with the spectral changes. An alternative is to adapt a hot-stage to fit the IR cell compartment. The expectation of sharp changes in the spectrum at the transition points is not always borne out in practice,[259] because the degradation of the resolution and signal-to-noise ratio at high temperatures may obscure the small changes being sought. Thermal emissivity, convection currents and change in focus may be the main causes of the problem. Detailed studies have established generally the decrease in intensity of IR bands of condensed phases with temperature[260] and a sudden decrease at transition points for alkanes.[261] It is important to make allowances for these variations when comparing spectra taken at different temperatures, as may be necessary when the polymorphs interconvert readily and so cannot be examined outside their range of stability. To overcome these problems and render small changes more visible, it was advantageous to record difference spectra,[262] but now chemometric methods have been brought to bear.[263] Gu[264] has used Malinowski's criteria of number of components to determine the number of transitions and temperature of transition points for glycerides. Two-dimensional correlation plots applied to variable temperature DRIFTS have also been used to pair-up bands in the spectra and so identify the spectroscopic components of the different phases.[265] Partial least squares computation has also been used in conjunction with variable temperature DRIFTS.[234]

The most exciting development in the application of IR spectroscopy to the study of polymorphism has been that of the IR microscope.[208,253,266–269] Normally a single crystal or crystalline powder of sufficient area to fill the sample aperture of an IR spectrometer cannot be examined by transmission because of excessive absorption and can be examined only with difficulty by reflectance because of the mixture of diffuse and specular reflectance components. Although there are techniques and computer programs for the transformation based on the Kronig–Kramers relationship[241] (Hilbert transformation[270,271]) the residual uncertainties make the technique unsatisfactory for comparing subtly differing spectra. With an IR microscope, however, individual small crystals can be examined directly in transmission. The pigment naphthazarin (5,8-dihydroxy 1,4-naphthoquinone) has been examined in this way.[225] Thicker crystals can be examined by seeking thinner areas of acceptable absorptivity near the edges.[272] Apart from the virtue of minimizing polymorphic transformation and of allowing measurements to be made on minimum sample quantities, the difference in the spectra of individual crystals can be ascertained, since it is not unknown for a crystallization to produce a mixture of polymorphs.[85,199,273] Microphases can also be examined.[274] Naturally a great deal more time and manipulation is required for IR microscopy, so in the usual instance, in which sufficient sample is available, an IR macro spectrum would normally be taken first under standard conditions.

Despite all the potential problems, many of which have been discussed above, in most cases IR spectroscopy provides a simple and reliable tool for the investigation of polymorphism. The distinction between spectra of different phases is rarely large, although there are exceptions.[160,275–277] Small changes in peak positions, peak shapes, and absence or presence of a few bands may be all that can be distinguished. This may be enough to characterize a whole series of polymorphs, for example all nine polymorphs and solvates of phenobarbitone prepared by Mesley *et al.* were clearly distinguishable by IR spectroscopy.[151] On the other hand, IR spectra of polymorphs have been frequently reported as virtually identical.[116,160,277–281] In some instances such indistinguishability may be an artefact[282] of interconversion. Reports of identity or difference in IR spectra and in X-ray diffraction patterns in many publications are not borne out upon examination of the accompanying spectra or diffractograms where these have been reproduced at sufficient size to make an informed comparison.

A valuable application of IR spectra (and X-ray diffractograms) of polymorphs is as the basis of a patent claim.[78,80] The use of the NH and OH stretching band positions in establishing stability relationships in hydrogen bonded polymorphic systems is discussed in the section on solubility and density measurement.

Near IR (NIR) spectra due to overtone and combination bands[283] are less resolved than spectra in the fundamental region in the mid-IR. The multivariate methods which are routinely used in this region[284,285] minimize this disadvantage and enable small differences between spectra to be distinguished. The spectra are also much less intense, but provided

that sufficient sample is available, this is an advantage, because saturation of the absorption will not occur and so neat samples can be used. NIR microscopy has also been tried[286] and should show the same advantages for polymorph investigation as IR microscopy. For the normal macro technique, the same problems of reproducible packing and effects of crystal size and orientation as discussed under diffuse reflection apply, but are reduced because of the larger illuminated area. The absence of diluent also removes three variables: the distribution of the analyte, the particle size of the carrier; and the bands due to the carrier or its impurities,[287] particularly moisture. The question of the particle size and reproducible packing discussed above for the mid-IR region are equally important here, although chemometric methods have been applied to try to minimize their effects.[288,289] Since the bands in the NIR region are due to OH, NH and CH stretching vibrations, it would be expected that the spectral changes would be most noticeable in hydrogen-bonded systems[290] and in conformational polymorphism. The published reports[291] are too few to confirm this, although the NIR spectra of many pharmaceutical polymorphs have been recorded. Therefore Fig. 3 shows the NIR spectra of a typical set of polymorphs of a substance, sulfathiazole, in which hydrogen-bonding networks play a significant role. Note that the differences in the spectra of polymorph III and polymorph IV, for example, are greater in the NIR region than in the mid-IR region, in line with the expectations expressed above. The technique is non-invasive, these spectra being obtained by placing a fibre optics probe on the outside of the glass tubes containing the samples. A further advantage of NIR spectra is the ease with which data manipulation, such as spectral differences, can be performed without generating unrealistic results.

Raman Spectroscopy

The Raman effect depends on the inelastic scattering, with loss of vibrational energy, of radiation in the near-UV, visible or NIR region of the spectrum.[292–294] It is inherently very weak and needs an intense, monochromatic excitation source and good filters to remove the excitation line from the collected radiation.[295]

Although commercial Raman spectrometers have been available for a long time, visible excitation sources tend to produce swamping fluorescence from many compounds.[296,297] Where this is due to impurities it may be possible to burn them out,[298] but otherwise the Raman spectrum is difficult or impossible to record against the background. In this case also there is a tendency to char the sample.[299]. There have been numerous mechanical[293] and electronic devices[299] proposed to minimize these effects, but they all have disadvantages. It is only since the advent of NIR Fourier transformation Raman (NIR–FT Raman) spectrometers using the Nd. YAG laser source at 1064 nm with efficient cut-off filters to remove Rayleigh scattering from the laser line,[300] that routine Raman spectra have become reliably available from most organic solids.[296] Although the spectra obtained are broadly similar to IR spectra, the difference in selection rules makes the information complementary.[294,301] Polar groups such as carbonyl and hydrogen-bonded hydroxy groups which are strongly apparent in the IR, are weak in Raman, whereas non-polar symmetrical or nearly symmetrical bonds such as carbon–carbon single and double bonds are strong in Raman.[292] Furthermore, the Raman effect, being a polarizability, falls off as the sixth power of the distance, whereas IR coupling, being a polarization, falls off only as the cube of the distance.[302] Therefore Raman spectra of molecular organic solids in the bond stretching and bending region would be expected to show little influence from neighbouring molecules. The effect is enhanced because the typical organic molecule consists of a non-polar backbone with polar groups on the periphery, so minimizing further the coupling of Raman active bands.

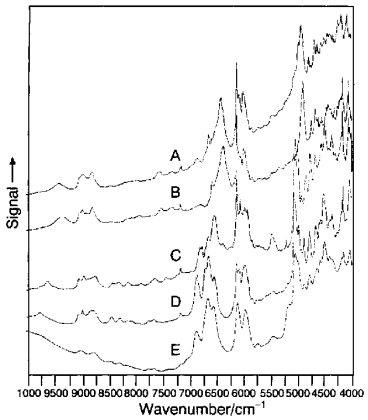


Fig. 3 Near IR spectra of sulfathiazole forms. A, Polymorph IV; B, polymorph III; C, polymorph II; D, polymorph I; and F, amorphous. The spectral differences appear larger than in the mid-IR region because NIR spectroscopy is insensitive to ring and chain modes and records only the XH modes, in this case particularly the NH stretchings.

The effect of this is firstly that Raman spectra of solids tend to have narrower bands than IR spectra. In one polymorphic set that we examined, the typical bands in the IR in the 700–1500 cm^{-1} region had bandwidths at half height of about 15 cm^{-1}, whereas the equivalent Raman bandwidth was about 11 cm^{-1}. Secondly, IR spectra are influenced by neighbouring molecules both directly by hydrogen bonding[303,304] and indirectly by the above spatial distance effect. One would therefore expect that conformational polymorphism would show up more distinctly in Raman spectroscopy and that packing effects especially of hydrogen-bonded molecules would show up most clearly in the IR spectra. There is little in the literature to test this, but we have encountered examples which support this contention. For rigid, non-hydrogen bonded molecules, the largest differences would be expected to occur in the region of the low-frequency lattice modes.[231,232] Comparison of coincidences in IR and Raman bands of symmetrical molecules can lead directly to a decision between alternative structures. The possible centrosymmetric structures for polymorphs B and C of naphthazarin were eliminated in this way.[305] This study shows that the Raman spectra of even deeply coloured solids can be obtained with NIR–FT Raman spectroscopy.[306]

The chief advantage of Raman spectroscopy is that no sample manipulation is required[294] and therefore in the case of polymorphs which are, or are suspected to be, susceptible to transformation, the spectra can be obtained with complete certainty of the identity of the sample under examination. The multiple scattering taking place in powder samples[307] tends to eliminate orientation effects in the same way as occurs in DRIFTS. Because glass is transparent to the excitation and emitted radiation and gives no interfering bands, spectra can even be obtained without removal of the specimen from the sample tube. Consequently, Raman spectra of polymorphs are now actually easier to obtain than IR spectra and deserve to be more widely recorded than the handful of papers[169,233,308,309] in the literature would indicate.

A disadvantage of the NIR–FT Raman system is that commercial instruments do not allow spectra to be recorded to very low frequencies, so that the region where the greatest difference between polymorphs might be expected to be seen,[231,232,310,311] is inaccessible. As this region is also outside

the range of most IR instruments, recourse must be made to conventional Raman spectrometers. As a result, there are few examples in the literature of the examination of organic polymorphs in this low-frequency region,[312–314] reflecting the difficulty of measurement.

Raman microscopy offers in principle even greater advantages than IR microscopy because the theoretical limit of resolution, related to the wavelength of the incident radiation, allows samples of an area less than 1 μm^2 to be examined.[296,297,315] The limit for IR is in the region of 50 μm^2 dependent on the wavelength range of interest.[316] However, in practice, the optical throughput due to the instrumental aperture characteristics, render it difficult to reach the theoretical limit of resolution with FT–NIR systems.[296,297] Conventional instrumentation with argon-ion laser sources at 488 nm, which can be used to examine smaller areas, produce the problems for organic compounds mentioned earlier of fluorescence and charring. The latter is particularly troublesome because of the high intensity at the focus of the beam. Even when charring is not observed, the possiblity of phase transition due to local heating needs to be taken into account.

Ultraviolet and Fluorescence Spectroscopy

Although electronic reflection spectroscopy has been rarely invoked for the examination of polymorphs, it has long been known that different polymorphs of coloured compounds[317–319] including certain dyes and pigments,[58,59] in particular, phthalocyanines,[149,320–323] display different hues. Bandshifts of up to 170 nm in the solid state as a result of packing differences of the molecules have been reported.[324–326] Furthermore, it is remarkable how many organic crystals deepen in colour on transformation to a higher melting polymorph,[98,122,155,159] so it must be presumed that many, probably most, uninvestigated colourless polymorphs would also show a spectral change in the UV region on transformation. The information that can be extracted from UV reflection is less than from the techniques whose spectral characteristics are more readily related to structure, and the measurements are more difficult. The electronic spectrum may, however, be recording more subtle solid-state changes. It has been recently ascertained that the yellow to red transformation of pyridinium picrate which has been known since 1929 does not occur at the temperature of the only transition point recorded by variable temperature X-ray diffraction studies.[327] The use of polarized near-normal UV spectral reflectance from different faces of single crystals has been applied to the conformational polymorphism of dichlorobenzylidene anilines to relate solution and crystal properties and to elucidate the relationship between molecular conformation and electronic properties.[4] The origin of these colour differences has been discussed only briefly, but must be presumed to be due to intermolecular charge-transfer effects.

Ultraviolet spectra of solids can also be obtained by transmission from the mull or KCl disk technique[328] (KCl is transparent to shorter wavelengths than KBr), provided that a thinner matrix is used and account is taken of the vast difference in molar absorption coefficients in the IR and UV regions. The UV spectra of polymorphs of 2(2-methyl-3-chloroanilino)nicotinic acid have been investigated by diffuse reflectance from Nujol mulls.[132]. A detailed comparison of the relative merits of photoacoustic spectroscopy and diffuse reflectance in the UV, visible and NIR regions has been made.[329]

The colour of cyanine dyes is related to the aggregated state in solution, concentrated solutions yielding the more deeply coloured solid-state forms containing the more extensive molecular aggregates.[330] The absorption spectra, the fluorescence spectra and the electronic properties of solid cyanines[331] display marked differences between the polymorphs. The fluorescence spectral differences in this and other cases[332] have been ascribed to a type of excimer formation. Fluorescence spectra have otherwise been little reported although they have been investigated for possible quantitative analysis of polymorph content.[333] Polymorphs may also differ in their thermoluminescent characteristics.[334,335]

Solid-state Nuclear Magnetic Resonance and Nuclear Quadrupole Resonance Spectroscopy

An NMR spectrum on a solid run under similar conditions to those used for solutions will result only in a broad hump of extremely low signal intensity. For the investigation of melting phenomena or of order–disorder transitions representing the onset of molecular rotation or libration this is advantageous: the phase yielding signals of moderate width as a result of orientational, positional or configurational freedom can be measured with little interference from the signals generated from the rigid solid phase.[336,337] For detailed observation and interpretation of the molecular structure, however, it is necessary to narrow the signals.[338,339]

The breadth and low sensitivity of the solid state signals in ^{13}C NMR spectroscopy is due to three separate effects, each of which must be minimized.[340–342] The lines are broadened firstly by anisotropic dipole–dipole coupling and the quadrupole field gradient. Secondly, the chemical field anisotropy which is normally averaged to zero in liquids cannot be averaged out by molecular tumbling in solids. Finally, the extremely long spin–lattice relaxation times require long pulse repetition times to build up the signal. The chemical field anisotropy can be averaged by magic-angle spinning (MAS) in which the sample is rotated at speeds of 4–15 kHz.[340–342] The dipolar and quadrupolar field effects can be removed by high-power heteronuclear decoupling. Finally, the spin–lattice relaxation time is reduced by cross-polarization involving pulse sequences which transfer energy between nuclei, thus involving the 1H nucleus in the mechanism of relaxation. The net result is that NMR spectra of solids are now routinely available of acceptable signal-to-noise ratio which show adequate resolution for structural interpretation,[343–345] although longer acquisition times than for solution spectra are necessary. The detail and information content of NMR spectra should be particularly valuable in distinguishing polymorphs and in understanding the sources of their differences.[64,313,342–345] The use of NMR spectra for examination of dosage forms has been canvassed.[345,346] In practice, relatively few descriptions of the NMR spectra of polymorphs are available in the literature and in several cases where phases which have proved to be very similar by other techniques have been examined, they have also proved to show few differences by NMR spectroscopy.[5,169,281,347] This illustrates that very small packing differences are sometimes characteristic of phases or polymorphs. The interpretation of the spectra in terms of molecular structure is normally by comparison with the solution spectrum, but the assignment of carbon type can be made in the solid state with the use of appropriate pulse-sequence techniques.[348] A promising use of solid-state NMR spectra is in investigating amorphous forms.[28,349,350] The amorphous form of testosterone was assumed to have ordered packing but disordered molecular orientation from examination of the features in the NMR spectrum associated with the different portions of the molecules.[116] Conclusions could therefore be drawn as to the probable mechanism of solidification. It is not clear why a solid with positional order but rotational freedom behaves as an amorphous phase rather than a disordered one. Solid-state NMR signals can sometimes be observed to be doubled as a result of non-equivalent crystallographic molecules in the unit cell.[116,340,351]

Nuclear quadrupole resonance spectroscopy[352] (NQR) is not troubled by the broadening effects encountered by NMR spectroscopy and has been widely used particularly for the examination of inorganic systems. It relies on the detection of the electric quadrupolar effects and is confined to those nuclei with suitable spins. For organic compounds these are principally ^{2}H, ^{14}N, ^{17}O, ^{19}F, ^{35}Cl, ^{37}Cl, ^{79}Br and ^{81}Br. It is relatively insensitive so large quantities of material are required. Chlorine and bromine can be detected by conventional radiofrequency spectroscopy but ^{14}N, which is probably the most generally useful nucleus for organic compounds,[353] requires sensitivity enhancement. Cross-relaxation experiments, similar to the cross-polarization experiments discussed above, are appropriate. ^{2}H and ^{17}O studies require isotopic enrichment. All these nuclei have been used to study phase transitions, particularly in relation to mechanism and molecular dynamics.[354,355] The use of ^{17}O to study order–disorder phenomena is discussed later. Phase transitions are detected by changes in relaxation times, couplings or multiplicity with temperature. Malononitrile[356,357] is particularly interesting, because the change in multiplicity of the ^{14}N NQR signals at −132 and 22 °C heralds a new phase in between those temperatures, although the phase below the lower temperature appears to be the same as that above the higher one. It can be seen from Fig. 4 that the Gibbs energy values for the two polymorphs are constrained to follow very similar paths. As might be expected from this, the intermediate phase has a structure which is only marginally different from the surrounding phase.

X-ray Crystallography

X-rays are reflected from crystals only when the angle between the ray and the planes in the crystal fulfil the Bragg condition $n\lambda = 2a\sin\theta$, where θ is the angle between the ray and the plane, λ is the wavelength of the radiation, a is the interplanar spacing and n is an integer. There is an infinite number of possible planes through the crystal, but only a limited number which give reflections within the accessible range $2 < \theta/\text{degrees} < 180$. With a single-crystal brought into all orientations with respect to the beam, a series of spots is generated on the surface of a sphere centred on the crystal. In the case of a powder sample a set of concentric cones is generated which can be recorded as a series of arcs on a photographic strip or as a diffraction trace *via* a detector.[358] Every molecular repetition will give a unique set of reflections and so generate a unique pattern. Any change in crystal packing will lead to changes in the form of the molecular repetition. In principle, then, any polymorph will give a distinctive X-ray powder pattern. X-ray powder crystallography is therefore of great value for distinguishing and identifying polymorphs.[359]

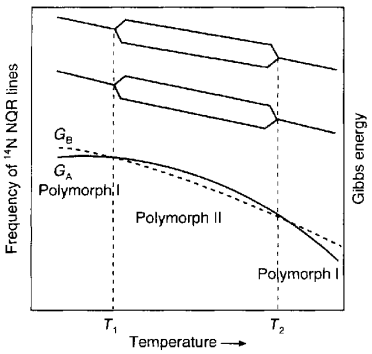


Fig. 4 Interpretation of the phase transitions of malononitrile in terms of Gibbs energy. The upper part of the diagram is a schematic representation of the variation of the ^{14}N NQR spectrum of malononitrile with temperature. T_1 and T_2 are the transition points at −132 and 22 °C, respectively. The lower part of the diagram represents the Gibbs energy situation. Instead of crossing once as in the enantiotropic system in Fig. 7, the Gibbs energy curves G_A and G_B (for polymorphs I and II, respectively) must cut twice (see text).

X-ray single-crystal diffraction is, of course, even more descriptive and in principle can lead to unique definition of the packing of the molecule, the molecular interconnectivity and the three-dimensional conformation of the molecule in the crystal. However, it often proves difficult in practice to grow crystals of sufficient size and perfection for an X-ray structural analysis to be carried out whereas a powder pattern can nearly always be obtained.[73] The difficulties which may be encountered in growing crystals of the polymorph stable at room temperature are much magnified when unstable polymorphs and enantiomeric polymorphs are required and particularly when crystals of unstable polymorphs of enantiomers are involved.[248,360–363] The evidence for this packing prejudice against optically active molecules has been undermined by a detailed comparison of the density measurements recorded in the literature for racemates and enantiomers and a consideration of the statistical bias,[124] but it remains a matter of common observation during crystallization experiments that optical isomers are difficult to produce as good crystals.[364] The problems with metastable forms are easy to understand as owing to the presence of crystal strain and defects. Some crystals show such a large change in volume on transition that they generate enough strain to shatter or move violently and are therefore sometimes characterized[275,312,347,365–367] as 'jumping crystals'. Variable-temperature X-ray diffractometers[368,369] are helpful and, of course, essential for the examination of polymorphs which have no existence at room temperature but the required apparatus is infrequently available in laboratories where polymorphs are encountered. It is good practice to look at a sample under the polarizing microscope for homogeneity and for appearance of individual crystals as single and perfect, free from twinning or unusual features, before submission for single crystal X-ray examination. Occasionally, even the most beautiful and transparent crystals may be twisted, too thin to produce an adequate signal, multiply twinned, polycrystalline or otherwise defective and hence fail to give an interpretable diffraction pattern.[370] Even if the diffraction pattern is too poor for a complete structural analysis, the unit cell dimensions are a criterion for the existence of distinctive phases and the derived density a further critical reference value for the polymorph. Regrettably, crystallographers often fail to record minimum physical characteristics of specimens of polymorphs such as melting point, range of stability or relative stability[371,372] or even origin[373,374] thus limiting the usefulness of their results. For this reason it proved impossible, by examination of the Cambridge Structural Database (Cambridge Crystallographic Data Centre), to check the reliability of the rule that the polymorph stable at higher temperature has the more symmetrical structure. The structure of over a thousand pairs of organic polymorphs has been recorded, but only a small portion have adequate accompanying physical information. The theoretical basis of the rule has been described by Kitaigorodski[375] and Desiraju.[376] The total energy of a crystal is the sum of the lattice energy and the vibrational energy. Close packing minimizes the lattice energy but interferes with vibrational motion increasingly at higher temperatures. The loss of lattice energy stabilization in a more open lattice can be compensated by the entropy gain resulting from the more symmetrical structure. The close packing requirement means that the majority of organic crystal structures reside in very few space groups ($P2_1/c$, $P\bar{1}$, $C2/c$, $P2_1$, $P2_12_12_1$).[32,33] The combined effect of the vibrational and close packing requirements on organic polymorphs is that

one of the commonest patterns for a dimorphic system on transition is monoclinic at low temperature to orthorhombic ($P2_12_12_1$) at higher temperatures. Higher symmetry space groups are adopted by disordered states.[275]. Plastic crystals generally adopt cubic space groups in the disordered phase,[8,377] reflecting the requirements for the molecular motions.

The development of area detectors for diffractometers for small molecule work means that crystals previously too small to examine can be successfully tackled, or areas of otherwise unsatisfactory crystals can be chosen.[378] This can be very effective in conjunction with the use of synchroton radiation.[312,379–382] Although there are occasional reports of incorrect conclusions being drawn from X-ray data[5,327,383,384] the most likely source of error in studying polymorphs is picking the wrong crystals.[385] As mentioned above, metastable forms often crystallize badly and in a sample of such a product it is not uncommon for the only satisfactory crystals to be interlopers of the stable polymorph. Computation of the correlation of X-ray single-crystal diffraction patterns with powder patterns is now possible and should capture such error at an early stage.[142,169,386] The contrary process, converting powder patterns of complex molecular crystals to structural information,[387] although an exciting prospect, is not yet applicable to sufficiently large molecules to be of general interest for studying polymorphs of commercially interesting compounds.

However, for the ordinary laboratory environment an X-ray powder diffractometer is of more general value. It will sometimes identify differences between samples which are too subtle to be detected up by thermal analysis[5,313] microscopy or IR spectroscopy,[388], although a few contrary examples are known.[312] One such general instance is where water or other small[389,390] molecules fill voids in a structure in a random fashion without altering the crystal packing itself as in the examples of antibiotics such as cefaloglycin and cefalexin.[391] A mixture of crystalline and amorphous material will be indistinguishable from a pure sample of the crystalline material except in absolute intensity which is rarely measured in normal use. There are other cases which are not so easy to explain.[282] For example, the X-ray patterns of the forms of D,L-norleucine are virtually identical, although the IR spectra are easily distinguishable.[160,392] Examination of the IR spectra excludes the possibility that a neutral $\longleftrightarrow$ zwitterionic transformation is involved.

A more common problem with X-ray powder diffraction is in the examination of samples consisting of larger crystals. These may produce a spotty pattern which is difficult to reduce to a series of line intensity measurements and is impossible to compare satisfactorily with diffractograms from other samples.[358] If the crystals are not roughly isometric, particularly if they are needles or platey, the pattern may show distinctive features from crystal orientation effects[169] as is shown in Fig. 5. Grinding is appropriate providing that the polymorph is stable. For soft crystals an inert powder may be mixed in,[393] in order to facilitate grinding. An alternative approach is the use of the Gandolfi camera which can be made to generate a simulated powder pattern from a single crystal. The orientational bias for platey crystals of polymorphs III and IV of sulfathiazole was eliminated in this way.[169] The calculation of powder patterns from single-crystal data mentioned above has been recommended by several groups as a means of obtaining the best reference X-ray powder pattern.[142,169,387,394]

Neutron diffraction, although of less general value than X-ray diffraction, has the advantage that the scattering factors for atoms vary little with atomic number.[395,396] Light atoms can therefore be detected and located accurately in the presence of heavy atoms, in contrast to X-ray studies. As such, it is of potential value in examining polymorphic systems for their hydrogen bonded networks[82,84,111,122,397] and in investigating tautomeric or zwitterionic polymorphism. The naphthazarin C polymorphs have been examined by neutron diffraction to establish their hydrogen-bonding characteristics and the order–disorder transition.[398] The deduced centrosymmetric structure, in contrast to the Raman results mentioned earlier, is the result of the averaging of the structure over a substantial time-scale. This factor also applies to X-ray structures[399] and needs to be borne in mind when comparing these with NMR and vibrational data. The comparative rarity of sources and the need for relatively large crystals means that neutron diffraction is likely to be infrequently used for investigation of polymorphs.

X-ray crystallography is well supported by texts at all levels, both for single-crystal work[400–404] and powder methods.[358,395,405,406]

Thermal Analysis

Although the term thermal analysis is sometimes considered to include hot-stage microscopy, it is convenient to deal with these methods separately. Microscopy is concerned with qualitative visual observations whilst instrumental thermal analysis is capable of giving quantitative measurements, but without necessarily identifying the nature of the processes responsible. Thus the techniques are complementary and best used in conjunction.[407] The main thermal techniques considered will be thermogravimetric analysis (TGA) and differential thermal analysis (DTA)/ DSC.[408] TGA measures the change in mass of a sample with temperature and is therefore particularly valuable in examining solvent loss from crystals and in identifying sublimation and decomposition processes. As it is recording dynamic processes, not only the temperature at which changes occur will vary with procedure but the very occurrence of those processes may depend on sample environment and heating conditions. The subtleties of thermal analysis are often overlooked. In the vivid words of Garn,[409] 'The apparent simplicity of the technique leads the uninformed to assume that satisfactory data may be obtained, for example, by sticking a pair of thermocouples into a sample and reference and lighting a fire under them.'

DSC and DTA are alternative ways of measuring heat capacity changes in a sample.[196,410] Although they may occasionally give significantly different thermal traces,[411] the term DSC will be used here without implying the method of acquisition of the data. Any compound will absorb heat in acquiring a higher temperature. During a transition, heat will be absorbed or emitted in effecting a change of phase. The remarks

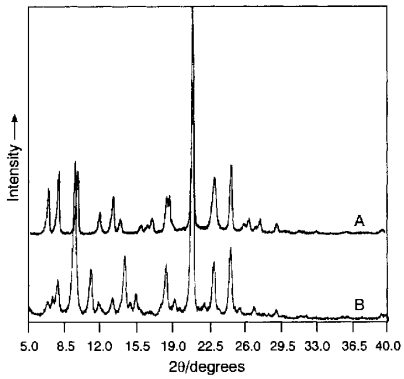


Fig. 5 Crystal orientation effects in X-ray powder diffraction. Traces due to A, the platey and B, acicular habits of the same polymorph of RP 54275 are shown. At high values of 2θ, the traces are similar, but at low values they are different. Reproduced with permission of Rhône-Poulenc Rorer Ltd.

made above regarding the dynamic nature of TGA apply equally to DSC. In most cases where the forms are stable to grinding and the transitions are rapid the resulting curves will be sensibly reproducible. In other cases, the thermograms obtained may depend on the heating rate,[412,413] sample packing,[414] crystal size,[415,416] the ambient atmosphere[417] and encapsulation[239,367,407,418] and interpretation needs appropriate care. In particular, it is often overlooked that the history of a polymorphic crystal may be critical, for example, a later run may differ because of tempering on standing with loss or gain of seed nuclei of other forms.[200,313,367,419–421] Many instruments now run TGA and DSC simultaneously. This is valuable in that it enables a clear distinction to be made between processes involving solvent loss, sublimation and decomposition on one hand and pure phase changes on the other. The principles of thermal analysis have been set out recently in a book[422] and in an introductory video.[423]

The features to be seen in a DSC trace (Fig. 6) are endotherms, representing absorption of heat, exotherms representing the emission of heat and the so-called second-order transitions representing a change in the heat capacity without either absorption or emission of heat. A sloping baseline could represent a continually changing heat capacity, but is often due to imbalance between sample and reference, or slow loss of mass from the sample during heating. During a heating cycle endothermic processes are the most common ones. Melting and sublimation are always endothermic as are transitions involving enantiomorphs at or above transition points. Desolvation is usually endothermic and chemical reactions can be, especially at lower temperatures. Monotropic transitions, crystallization and most decomposition reactions are exothermic. On cooling, crystallization and enantiotropic transitions are exothermic, so cooling cycles normally contain only exotherms. Despite this there is often value in running the sample under both heating and cooling modes.[414] Although this has long been recommended, it is rarely indicated in the thermal analysis literature on small molecules that this has been considered.[208] By contrast it is common in lipid and polymer work to run both heating and cooling curves.[89] If it is intended to identify the material at room temperature after a phase transition, it is imperative to check on the cooling cycle that no reverse change has occurred. Heats of transformation and melting can be evaluated from the area under a DSC curve,[424,425] although not, of course, as satisfactorily as from a precision adiabatic calorimeter.[426] Conditions need to be chosen carefully in order to obtain reliable results. The greatest difficulty is in determining the most suitable base line.[427]

It is common for a polymorph to show a transition to a higher melting polymorph at the appropriate transition temperature when heated slowly, but to overshoot and melt at its own melting point under more rapid heating conditions.[194] This is often followed immediately by re-solidification to the higher melting polymorph giving a characteristic curve shape (Fig. 6, c). The polymorph thus produced may or may not be the same as that resulting from the transition at the proper transition point and in other instances the re-solidification may be delayed.[224] Dependent on the complexity of the polymorphic set, a whole series of such events may take place. Finally, the form with the highest melting point will melt if it has not previously decomposed. Several meltings may take place in the case of a

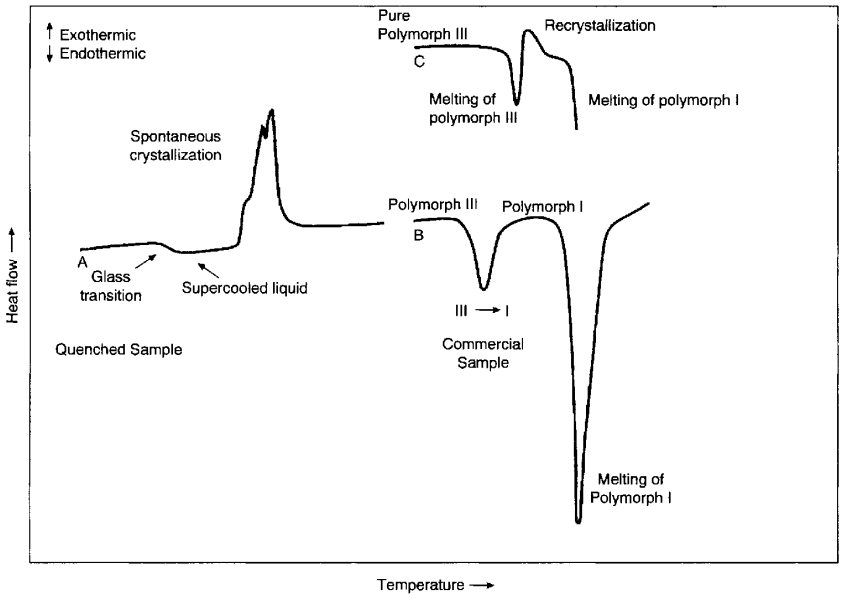


Fig. 6 Typical features in the DSC of a polymorphic system. A, Quenching the melt of sulfathiazole gives an amorphous solid, which on heating undergoes a second-order transition (glass transition) to a supercooled liquid (see refs. 422, 542–544). In a second order transition no heat is evolved or absorbed and only the heat capacity alters. This is seen as a drop in the base line. A supercooled liquid always represents an unstable phase and on heating spontaneous crystallization of this can occur. In this case it happens suddenly, causing the rapid movement away from this new base line. Irreversible processes are exothermic, but the complex exotherm which follows is unusual and probably represents several overlapping transitions. As described by Ostwald's Principle (see refs. 258 and 436) this is a cascade of transitions to successively more stable forms at that temperature. The resulting phase must be polymorph I, since it melts at 201 °C without further thermal events occurring, B, a specimen of polymorph III shows an endotherm due to the transition from polymorph III to polymorph I, followed by melting. The fact that it is endothermic indicates that polymorph I and polymorph III are enantiotropic. This endotherm always occurs around 150–175 °C although it is known that the true transition point lies many degrees below this; and C, a specimen of polymorph III which is free from seeds of polymorph I (see refs. 194 and 242), may overshoot the transition point and melt at its own melting point. This is often followed immediately by recrystallization, which is an exothermic process, of the higher melting polymorph I giving the characteristic trace shown.

compound with liquid crystal phases, but finally a clear melt will form.

The literature on the investigation of the behaviour of phenylbutazone[239,428–432] provides an instructive example of the role of thermal analysis in polymorphism. Early work produced the not untypical situation of conflicting data on the number and properties of polymorphs.[239,428] Subsequent application of thermogravimetric analysis showed that two of the reported polymorphs were in fact solvates.[429] In a substantial re-investigation, five polymorphs were identified and characterized.[430] The IR spectra were not very useful for differentiating between the crystal forms because of their similarity.[430] The X-ray diffractograms were also reported as somewhat similar, although the earlier work[429] had relied on these to distinguish forms. The published patterns look distinguishably different[239,429,431] but it is reported that phenylbutazone shows orientation effects and is sensitive to grinding,[239] which is undoubtedly the reason for the reported similarity of the IR spectra. Dissolution rate data were also acquired, but in the absence of surface area information (see later) they cannot be regarded as definitive evidence for polymorphism. Distinction between the polymorphs relies then in this study[430] on thermal analysis. The temperatures of peak maxima are quoted for all polymorphs as well as onset temperatures of melting, the latter agreeing closely with the melting point as determined on a hot-stage microscope. The two highest melting polymorphs, A and B, show only a single peak due to melting at all heating rates, with onset temperatures of 105 and 103 °C, respectively. The remaining three polymorphs, C, D and E, each show a single melting endotherm at 96, 94 and 92.5 °C under rapid heating rate conditions of 32 °C min^{-1}. At lower heating rates they all display a melting endotherm adjacent to a recrystallization exotherm (similar to that shown in Fig. 6, c) followed by a melting endotherm at 105 °C. This was interpreted as the formation of polymorph A from the melt. Grinding or compressing the polymorphs C, D and E caused an increase in the area under this higher melting peak and a small reduction in the observed temperature of all the endotherms. In view of this and the closeness of the melting points it is difficult to be sure that A and B do not represent only one polymorph and C, D and E another, although there is some evidence of a third endotherm in some of the thermograms and evidence from the other papers of at least four forms. Subsequent studies have identified other forms[431] and confirmed the sensitivity of the results to the thermal history of the sample.[432]

By contrast, the melting points of the three polymorphs of gepirone hydrochloride[433] are substantially different and the conclusions from thermal analysis about the relationship between them unambiguous. Under slow heating conditions, samples of the low melting polymorph (mp 180 °C) showed an endotherm due to the transformation to the higher melting polymorph. At faster heating rates, a melting endotherm followed immediately by an exotherm representing re-solidification of the higher melting polymorph was observed. The higher melting polymorph then melted at 220 °C. This interpretation of the DSC measurements was confirmed by hot-stage microscopy. By prolonged heating of the lower melting polymorph it could be converted entirely to the higher melting form. The sample then showed a single endotherm at 220 °C. The endotherms of mixtures showed the disproportionate effect of small quantities of the higher melting form. The third polymorph could only be produced by crystallization as a minor component of a mixture. From DSC supported by thermomicroscopy the melting endotherm could be identified at 212 °C. Consideration of the relative thermal stabilities allowed small samples of the pure polymorph to be produced by heat treating mixtures in the calorimeter; the pure polymorph so produced showed only a single endotherm at 212 °C whereas the mixture had shown endotherms at all three melting points. From these experiments it was possible to decide on the relative thermal stabilities of the polymorphs and to calculate their heats of fusion.

The most important advance in understanding of the thermodynamic relationships between polymorphs and in interpretation of DSC curves has been through the formulation of Burger's rules.[136,434] Two of these will be discussed here and the other two in Solubility and Density Measurement. Burger's heat of transition rule implies that (*i*) if an endothermic transition is observed at a certain temperature on heating, then there must be an enantiotropic transition point at or below that temperature; but (*ii*) if an exothermic transition is observed, then the transition point must lie above that temperature, or the two forms are related monotropically.

Burger's heat of fusion rule is of value when the heat of transition cannot be observed, owing to the failure of the polymorphs to transform readily. This states that the higher melting polymorph will have the lower heat of fusion if the polymorphs are in an enantiomorphic relationship, otherwise they are monotropically related. Because of the misunderstanding of these rules which is apparent from the literature, and because of the insight into the stability relationships between polymorphs which they yield, a simplified derivation will be given here.

Fig. 7(*a*) and (*b*) are representations of the Gibbs–Helmholtz equation for enantiotropic and monotropic cases, respectively. The shape of the H (enthalpy) curves is determined by $H = H_o + \int c_p dT$. Since the specific heat C_p is always positive, they must slope upwards at an increasing rate with temperature, as shown. G, the Gibbs energy, is related to the negative summation of all the entropies, S. The value of S is again dependent on C_p. The value of S must be positive, therefore the G curves must slope increasingly downwards, again as shown. At absolute zero, $H = G$ and the curves meet. The lowest energy crystalline structure at absolute zero will have the strongest intermolecular bonds. Strong bonds imply high lattice vibration frequencies (phonon modes[396,435]) which make the smaller contribution to C_p. Therefore, the angle of divergence of the G and H curves of the polymorph most stable at low temperatures will be less than that of the less stable polymorph. Hence the G curves will tend to cross, but the H curves will not. The heat of transformation rule can be ascertained by concentrating on the H curves and noting the enthalpy consequences on going from H_a to H_b or *vice versa*, remembering that this is only possible by lowering the Gibbs energy, *i.e.*, ΔG must be positive. Hence processes which are exothermic on raising the temperature are spontaneous ones and are irreversible at or below that temperature, and *vice versa* for endothermic processes. The heat of fusion rule depends on the enthalpy curves for the polymorphs and the liquid phase being approximately parallel over the relevant region, so that the differences in C_p do not obscure differences in the heats of transition. These rules are extra-thermodynamic, in that they involve structural considerations, so they are not 100% certain. It is not clear whether there are any exceptions in practice as re-evaluation of the literature data has eliminated many of the apparent exceptions.[42]

These rules, as already implied, can be helpful in sorting out DSC results. The concept of enantiotropism as reversibility needs to be approached with caution. Mirror image curves cannot be expected on heating and cooling. Apart from Ostwald's rule[257,436] and hysteresis due to high energy barriers,[194,434] leading to offset of heating and cooling events, consider the energy–temperature diagram for a trimorphic enantiotropic system, Fig. 8(*a*). The heating cycle might produce transformations at A, B and C whilst the cooling cycle might proceed *via* any of the many paths on the diagram. A form such as polymorph II in Fig. 8(*b*) which is metastable at any temperature would be most unlikely to form on heating, but could well be the product of cooling the melt.

For investigation of melting by DSC, small samples are usually appropriate and the temperature of melting is taken as either the peak maximum, or more precisely as a peak maximum corrected for heat flow,[425] or as the extrapolation of the leading edge back to the base line.[437] Because solid–solid transformations are often sluggish[157,438] and may reflect very small enthalpy changes, the use of larger quantities of compacted sample has been recommended, together with low heating rates and the assignment of the first discernible movement away from the base-line as the transition temperature.[197] The appropriateness of this may depend on the thermal stablity of the material under examination. Similar treatment of cooling curves then yields a transition range dependent on the hysteresis of the system. Organic compounds may be more appropriate calibrants than the almost universally used indium, as they are likely to have conductivity characteristics similar to the sample.[197,439]

It is often implied in accounts of the determination of purity by DSC that the true melting endotherm of a pure substance will be infinitely sharp,[440] but of course this cannot be so for organic powders. Apart from practical considerations of thermal conductivity, edges and surfaces are less stable than bulk and will melt first and so small crystals will melt before larger ones.[441] Melting normally starts at crystal defect sites. The observed melting will also be affected by a polymorphic transition very near to the melting temperature or decomposition at the melting point and, of course, impurities. Although it was generally thought that the melting temperature could not be exceeded without melting occurring, there are scattered reports of slow melting[442,443] and superheating[444] and increasing acceptance of the existence of this phenomenon.[445] In addition there are instrumental factors. Different instruments (DSC, DTA, melting point apparatus, hot stages, thermal photometers) measure different manifestations of the melting process and so will not necessarily give the same value.[196,199] All these factors apply also to solid-solid transformations. Even after the elimination of the possible effects, there still remain unexplained examples of anomalous melting behaviour. For obvious reasons most of these never appear in the literature but there are a few[446–449] and further examples are known to the author. Note that whilst examples of curious melting and transition behaviour ought to be carefully checked, they are not necessarily the result of inaccurate observation.

A large endotherm followed by a small melting endotherm is characteristic of the formation of a disordered phase in which the positional order of the crystal is retained, but the orientational order is lost.[8,275,426,438] This may be due to random orientation of molecules, but is most often associated in organic systems with the onset of 'free' rotation. Molecules of roughly spherical shape are particularly likely to show an order–disorder transition to a plastic crystal state.[8,224,426,450,451] At lower temperatures, crystals of such molecules sometimes show a glass transition in the crystalline state.[452,453] Order–disorder transitions have been regarded as second-order transitions,[154,180,454] but organic examples are not characterized by 'second-order' DSC traces. Although second-order transitions are widely discussed in the literature, the concept presents certain difficulties as has been well addressed by West.[154] On the whole the term is better avoided, except in reference to glass transitions, in considering the inter-relationships of organic polymorphs.

From a study involving a selection of appropriate techniques it should be possible in most cases to acquire a reliable listing of the polymorphs, their relative stabilities and their transition points, which is as far as present day economics of industry may allow. However, a study is incomplete without the drawing of a semi-schematic energy–temperature or the equivalent pressure–temperature diagram.[433] If all the relevant data have been assembled such a figure takes, except in complicated cases, only a few minutes to prepare. The discipline of setting out the results in this form leads to a great confidence that the system is understood and avoids the erroneous descriptions of polymorphic systems sometimes presented in the literature.[35] Whilst the unwelcome appearance of a further polymorph at a late stage of investigation cannot thereby be excluded, it is rendered less likely.

A development which offers greater sensitivity as well as enabling overlapping spontaneous and reversible processes to be separated is oscillating, alternating or modulated DSC.[455] The superposition on the temperature ramp of a periodic temperature function allows a computational separation *via* a Fourier transform. Although the rate of modulation in commercial instrumentation is too slow for many polymorphic transitions, it is already being found useful in pharmaceutical investigations.

Thermosonimetry[456] is a relatively unexplored technique owing to the lack of convenient instrumentation and the dearth of applicable theory. It is mentioned here because it would appear to have considerable potential for the identification of phase changes and possibly for the understanding of the crystal structure changes accompanying these. The frequency spectra of the sonic emission of solids on heating are very rich, although it is only possible to use these at present as a signature.[457,458] Phase changes are accompanied by increased activity and a change in the spectrum.

Solubility and Density Measurement

These are two of the measurements traditionally used to identify polymorphic behaviour. They remain important today: solubility because that is often the target property which is required of the polymorph in practice: and density because of its reliability and theoretical linkage with crystal structure and with stability. A pigment which bleeds, a solution of an agrochemical* which is liable to precipitate and block spray nozzles or a suspension of any product which cakes[47–49,461] during storage is probably unmarketable. The solubility also has an important thermodynamic feature: it is inversely related to the stability of the polymorph such that the most stable polymorph is always the least soluble at a given temperature.[19,34] At a transition point, the interconverting polymorphs are equally soluble. There is an implicit assumption bchind these assertions that the solutions prepared from either of the polymorphs are identical. There is limited evidence against this in some cases. For example, in the case of sulfonamides the polymorph crystallizing from solution is dependent on that dissolved.[462] In principle then, the determination of the solubility over a temperature range for two or more forms of a substance will readily establish the transition points and thermodynamic stabilities.[463] It is the author's experience, however, that the measurement of solubility gives rise to more difficulty and more erroneous data than any other connected with polymorphism. The problem is threefold.

(i) The attainment of equilibrium is often slow, particularly with poorly soluble or poorly wettable substances,[464] for which several days' agitation may be required to establish a consistent value. Either through system instability, lack of awareness or time constraints this is often not done and the measured solubility is then effectively a dissolution rate measurement. This latter, whilst related to solubility *via* the Noyes–Whitley equation[465] and so roughly parallelling it in many cases, is also a direct function of surface area and therefore of particle size.[36,466] If particle size is checked only instrumentally

* Examples of polymorphs of agrochemicals in the open literature are few, *e.g.*, Borka.[459] Instability of formulations is more often related to supersaturation than to polymorphism and problems are often solved pragmatically. However, the more sophisticated formulations now being introduced demand attention to polymorphism.[460]

(Coulter counter, Malvern analyser) over-all aggregate size rather than individual grain size may well be measured.[467] Any differences in grain and aggregate size can then result in erroneous solubility comparisons. A preliminary microscopic examination will give forewarning of such a situation, but may not indicate how to solve it. Intrinsic dissolution measurements[464,468] may provide a surrogate solution to the problem. 'Surrogate' because there are both practical and theoretical reasons why the intrinsic dissolution rate ratio of polymorphs will only approximate the relative solubilities. (For an example see Table 1 in the study by Buxton, *et al.*[469]). Wettability differences can totally destroy any correlation.[470,471] Nor can slow equilibrium be overcome by working at higher temperatures followed by cooling, because the temperature–solubility hysteresis usually determines an even longer equilibration time. The second factor is the susceptibility of the polymorphs to transformation when examined outside their stability ranges.[472] As indicated earlier, the presence of a solvent can be particularly efficacious at promoting a polymorphic transition. It is often possible to measure the solubility of a polymorph below its lower transition point, but rarely many degrees above its upper one.

(ii) The possibility of a transformation to a solvate,[473] or hydrolysis[146] or other chemical reaction. Sometimes the shape of a solubility–time curve will indicate whether a transformation is occurring, but whether or not this is so depends on the relative kinetics of the dissolution and transformation processes. One solution is to measure the solubility of the polymorphs in an inert solvent and then measure the partition coefficient rapidly.[474]

(iii) There are the consequences of pH variation in the measurement of the solubility of ionizable species.[463,475] The self-buffering capacity of organic acids and bases can often make a dramatic difference to the observed solubility. The need to match buffer capacity to the expected solubility is rarely considered.[476] Trace ionic[477] or other (oxygen, carbon dioxide) contamination can occasionally present a source of error. If the solubilities are being measured spectrophotometrically the effect of pH or complexation on the absorption spectrum also needs to be taken into account.[36,478]

When the solubilities cannot be determined in the region of the supposed transition point, it is possible to extrapolate from other temperatures using the van't Hoff isochore. This procedure needs to be applied with caution as the experimental inaccuracies and theoretical assumptions are often not appreciated.[77,162,463,479]

For molecular solids in which hydrogen bonding is not a structural feature, the stability of a form is nearly always closely related to the density. Although this relationship, as a consequence of the rapid reduction of intermolecular attractive forces with distance, has been understood for a long time, the structural implications were first explored in detail by Kitaigorodski.[480] Dipole–dipole interactions can contribute to the structural stability (surprisingly, however, they do not appear to contribute to the preferential formation of polymorphs[481]), but the only common and significant attractive force other than van der Waal's forces is hydrogen bonding. This can produce more open structures in which the loss of polarizability energy is matched by favourable disposition of the strong hydrogen bonds. This is the basis of the other two of Burger's rules,[136] namely the density rule 'the more stable polymorph at absolute zero will possess the highest density' and the IR rule 'the highest frequency OH or NH stretching band will be associated with the form least stable at absolute zero'. The highest frequency OH or NH stretching will be associated with the weakest hydrogen bond. Juxtaposition with the heat of transformation and heat of fusion rules will usually allow the deductions to be generalized to working temperatures. Consideration of the circumstances pertinent to these rules could lead to the expectation of exceptions. It is found in practice that whilst there is a small proportion of exceptions to each rule, their complementarity makes the concurrent failure of both rules less likely.[42]

Density can be measured by flotation,[482,483] by volumenometry, or by pyknometry.[483] All are time consuming. Alternatively the true density* can be calculated from the unit cell dimensions.[485] The latter must always be marginally greater than the measured density, as the crystal voids and other defects always lower the overall density of the crystals. Any discrepancy is a warning of solvates or other incorrectly assumed molecular structure. Generally, the measured density will increase marginally on grinding as a result of cracking occurring preferentially at crystal pores and defects, but on prolonged grinding it may begin to decrease owing to increased surface area and amorphization.[42,486] An attempt to check Burger's density rule against the true densities by using the Cambridge Crystallographic Data Centre data base for X-ray structures failed for the reasons mentioned earlier.

The air comparison pyknometer represents an instrumental method of measuring densities with enhanced sensitivity. Flotation is best carried out with centrifugation and it may detect the presence of interloper crystals of a different polymorph in a specimen. The main problems with flotation are in finding a liquid mixture of suitable density that does not dissolve the sample and in maintaining that density through adequate temperture control. The first requirement is particularly critical for organic polymorphs.

Solvates

Hydrates or other solvates often produce a further level of complexity in a polymorphic system.[487,488] There is the expectation of a monohydrate or monosolvate but, in fact, the accommodation in a unit cell for a small molecule can produce multiple,[489,490] fractional,[282] irrational[412] or variable[469,491] molar ratios. Amongst the polymorphs of a molecule some can be hygroscopic and others stable to water or water vapour.[489] Different hydrates can be produced from different polymorphs.[45] This is probably related to the 'stuffing' effect of impurities described by Buerger.[3] Where there are two or more hydrates of the same composition, these are in a polymorphic relationship with each other.[138] In practice it may be difficult to interconvert polymorphic solvates, because of the likelihood of preceding desolvation.[389,469] The desolvation of a solvate can sometimes produce a polymorph not obtainable in any other way.[138,389] A detailed study of celiprolol hydrochloride has shown that the hydrate is not a true one in the usual sense but appears to be a solid solution of the drug in water.[492] This leads to speculation about the exact nature of the crystal structure involved.

Thermomicroscopy in silicone oil will reveal desolvation on heating by bubble formation.[178] DSC will show features corresponding to solvent loss, but such features are notoriously sensitive to heating rate, crystal size, mass of sample, sample packing, and to the use of open as against closed or sealed pans or even pan shape.[427] When the transitions are accompanied by inhomogeneous melting (dissolution) or a mixture of inhomogeneous and homogeneous melting[282] or when the desolvation overlaps the normal melting or a phase transition, the DSC can become difficult on interpret. Another phenomenon which leads to confusion when the DSC trace is viewed in isolation is stepwise loss of solvent, especially when this occurs in irrational proportions.[492] A simultaneous TGA is of unique

* The term 'true density' is used by other authors in contrast with bulk density to describe what is here called the 'measured density'. For a discussion of different measures of density, see Lowell and Shields.[484]

value in these cases in pinpointing the temperature or temperatures of solvent loss in the particular run. It cannot be necessarily assumed that the form resulting from recrystallization from an 'anhydrous' solvent will be the anhydrate.[494] In contrast, the anhydrous form III of cortisone acetate is reported as only obtainable in the presence of water, whilst the hemihydrate is produced from wet solvents and the monohydrate from dry solvents.[488] Erythromycin dihydrate is said to dehydrate when heated in water at lower temperatures than in air.[417,487]

Whilst X-ray powder diffraction patterns will distinguish a solvate except for the rare examples discussed earlier, they do not display any characteristic features of the solvent as such. By contrast, all of the common solvents have strong and distinct bands in the IR spectrum which generally reappear at the same or similar wavelengths in the solvate.[495] Those bands sensitive to hydrogen bonding will shift, but these shifts are again very characteristic. It could be supposed that except for very low molar ratios of solvent or high molecular mass compounds, IR spectra would be a totally reliable reflection of the presence of a solvate. The bands due to water are often difficult to distinguish from those due to hydrogen-bonded hydroxy groups in the host molecules and there are occasional reports of the indistinguishability of IR spectra of hydrates and other solvates.[365,430,496,497] There is the danger of pumping off the solvent if the sample is prepared as a KBr disk, or of rehydration.[365] Some of the literature reports may well reflect this. Hydrates have occasionally been mistaken for enolic tautomers[498] and frequently for simple polymorphs. A microanalysis, Karl–Fischer or mass loss determination will avoid such misinterpretation. Quantitative DSC has also been used to determine the degree of hydration, based on assumptions of the energy of binding of the water molecules.[499] Solid-state ^{13}C NMR spectra will show bands due to solvate guest molecules but not, of course, to water. The presence of the latter will affect the positions of other signals,[349,500] except presumably in those cases where X-ray diffraction shows no change in packing. In one such case of spectral indistinguishability, resort was made to differences in spin–lattice relaxation times.[346]

The solubility of a hydrate in water or a solvate in its own solvent is always less than that of the unsolvated form, for thermodynamic reasons. On the other hand, the solubility of the hydrate in ethanol or of an ethanolate in water will be always greater than that of the unsolvated form.[463] The vacuum microbalance which measures the mass of a sample under different pressure and humidity conditions is a valuable way of quantifying the stepwise loss and gain of solvent.[501]

Quantitative Aspects

The requirement of analytical control implies reliable methods of detecting, distinguishing and quantifying polymorphs. All the caveats in the examination of polymorphs referred to previously apply with greater force when quantification is required. A method needs to be selected in which the differences between the polymorphs is maximal, yet unlikely to be interfered with by the presence, in particular, of other potential polymorphs or solvates. X-ray powder crystallograpy,[359,393,502] IR,[234,469] NIR[291] and Raman[308] spectroscopy, DSC[234] and DTA[503] have all been investigated for the determination. They have a common feature, namely that the transfer of energy to and through the powdered sample is one of the critical factors with respect to the precision of the measurement. Whilst solution transmission properties are capable of being dealt with theoretically, powder absorption can only be tackled when simplifying assumptions are made.[251,504] The critical features are the particle size and shape of the sample and of the diluent, if one is present, and the homogeneity.[505] It is therefore necessary to grind, and to grind reproducibly. The sample then needs as a minimum requirement to be stable under the grinding conditions. Again microscopy comes into play to check whether the sample is dispersed. Care must be taken to ensure that the sample is quantitatively transferred with the matrix powder, rather than left coating the vessel.[505] This applies particularly to greasy, low melting or plastic crystals. Each compound will present its own problems. It is unlikely that any one technique will prove universally suitable. Because of the small differences that are commonly encountered, realistic limits of quantification even with the use of chemometric methods will probably be 1–10%, dependent on the individual problem. The few examples in the literature on the determination of polymorphic mixtures support most of these contentions. The precautions needed to obtain reliable results in DRIFT spectra have been explored in detail in the case of sulfamethoxazole[234] and of a new anti-inflammatory drug.[226] The potential of X-ray methods have been explored on a model system.[394] Although it has a long history,[359] quantitative X-ray analysis has often been used without attention to possible sources of error. The α-inosine content of mixtures of α- and β-inosine has been investigated by both X-ray powder diffraction and IR spectroscopy.[393] The limit of detection by the X-ray method was decidedly superior to that by IR spectroscopy, but the IR spectra display some curious features. X-ray diffraction has also been used for the detection of α-prasosin in γ-prasosin. Using a profile fitting analysis, a detection limit of 0.5% was achieved.[506] Possible interference from other polymorphs was not considered. The polymorphic composition of cortisone–acetate mixtures and of a candidate hypolipidaemic drug have been determined by Raman spectroscopy,[309] as has chlorpropamide.[507] DTA was found to be superior to X-ray powder diffraction for the determination of fatty acid polymorphs.[503]

If the enthalpy of solution of two polymorphs is sufficiently different, then solution calorimetry can be used for their determination in a mixture.[508,509] The solution obtained by dissolution of one polymorph must be the same by definition, as that obtained from another polymorph of the same substance.[19,462] The difference in heat (enthalpy) of solution therefore determines the relative enthalpies of the polymorphs.[463] the polymorph stable at lower temperatures will have the lower enthalpy (see Fig. 7). The determination can be made indirectly from solubility measurements over a temperature range with the application of the van't Hoff isochore or preferably, directly by measuring the heat of solution in an adiabatic calorimeter.[463] The enthalpy difference will be the same whatever solvent is chosen: therefore it is possible to select one in which adequate solubility is shown. The occurrence of polymorphic change during dissolution will not affect the calorimetric result, as the heat of transition will be summed in the measured heat of dissolution.[463] X-ray powder studies are most commonly used to determine the degree of crystallinity.[510] Solution calorimetry has also been applied to the determination of degree of crystallinity of partly amorphous antibiotics, proving more reliable than X-ray powder methods.[512] The values of crystallinity determined by the two methods were substantially different. The polymorphic composition of phenobarbitone[411] and phenylbutazone[512] by X-ray powder diffraction and by DSC have also been reported to be different, but no explanation of either of these observations has been offered.

Amorphous and Crystalline Solids

There are different schools of thought as to whether amorphous states ought or ought not to be included in the definition of polymorphism.[513] Crystalline solids are distinguished by the presence of periodic pattern repetition in three dimensions

leading to long-range order*: this can be defined as the expectation of finding an identical pattern repeated at regular intervals in any direction throughout the solid.[514] Isotropic liquids and amorphous solids, on the other hand, have no long-range order so the most that can be said about the structure is that the probability of finding a particle distant from any point is given by the particle density.

The neatness of this distinction has been obscured firstly by the existence of liquid crystals[515] with one- or two-dimensional long-range order and incommensurate phases[516] and more recently by the discovery of quasicrystals[517,518] with long-range non-periodic order,[519] often characterized by pseudo five-fold crystallographic axes,[520,521] some of which enjoy greater stability than the equivalent crystalline state.[522] The term non-crystalline therefore does not imply total randomness and there

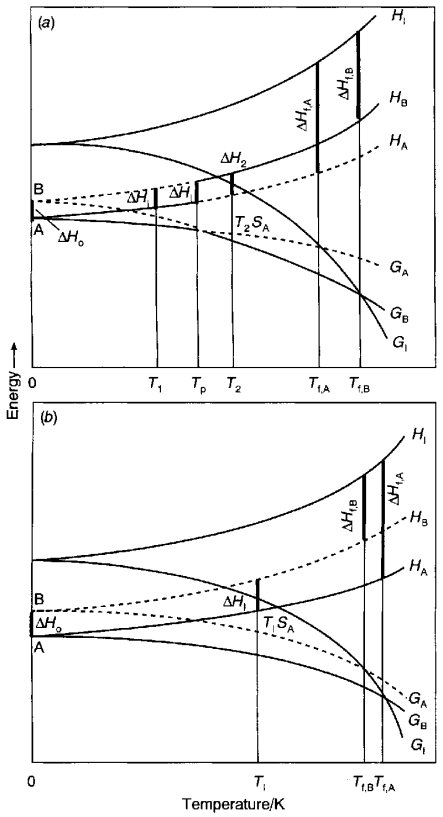


Fig. 7 Energy–temperature diagrams of dimorphic systems. Reproduced from Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1979, **II**, 261 by permission of Springer–Verlag, Vienna (*a*) Enantiotropic systems and (*b*) monotropic systems. (T_p, transition point; T_f, fusion point; H, molar enthalpy; G, molar free energy; S, molar entropy; A, B: crystalline modifications; l, liquid phase).

* More precisely, the definition of a crystalline array is given by:

$$\lim \mid x - x' \mid \rightarrow \infty < \rho(x)\,\rho(x') > = F(x - x')$$

Where $< \rho(x)\,\rho(x') >$ is the density-density correlation between two points x and x' related by a basis factor. Isotropic liquids and amorphous solids, on the other hand, have no long-range order, so the probability of finding a particle distant from x is given by

$$\lim \mid x - y' \mid \rightarrow \infty < \rho(x)\,\rho(x') > \simeq \bar{\rho}^2,$$

where $\bar{\rho}$ is the average particle density.

is an increasing awareness of the possibility of different amorphous structures.[523–524] For example, the amorphous and liquid state are generally considered to represent the same phase, yet there are substances which exist in two amorphous forms separated by what appears to be a phase transition.[131,524] Different amorphous structures may arise from different processes of production.[525,526] In practice many of the organic materials usually described as amorphous are the 'meringues' produced by evaporation of solvent from solutions of substances which do not crystallize readily, or the powders produced by precipitation, transition,[487] freeze drying,[527] spray drying[259,528] or grinding,[449] although the terms microcrystalline or colloidal might be more appropriate, dependent on the size of the crystalline volume.

The concept of an amorphous solid as microcrystallite clusters rather than as a continuous random network or dense random packing has fallen into disfavour, but most of the work has been done with semiconductor materials, and the conclusion may not apply to organic molecular solids. Quasicrystal clusters or 'amorphons' may need to be considered for organic states.[8,9,529] However, there is limited possibility with the analytical tools presently at our disposal of deciding the nature of the detailed structure of amorphous materials. X-ray crystallography has been the most used technique for establishing structure both in terms of long- and short-range order,[9,358,530], although calorimetric methods, vibrational spectroscopy, and increasingly NMR spectroscopy[531,532] provide structural information. Solid-state ^{13}C NMR spectroscopy can show, for example, conformational preferences of molecules even when there is no discernable X-ray pattern.[28,349] Despite this, there has been an almost total neglect of the study of organic amorphous materials. When they are reported they are usually characterized inadequately, if at all. It is not always possible even to ascertain if the reported lack of crystallinity is derived from visual examination, polarized light microscopy or X-ray examination. The significant advances in our understanding of the amorphous solid-state have come recently not in the area of structure but in recognizing the entropic relationships between liquids, crystals and the amorphous state.[533–537]

The most investigated amorphous materials are polymers[364] and inorganic glasses formed by cooling silicate melts[538] although amorphous metals and semiconductors have become the subject of intense research activity in recent years.[320,539] The solids most typically and traditionally regarded as amorphous are those produced by cooling a liquid in the absence of crystallization. During this process the material passes by continual change from a liquid state though the glass transition to a solid state, *via* a more viscous, possibly rubbery or malleable state.[540,541] The term 'supercooled liquid' gives rise to some confusion.[542] A solid is usually arbitrarily defined as a material whose shear viscosity exceeds $10^{14.6}$ poise ($10^{13.6}$ N s m^{-2}).[515] Amorphous materials have therefore been described as having the rheological properties of a solid but the structure of a liquid.[543] Given the limited knowledge of the structure of either liquids or amorphous materials, it may be felt that the latter half of that statement is ambitious. The glass transition temperature is the point at which the melt sets, accompanied by changes in many other properties. There are several methods of investigating the glass transition, including DSC.[544,545] In the idealized case, the DSC trace shows no peak, but only a step representing a change in the heat capacity. This occurs only when the heating rate is the same as the cooling rate which has produced the glass. If the heating rate is faster than the cooling rate, an exotherm is superimposed and if the cooling rate is faster, the usual case, an endotherm is superimposed.[546] These effects are due to strain as a result of the structure failing to reach equilibrium within the experimental time-scale.[9,531,540] In either case the underlying heat capacity change can be

obscured. The temperature of the glass transition is not fixed, but is lower the slower the cooling and heating rates.[422,546] Amorphous solids are always less stable than crystalline forms and so on heating will normally show an exothermic transition to a crystalline phase, although this may be preceded by a glass transition.[242,422] There are a few compounds which, as solids, are only known in the amorphous state and these display only a step corresponding to the glass transition.[547]

Many organic materials can be prepared as glasses by rapid cooling.[162] Molecules with myriad conformational possibilities, particularly polysaccharides and synthetic polymers, tend to occur as amorphous forms. Molecules whose shape precludes a packing density, that is, the ratio of the volume occupied by the molecules as such to the volume of the space in which they reside, of at least 0.60 also solidify most easily as glasses.[85,548] Directed bonds favour the more open structure implied by these low densities, so that multiply hydrogen-bonded molecules, for example, carbohydrates, are notoriously difficult to crystallize.[73,549,540]

The industrial significance of amorphous organic materials has increased enormously. Polymers are, of course, ubiquitous. In the pharmaceutical industry there are compounds, particularly antibiotics, which have long been used in that form because of the difficulty of crystallization and solubility

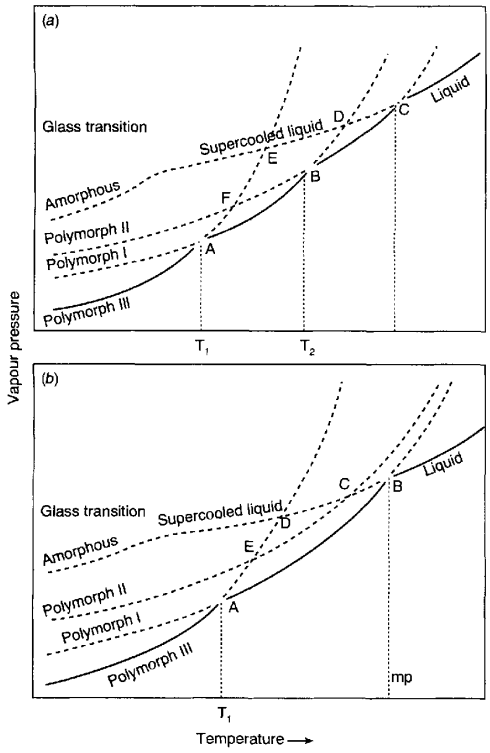


Fig. 8 Vapour pressure–temperature diagrams for trimorphic systems showing that heating and cooling curves can follow different paths *via* different polymorphs. Dashed lines represent metastable equilibria and full lines stable equilibria. The heating cycle in the system shown in (*a*) will probably proceed *via* A, B and C (but see ref. 194 and the caption to Fig. 6 whilst any propensity to undercool might give routes to polymorph III *via* CBF, CDB or CEA. In addition the paths may well end at the amorphous form or polymorphs I or II. Similarly in (*b*) heating will probably proceed *via* A and B, but cooling could follow several paths. In either case spontaneous transitions (vertical drops) are also possible.

problems of the crystalline forms.[43,512,551] More recently attention has been paid to the deliberate use of amorphous forms with a crystallization inhibitor as a means of more rapid drug delivery.[521] Interest in amorphous forms relates not only to active ingredients but to excipients including sugars[550,552] and polymers. In the food industry, carbohydrates often need to be used in amorphous forms and many food constituents exist naturally in an amorphous state.[66–73,553,554]

Amorphous material may result from grinding[449,555], deliberately or inadvertently. The effect of comminution of a crystal is to reduce the long-range periodicity and broaden the signals in X-ray diffraction patterns until in the limit the pattern is so diffuse as to be indistinguishable from that of an amorphous form produced from the melt.[524] On this argument there is no break between a crystalline and an amorphous form. If by contrast, one cools a melt so as to produce a glass, then by this process there is no break between the liquid state and the amorphous form. There may be distinction between the products of the two processes. It may be possible in principle, or in practice in favourable cases, to distinguish between limitingly small crystalline domains and large non-crystalline domains, for example by analysis of the shapes of X-ray powder diffraction lines,[358,405,556] but it would be very artificial to draw the boundaries of the coverage of this review between the two, especially as their properties for all practical purposes are likely to be identical. On balance then, the wider definition is adopted here, intended to allow the reader to decide on the inclusion of amorphous states or otherwise in the term polymorphism. On this wider definition, McCrones' view[1] that every system will be discovered to be polymorphic if studied enough, comes much nearer to verification.

The author thanks numerous colleagues for their help in locating references. The IR spectra in Figs. 1 and 2 and DSC measurement in Fig. 6 were provided by P. Elliott and S. Taramer, University of York. I am grateful to G. Nichols of Pfizer, Sandwich, and Dr. B. Slater of Rhône–Poulenc Rorer, Dagenham, for suggestions about the manuscript and I am particularly indebted to Professor M. Hursthouse, University of Wales, Cardiff, for his comments on crystallographic aspects of the manuscript and for help in so many ways over many years.

References

1 McCrone, W. C. in *Physics and Chemistry of the Organic Solid State*, ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1965, vol. II, p. 725.
2 Deffet, L., *Répertoire des Composés Organiques Polymorphes*, Desoer, Liege, 1942.
3 Buerger, M. J., *Trans. Am. Crystallogr. Assoc.*, 1971, **7**, 1.
4 Bernstein, J. in *Organic Crystal Chemistry*, ed. Garbarczyk, J. B. and Jones, D. W., International Union of Crystallography, Oxford University Press, 1991.
5 Dunitz, J. D., *Pure Appl. Chem.*, 1991, **63**, 177.
6 Bayard, F., Decoret, C., and Royer, J., *Stud. Phys. Theort. Chem.*, 1990, **69**, 211.
7 Rao, C. N. R., and Rao, K. J., *Phase Transitions in Solids*, McGraw–Hill, New York, 2nd edn., 1978.
8 Ubbelhohde, A. R., *The Molten State of Matter*, Wiley, Chichester, 1978.
9 Elliott, S. R., *The Physics of Amorphous Materials*, Longmans, Harlow, 2nd edn., 1990.
10 Verma, A. R., and Krishna, P., *Polytypism and Polymorphism in Crystals*, Wiley, New York, 1966.
11 Amelinkx, S., *Acta Cryst.*, 1955, **8**, 53.
12 Amelinkx, S., *Acta Cryst.*, 1955, **9**, 16.
13 Amelinkx, S., *Acta Cryst.*, 1955, **9**, 217.
14 Dunitz, J. D., *Pure Appl. Chem.*, 1991, **63**, 177.
15 Maliniak, A., Greenbaum, S., Poupko, R., Zimmermann, H., and Lutz, Z., *J. Chem. Phys.*, 1993, **97**, 4832.

16 Bhide, V. G., Agrihotri, S. A., and Chaudra, S., *Indian J. Pure Appl. Phys.*, 1981, **19**, 821.
17 Gruger, A., Romain, F., and Le Calvé, N., *Thermochim. Acta*, 1984, **116**, 57.
18 Reinke, H., Heinz, D., and Hans, M., *J. Chem. Ed.*, 1993, **70**, 101.
19 Campbell, A. N. and Smith, N. O., *Alexander Findlay's The Phase Rule*, Dover, New York, 9th edn., 1951.
20 Sharma, B. D., *J. Chem. Ed.*, 1987, **64**, 404.
21 Sirota, N. N., *Cryst. Res. Technol.*, 1987, **22**, 1343.
22 Sirota, N. N., *Cryst. Res. Technol.*, 1982, **17**, 661.
23 Doyle, B. B., Hukins, D. W. L., Hulnes, J. S., Miller, A. and Woodhead-Galloway, J., *J. Mol. Biol.*, 1975, **91**, 79.
24 Oxford, G. S., and Rollinson, D., *Protein Polymorphism: Adaptive and Taxonomic Significance*, Academic Press, London, 1983.
25 Jackson, A. P., Maxwell, A., and Wigley, D. B., *J. Mol. Biol.*, 1991, **217**, 15.
26 Knobler, C. M., and Desai, R. C., *Annu. Rep. Phys. Chem.*, 1992, **43**, 207.
27 Chapman, D., Urbino, J. and Keough, K. M., *J. Biol. Chem.*, 1974, **249**, 2512.
28 Saito, H., *Yuki Gosei Kagaku Kyokaishi*, 1992, **50**, 488.
29 Burden, C. H., PhD Thesis, University of Leeds, 1987.
30 Corradini, P. and Guerra, G., *Adv. Polymer Sci.*, 1992, **100**, 182.
31 *CRC Handbook of Chemistry and Physics*, ed. Weast, R. C., CRC Press, Cleveland, 61st edn., 1981, p. B-69.
32 Wilson, A. J. C., *Acta Cryst., Sect. A*, 1988, **44**, 715.
33 Wilson, A. J. C., *Acta Cryst., Sect. A*, 1990, **A46**, 742.
34 Kuhnert-Brandstätter, M. and Riedmann, M., *Mikrochim. Acta*, 1987, **II**, 107.
35 Burger, A., *Acta Pharm. Tech.*, 1979, **Suppl. 7**, 107.
36 Burger, A., in *Topics in Pharmaceutical Sciences*, ed. Bremer, D. D., and Speiser, P., Elsevier, Amsterdam, 1983, p. 347.
37 Halebian, J. K., and McCrone, W. C., *J. Pharm. Sci.*, 1969, **58**, 911.
38 Halebian, J. K., *J. Pharm. Sci.*, 1975, **64**, 1269.
39 Kuhnert-Brandstätter, M., *Thermomicroscopy in the Analysis of Pharmaceuticals*, Pergamon Press, Oxford, 1974.
40 Byrn, S. R., *Solid State Chemistry of Drugs*, Academic Press, New York, 1982.
41 Aguiar, A. J., Krc, J., Kinkel, A. W., and Samyn, J. C., *J. Pharm. Sci.*, 1967, **56**, 847.
42 Burger, A., *Pharm. Int.*, 1982, **3**(5), 158.
43 Mullins, J. D. and Macek, T. J., *J. Pharm. Sci.*, 1960, **49**, 245.
44 Bavin, M., *Chem. Ind.*, 1989, 527.
45 Nakamachi, H., Yamaoka, T., Wada, Y. and Miyaka, F., *Chem. Pharm. Bull.*, 1982, **30**, 3685.
46 Chan, H. K., and Doelker, E., *Drug Dev. Ind. Pharm.*, 1985, **11**, 315.
47 Macle, C. H. G., and Grant, D. J. W., *Pharm. Int.*, 1986, **September**, 233.
48 Thoma, K., and Serno, P., *Deut. Apotek. Z.*, 1984, **124**(43), 2162.
49 Liversidge, G. G., Grant, D. J. W., and Padfield, J. M., *Anal. Proc.*, 1982, 549.
50 Woodard, G. D., and McCrone, W. C., *J. Appl. Crystallog.*, 1975, **8**, 342.
51 Kohlbeck, J. A., *Microscope*, 1982, **30**, 249.
52 Teetsov, A., and McCrone, W. C., *Microscope*, 1965, **15**, 13.
53 Kendall, D. N., *Anal. Chem.*, 1952, **24**, 382.
54 Susich, G., *Anal. Chem.*, 1950, **22**, 425.
55 Ebert A. A., and Gottlieb, H. B., *J. Am. Chem. Soc.*, 1952, **74**, 2806.
56 Whitaker, A., *J. Soc. Dyers Colour*, 1992, **108**, 282.
57 Thomas, A., and Ghode, P. M., *Paintindia*, 1989, **39**, 25.
58 Kuhnert-Brandsttter, M. and Riedmann, M., *Mikrochim. Acta*, 1989, **I**, 373.
59 Warwicker, J. O., *J. Text. Inst.*, 1959, **50**, T443.
60 Etter, M. C., Kress, R. B., Bernstein, J., and Cash, D. J., *J. Am. Chem. Soc.*, 1984, **106**, 6921.
61 *The Physical Chemistry of Lipids*, ed. Small, D., Plenum, New York, 1986.
62 *Crystallization and Polymorphism of Fats and Fatty Acids*, ed. Garti, N. and Sato, K., Marcel Dekker, New York, 1988.
63 Gupta, S., *J. Am. Oil Chem. Soc.*, 1991, **94**, 450.
64 Srivastara, S. P., Handoo, J., Agrawal, K. M., and Joshi, G. C., *J. Phys. Chem. Solids*, 1993. **54**, 639.
65 Ungar, G., *J. Phys. Chem.*, 1983, **87**, 689.
66 Cebula, D. J., and Ziegleder, G., *Fett Wiss. Technol.*, 1993, **95**, 340.
67 deMan, L., Shen, C. F., and deMan, J. F., *J. Am. Oil Chem. Soc.*, 1991, **68**, 70.
68 Saltmarsh, M., and Labuza, T. P., *J. Food Sci.*, 1980, **45**, 1231.
69 Roos,Y., and Karel, M., *Int. J. Food Sci. Technol.*, 1991, **26**, 55.
70 Herrington, T. M. and Branfield, A. C., *J. Food Technol.*, 1984, **19**, 427.
71 Cammenga, H. K., and Steppuhn, I. D., *Thermochim. Acta*, 1993, **229**, 253.
72 Siniti, M., Carré, J., Bastide, J. P., Létoffé, J. M., and Claudy, P., *Thermochim. Acta*, 1993, **224**, 105.
73 Imberty, A., Buleon, A., Vihn, T., and Perez, S., *Starch/Staerke*, 1991, **43**, 375.
74 Bothe, H., and Cammenga, H. K., *J. Therm. Anal.*, 1979, **16**, 267.
75 Pirttimäki, J., Laine, E., Ketolainen, J., and Parrien, P., *Int. J. Pharm.*, 1993, **95**, 93.
76 Gunstone, F. D., Harwood, J. L., and Padley, F. B., *The Lipid Handbook*, Chapman and Hall, London, 2nd edn., 1994.
77 Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1980, **I**, 17.
78 Bavin, P. M. G., Sly, J. C. P., Tovey, G. D., and Ward, R. J., *Ger. Offenlegung* 1978, **742**, 2, 531.
79 Cholerton, T. J., Hunt, J. H., Klinkert, G., and Martin-Smith, M., *J. Chem. Soc., Perkin Trans. 2*, 1984, 1761.
80 Crookes, D. L., *UK Pat.* 2084580B, 1982.
81 *Guardian*, September 9, 1994.
82 Reutzel, S. M., and Etter, M. C., *J. Phys. Org. Chem.*, 1992, **5**, 44.
83 Hammond, R. B., Roberts, K. J., Singh, D., and York, P., in preparation.
84 Bernstein, J., *J. Phys. D; Appl. Phys.*, 1993, **26**, 1366.
85 Bernstein, J., and Chosen, E., *Mol. Cryst. Liquid Cryst.*, 1988, **164**, 213.
86 Sato, K., *J. Phys. D; Appl. Phys.*, 1993, **26**, B77.
87 Desiraju, G. R., *Crystal Engineering*, Elsevier, Amsterdam, 1989.
88 Gavezzotti, A., *Acc. Chem. Res.*, 1994, **27**, 309.
89 Mannock, D. A., Lewis, R. N. A. H., Sen, A. and McElhaney, R. N., *Biochemistry*, 1988, **27**, 6852.
90 Hartshorne, N. H., and Stuart, A., *Crystals and the Polarising Microscope*, Edward Arnold, London, 4th edn., 1970, p. 20.
91 Stobbe, H., *Ber. Deut. Chem. Ges.*, 1905, **44**, 2732.
92 Bernstein, J., and Hagler, A. J., *J. Am. Chem. Soc.*, 1978, **100**, 673.
93 Bancroft, *J. Phys. Chem.*, 1898, **2**, 143.
94 Bancroft, *J. Phys. Chem.*, 1899, **3**, 44.
95 Rao, B. R., Rao, G. R., and Avadharmlu, A. B., *J. Sci. Ind. Res.*, 1987, **46**, 450.
96 Byrn, S. R., Curtin, D. Y., and Paul, I. C., *J. Am. Chem. Soc.*, 1972, **94**, 890.
97 Elguero, J., Marzin, C., Katritzki, A. R., and Linda, P., *The Tautomerism of Heterocycles*, Academic Press, London, 1966.
98 Desiraju, G. R., *J. Chem. Soc., Perkin Trans. 2*, 1983, 1025.
99 Gassim, A. E. H., Takla, P. G., and James K. C., *Int. J. Pharm.*, 1986, **34**, 23.
100 Costakis, E., Canone, P., and Tsala, G., *Can. J. Chem.*, 1969, **47**, 4483.
101 Annese, M., Corradi, A. B., Forlani, L., Rizzoli, C., and Sgarabotto, P., *J. Chem. Soc., Perkin Trans. 2*, 1994, 614.
102 Terol, A., Pauvert, B., Bouasseb, A., Chevallet, P., and Cassaneus, G., *J. Therm. Anal.*, 1992, **38**, 1545.
103 Kehrmann, F., and Matusinsky, Z., *Ber. Deut. Chem. Ges.*, 1912, **45**, 3498.
104 Elguero, J., Marzin, C., Katritzki, A. R., and Linda, P., *The Tautomerism of Heterocycles*, Academic Press, London, 1966, p. 57.
105 Cleverly, B., and Williams, P. P., *Tetrahedron*, 1959, **7**, 277.
106 Kuhnert-Brandstätter, M., and Sollinger, H. W., *Mikrochim. Acta*, 1990, **III**, 247.
107 Katrusiak, A., *J. Mol. Struct.*, 1992, **269**, 329.
108 Elguero, J., Marzin, C., Katritzki, A. R., and Linda, P., *The Tautomerism of Heterocycles*, Academic Press, London, 1966, p. 5.
109 Threlfall, T. L., PhD Thesis, University of London, 1972.
110 Ojala, W. H., and Etter, M. C., *J. Am. Chem. Soc.*, 1992, **114**, 10288.
111 Bernstein, J., *Acta Cryst Sect. B*, 1991, **47**, 1004.

112 Bernstein, J., *Acta Cryst Sect. C*, 1988, **44**, 900; Etter, M. C., *Acc. Chem. Res.*, 1990, **23**, 120; Aakeroy, C. B. and Seddon, K. R., *Chem. Soc. Rev.*, 1993, **22**, 397.
113 Bernstein, J., in *Organic Solid State Chemistry, Studies in Organic Chemistry, Vol. 32*, ed. Desiraju, G. R., Elsevier, Amsterdam, 1987.
114 Duax, W. L., *J. Chem. Ed.*, 1988, **65**, 502.
115 Blake, A. J., Gould, R. O., Halerow, M. A., and Schroeder, M., *Acta Cryst., Sect. B*, 1993, **49**, 773.
116 Fletton, R. A., Harris, R. K., Kenwright, A. M., Lancaster, R. W., Packer, K. J., and Sheppard, N., *Spectrochim. Acta, Part A*, 1987, **43**, 1111.
117 Phillips, D. C., *Acta Cryst.*, 1956, **9**, 237.
118 Senge, M. O., Hope, H., and Smith, K. M., *J. Chem. Soc., Perkin Trans. 2*, 1993, 11.
119 Barikigia, K. M., Renner, M. W., Furentio, L. R., Medforth, C. J., Smith, K. M., and Fajer, J., *J. Am. Chem. Soc.*, 1993, **115**, 3627.
120 Williams, P. P., *Acta Cryst., Sect. B*, 1974, **30**, 12.
121 McDowell, J. J. H., *Acta Cryst., Sect. B*, 1977, **33**, 5.
122 Desiraju, G. R., Paul, I. C., and Curtin, D. Y., *J. Am. Chem. Soc.*, 1977, **99**, 1594.
123 Perrin, R., Lamartine, M., Perrin, R., and Thozet, A., in *Organic Solid State Chemistry, Studies in Organic Chemistry, Vol. 32* ed. Desiraju, G. R., Elsevier, Amsterdam, 1987.
124 Brock, C. P., Schweizer, W. B., and Dunitz, J. D., *J. Am. Chem. Soc.*, 1991, **113**, 9811.
125 Eistert, B., Weygand, F., and Csenides, E., *Chem. Ber.*, 1952, **85**, 164.
126 Okada, Y., Takebayashi, T., Mashimoto, M., Kasiga, S., Sato, S., and Tamura, C., *J. Chem. Soc., Chem. Commun.*, 1983, 784.
127 Wilson, K. R., and Pincock, R. E., *Can. J. Chem.*, 1977, **55**, 889.
128 Matthews, T. H., Paul, I. C., and Curtin, D. Y., *J. Chem. Soc., Perkin Trans. 2*, 1991, 113.
129 McBride, J. M., *Angew. Chem., Int. Ed. Eng.*, 1989, **28**, 377.
130 David, R., and Giron, D., *Handbook of Powder Technology*, 1994, **9**, 193.
131 Stezowski, J. J., Biedermann, P. U., Hildenbrand, T., Dorsch, J. A., Eckhardt, C. J., and Agranat, I., *J. Chem. Soc. Chem. Commun.*, 1993, 213.
132 Takasuka, M., Nakei, H. and Shiro, M., *J. Chem. Soc., Perkin Trans. 2*, 1982, 106.
133 Zhang, Z., Rettig, S. J. and Orvig, C., *Can. J. Chem.*, 1992, **70**, 763.
134 Nelson, W. O., Karpishin, T. B., Rettig, S. J., and Orvig, C., *Can. J. Chem.*, 1988, **66**, 123.
135 Kessler, H., Zimmermann, G., Foerster, H., Engel, J., Oepen, G., and Sheldrick, W. S., *Angew. Chem., Int. Ed. Eng.*, 1981, **20**, 1053.
136 Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1979, **II**, 259.
137 Mnyukh, Y. U., and Panfilova, N. A., *J. Phys. Chem. Solids.*, 1973, **34**, 159.
138 McCauley, J. A., Varsolona, R. J., and Levorse, D. A., *J. Phys. D; Appl. Phys.*, 1993, **26**, B85.
139 Westrum, E. R. and McCullough, J. P., in *Physics and Chemistry of the Organic Solid State*, ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1963, vol. 1, p. 76.
140 Krc, J., *Microscope*, 1977, **28**, 25.
141 Gunning, S. R., Freeman, M., and Stead, J. A., *J. Pharm. Pharmacol.*, 1976, **28**, 758.
142 Bar, I., and Bernstein, J., *J. Pharm. Sci.*, 1985, **74**, 255.
143 Cleverley, B., and Williams, P. P., *Chem. Ind.*, 1959, 49.
144 Smakula, E., Gori, A., and Wotiz, H. H., *Spectrochim. Acta*, 1957, **9**, 346.
145 Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1979, **II**, 271.
146 Threlfall, T. L., and Slater, B. J., in preparation.
147 Sukenik, C. N., Bonapace, J. A., Mandel, N. J., Land, P. Y., Wood, G., and Bergin, R. J., *J. Am. Chem. Soc.*, 1977, **99**, 851.
148 Katritsky, A. R., and Lagowski, J. M., *Adv. Heterocyclic Chem.*, 1963, **1**, 382.
149 Iwatsu, F., *J. Phys. Chem.*, 1988, **92**, 1678.
150 Dumas, J. P., Tounsi, F., and Babin, L., *J. Dispersion Sci. Technol.*, 1987, **8**, 29.
151 Mesley, R. J., Clements, R. L., Flaherty, B., and Goodhead, K., *J. Pharm. Pharmacol.*, 1968, **20**, 239.
152 Kuhnert-Brandstätter, M., and Moser, I., *Mikrochim. Acta*, 1979, **I**, 125.
153 Mnyukh, Y. V., and Petropavlov, N. N., *J. Phys. Chem. Solids*, 1972, **33**, 2079.
154 West, A. R., *Solid State Chemistry and its Applications*, Wiley, Chichester, 1984.
155 Theocharis, C. R., and Jones, W., *J. Chem. Soc. Chem. Commun.*, 1984, 369.
156 Theocharis, C. R., Jones, W., and Rao, C. N. R., *J. Chem. Soc. Chem. Commun.*, 1984, 1291.
157 Jones, W., Thomas, J. M., and Williams, J. O., *Philos. Mag.*, 1975, **32**, 1.
158 Kuhnert-Brandstätter, M., and Sollinger, H. W., *Mikrochim. Acta*, 1990, **III**, 233.
159 Barbour, R. H., Freer, A. A., and MacNichol, D. D., *J. Chem. Soc., Chem. Commun.*, 1983, 362.
160 Kuhnert-Brandstätter, M., and Moser, I., *Mikrochim. Acta*, 1981, **I**, 421.
161 Kuhnert-Brandstätter, M., and Friedl, L., *Mikrochim. Acta*, 1979, **II**, 97.
162 Burger, A., and Schulte, K., *Arch. Pharm.*, 1981, **314**, 398.
163 Carter, P. W., and Ward, M. D., *J. Am. Chem. Soc.*, 1994, **116**, 769.
164 Royer, J., Decoret, C., Tinland, B., Perrin, M., and Perrin, R., *J. Phys. Chem.*, 1989, **93**, 3393.
165 Etter, M., Britton, D., and Reutzel, S. M., *Acta Cryst., Sect. C*, 1991, **47**, 556.
166 Di, L., and Small, D. L., *J. Lipid Res.*, 1993, **34**, 1611.
167 Dunitz, J. D., and Bernstein, J., *Acc. Chem. Res.*, 1995, **28**, 193.
168 Pfeiffer, R. R., *J. Pharm. Pharmacol.*, 1971, **23**, 75.
169 Anwar, J., Tarling, S. E., and Barnes, P., *J. Pharm. Sci.*, 1989, **78**, 337.
170 Harris, R. K., Kenwright, A. M., Say, B. J., Yeung, R. R., Fletton, R. A., Lancaster, R. W. and Mangrove, G. L., *Spectrochim. Acta, Part A*, 1990, **46A**, 927.
171 Snévity, D., Vancso, J., and Rutledge, G. C., *Macromolecules*, 1992, **25**, 7037.
172 Kaneko, F., Sakashita, H., Kobayashi, M., and Suzuki, M., *J. Phys. Chem.*, 1994, **98**, 3801.
173 Jones, W., and Thomas, J. M., *Prog. Solid State Chem.*, 1980, **12**, 101.
174 Lourdin, D., Roux, A. H., Grolier, J. P. E., and Butsin, J. M., *Thermochim. Acta*, 1992, **204**, 99.
175 Lumley, C. E. and Walker, S. R., in *Medicines: Regulation, Research and Risk*, ed. Griffin, J. P., Greystone, Antrim, 1989, p. 157.
176 Kofler, L., and Kofler, A., *Thermomikromethoden*, Wagner, Innsbruck, 1952.
177 Hartshorne, N. H., and Stuart, A., *Crystals and the Polarising Microscope*, Edward Arnold, London, 4th edn., 1970.
178 Kuhnert-Brandstätter, M., in *Comprehensive Analytical Chemistry*, ed. Svehla, G., Elsevier, Amsterdam, 1982, vol. XVI.
179 McCrone, W. C., *Fusion Methods in Chemical Microscopy*, Interscience, New York, 1957.
180 Bloss, F. D., *Crystallography and Crystal Chemistry*, Holt, Reinhart and Winston, New York, 1971.
181 Jordan, D. D., *J. Pharm. Sci.*, 1993, **82**, 1269.
182 Ojena, S. M., and DeForest, P. R., *J. Forensic Sci. Soc.*, 1972, **12**, 315.
183 Ojena, S. M., and DeForest, P. R., *J. Forensic Sci.*, 1972, **17**, 409.
184 Holik, A. S. and Taylor, D. F., *Microscope*, 1977, **28**, 265.
185 Bloss, F. D., *The Spindle Stage*, Cambridge University Press, 1981.
186 Hartshorne, N. H., *Microscope*, 1975, **23**, 177.
187 Hartshorne, N. H., *Microscope*, 1976, **24**, 102.
188 Hartshorne, N. H., *Microscope*, 1976, **24**, 215.
189 McCrone, W. C., *Microscope*, 1991, **39**, 43.
190 Watanabe, A., Tanaku, Y. and Tanaku, Y., *Chem. Pharm. Bull.*, 1977, **25**, 2239.
191 Saylor, C. P., *Anal. Chem.*, 1975, **47**, 1114.
192 Chao, E. C. T., *Am. Mineral.*, 1976, **61**, 212.
193 Craven, B. M., and Vizzici, E. A., *Acta Cryst., Sect. B*, 1971, **27**, 1917.
194 Kuhnert-Brandstätter, M., *Thermomicroscopy in the Analysis of Pharmaceuticals*, Pergamon Press, Oxford, 1974, p. 19.
195 McCrone, W. C., *Discuss. Faraday Soc.*, 1949, **5**, 158.
196 Wiedermann, H. G., and Bayer, G., *J. Therm. Anal.*, 1985, **30**, 1273.
197 Burger, A., *Pharm. uns. Zeit*, 1982, **11**, 177.

198 Kuhnert-Brandstätter, M., and Sollinger, H. W., *Mikrochim. Acta*, 1990, **III**, 137.
199 Richardson, M. F., Yang, Q. C., Novotny-Bregger, E., and Dunitz, J. D., *Acta Cryst., Sect. B*, 1990, **46**, 653.
200 Kuhnert-Brandstätter, M., and Proell, F., *Mikrochim. Acta*, 1983, **III**, 287.
201 Reffner, J. A., and Ferrillo, R. G., *J. Therm. Anal.*, 1988, **34**, 19.
202 Cammenga, H. K., and Hemminger, W. F., *Labo*, 1990, **21**, 7.
203 Willis, H. A., van der Maas, J. H., and Miller, R. G. J., *Laboratory Methods in Vibrational Spectroscopy*, Wiley, Chichester, 3rd edn., 1987.
204 Duyckaerts, G., *Analyst*, 1959, **84**, 201.
205 Rosenkrantz, H., and Zablow, L., *Anal. Chem.*, 1953, **25**, 1025.
206 Baker, A. W., *J. Phys. Chem.*, 1957, **61**, 450.
207 Sharpless, N. E. and Gregory, D. A., *Appl. Spectrosc.*, 1963, **17**, 47.
208 Griesser, U. J., and Burger, A., *Sci. Pharm.*, 1993, **61**, 113.
209 Kobayashi, M., Matsumoto, Y., Ishida, A., Ute, K., and Hatada, K., *Spectrochim. Acta, Sect. A*, 1994, **50**, 1605.
210 Farmer, V. C., *Spectrochim. Acta*, 1957, **8**, 374.
211 Stewart, J. E., *J. Chem. Phys.*, 1957, **26**, 248.
212 Kuhnert-Brandstätter, M., and Riedmann, M., *Mikrochim. Acta*, 1989, **I**, 81.
213 Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1979, **II**, 271.
214 Free, M. L., and Miller, J. D., *Appl. Spectrosc.*, 1994, **48**, 891.
215 De Faubert Maunder, M. J., *Practical Hints on Infrared Spectroscopy*, Adam Hilger, London, 1971.
216 Potts, W. F., *Chemical Infrared Spectroscopy*, Wiley, New York, 1963, vol. 1.
217 Bradley, K. B., and Potts, W. J., *Appl. Spectrosc.*, 1958, **12**(3), 77.
218 Roberts, G., *Anal. Chem.*, 1957, **29**, 911.
219 White, R. G., *Handbook of Industrial Infrared Analysis*, Plenum, New York, 1964.
220 Fuller, M. P., and Griffiths, P. R., *Anal. Chem.*, 1978, **50**, 1906.
221 Krishnan, K., and Ferraro, J. R. in *Fourier Transform Infrared Spectroscopy*, ed. Ferraro, J. R. and Basile, L. J., Academic Press, New York, 1982, vol. 3, p. 149.
222 Harrick, N. J., *Internal Reflection Spectroscopy*, Wiley, New York, 1967.
223 Mirabella, F. M., *Internal Reflectance Spectroscopy*, Marcel Dekker, New York, 1992.
224 Kuhnert-Brandstätter, M., and Riedmann, M., *Mikrochim. Acta*, 1989, **II**, 173.
225 Schutte, C. J. H., and Paul, S. O., *S. Afr. Tydskr. Chem.*, 1986, **39**, 252.
226 Roston, D. A., Walters, M. C., Rhinebarger, R. R., and Ferro, L. J., *J. Pharm. Biomed. Anal.*, 1993, **11**, 293.
227 Barker, S. A., Bourne, E. J., Weigl, M., and Whiffen, D. H., *Chem. Ind.*, 1956, 318.
228 Ford, M. A., and Wilkinson, G. R., *J. Sci. Instr.*, 1954, **31**, 338.
229 Price, W. C., and Tetlow, K. S., *J. Chem. Phys.*, 1948, **16**, 1157.
230 Kuhnert-Brandstätter, M., and Bachleitner-Hoffman, F., *Spectrochim. Acta, Part A*, 1971, **27**, 191.
231 Kirov, N., Fontana, M. P., and Cavatorte, F., *J. Mol. Struct.*, 1980, **59**, 147.
232 Gruger, A., Romain, F., and le Calvé, N., *Thermochim. Acta*, 1984, **116**, 85.
233 Neville, G. A., Beckstead, H. D., and Shurvell, H. F., *J. Pharm. Sci.*, 1992, **81**, 1141.
234 Hartauer, K. J., Miller, E. S., and Guillory, J. K., *Int. J. Pharm.*, 1992, **85**, 163.
235 Yeboah, S. A., Wong, S-H., and Griffiths, P. R., *Appl. Spectrosc.*, 1984, **38**, 259.
236 Brimmer, P. J., and Griffiths, P. R., *Appl. Spectrosc.*, 1988, **42**, 242.
237 TeVrucht, M. L. E., and Griffiths, P. R., *Appl. Spectrosc.*, 1989, **43**, 1492.
238 Marabella, L. J., *Appl. Spectrosc. Revs.*, 1985, 21, 45.
239 Ibrahim, G., Pisano, F., and Bruno, A., *J. Pharm. Sci.*, 1977, **66**, 669.
240 Hartauer, K. J., and Guillory, J. K., *Pharm. Res.*, 1989, **6**, 608.
241 Katon, J. E., and Sommers, A. J., *Anal. Chem.*, 1992, **64**, 931A.
242 Lagas, M., and Lerk, C. F., *Int. J. Pharm.*, 1981, **8**, 11.
243 Burger, A., and Dialer, R. D., *Pharm. Acta Helv.*, 1983, **58**, 72.
244 Vidine, D. W., in *Fourier Transform Infrared Spectroscopy* ed. Ferraro, J. R., and Basile, L. J., Academic Press, New York, 1982, vol. 3.
245 Graham, J. A., Grim, N. M., and Fateley, W. G., in *Fourier Transform Infrared Spectroscopy*, ed. Ferraro, J. R. and Basile, L. J., Academic Press, New York, 1985, vol. 4, p. 346.
246 Belton, P. S., Saffron, A. M., and Wilson, R. H., in *Analytical Applications of Spectroscopy*, ed. Creaser, C. S. and Davies, A. M. C., The Royal Society of Chemistry, London, 1988.
247 Rockley, N. L., Woodard, M. K., and Rockley, M. G., *Appl. Spectrosc.*, 1984, **38**, 329.
248 Ashizawa, K., *J. Pharm. Sci.*, 1989, **78**, 256.
249 Griffiths, P. R., and Fuller, M. P. in *Advances in Spectroscopy*, ed. Clark, R. J. H., and Hester, R. E., 1982, **9**, 63.
250 Huvenne, R., Depecker, C., and Legrand, P., *S.T.P. Pharma*, 1989, **5**, 350.
251 Chalmers, J. M., and Mackenzie, M. W. in *Advances in Applied Fourier Transform Infrared Spectroscopy*, ed. Mackenzie, M. W., Wiley, New York, 1988.
252 Brickell, W. S., *Proc. Anal. Div. Chem. Soc.*, 1978, 343.
253 Yano, J., Ueno, S., Arishima, T., Sagi, N., Kaneko, K., and Kobayashi, M., *J. Phys. Chem.*, 1993, **97**, 12967.
254 Kaneko, F., Sakashita, H., and Kobayashi, M., *Acta Cryst., Sect. C*, 1994, **50**, 245, 247.
255 Kaneko, K., Shirai, O., Miyamoto, H., Kobayashi, M., Kitagawa, Y., Matsuura, Y., and Sasaki, M., *J. Phys. Chem.*, 1994, **98**, 2185.
256 Kuhnert-Brandstätter, M., and Junger, E., *Spectrochim. Acta, Part A*, 1976, **23**, 1453.
257 Ostwald, W., *Z. Phys. Chem.*, 1897, **22**, 306.
258 Cocks, G. G., and Jelley, E. E., in *Physical Methods of Chemistry*, ed. Weissberger, A., and Rossiter, B. W., Wiley, New York, 1972, p. III
259 Matsuda, Y., and Tatsumi, E., *Int. J. Pharm.*, 1990, **60**, 11.
260 George, W. O., Hassid, D. V., and Maddams, W. F., *J. Chem. Soc., Perkin Trans. 2*, 1973, 957.
261 Snyder, S. G., Maroncelli, S., and Hallmark, V. M., *J. Phys. Chem.*, 1986, **90**, 5623.
262 Chapman, D., *J. Chem. Soc.*, 1958, 3186.
263 Rao, G. R., and Zerbi, G., *Appl. Spectrosc.*, 1984, **38**, 795.
264 Gu, W., *Anal. Chem.*, 1993, **65**, 827.
265 White, R. L., *Appl. Spectrosc.*, 1993, **47**, 1492.
266 Bergin, F. J., *Appl. Spectrosc.*, 1989, **43**, 511.
267 Humecki, H. J., *Practical Guide to Infrared Microspectrometry*, Marcel Dekker, New York, 1988.
268 Nguyen, N. A. T., Ghosh, S., Gatlin, L. A., and Grant, D. J. W., *J. Pharm. Sci.*, 1993, **83**, 1116.
269 *The Design, Sampling, Handling and Application of Infrared Microscopes*, ASTM Special Technical Publication 9494, ed. Roush, P. B., American Society for Testing and Materials, Philadelphia, 1987.
270 Marshall, A. G., *Fourier, Hadamard and Hilbert Transformations in Chemistry*, Plenum, New York, 1982.
271 Marshall, A. G., *Chemom. Intell. Lab. Syst.*, 1988, **3**, 261.
272 Turner, P. M., *Anal. Proc.*, 1986, **23**, 268.
273 Okada, Y., Takebayashi, T., and Sato, S., *Chem. Pharm. Bull.*, 1989, **37**, 5.
274 Kellner, R., Kuhnert-Brandstätter, M., and Malissa, H., *Mikrochim. Acta*, 1988, **III**, 153.
275 Kuhnert-Brandstätter, M., and Moser, I., *Mikrochim. Acta*, 1980, **II**, 333.
276 Todori, K., Sano, K., and Mori, Y., *Spectrochim. Acta, Part B*, 1994, **50**, 1201.
277 Geiger, W., *Spectrochim. Acta*, 1963, **10**, 655.
278 Kuhnert-Brandstätter, M., Wurian, I., and Geiler, M., *Sci. Pharm.*, 1982, **50**, 91.
279 Kuhnert-Brandstätter, M., and Wurian, I., *Sci. Pharm.*, 1982, **50**, 3.
280 Borka, L., *Acta Pharm. Suec.*, 1977, **14**, 205.
281 Bettinetti, G., Giordiano, F., Fronza, G., Italia, A., Pellegrata, R., Villa, M., and Ventura, P., *J. Pharm. Sci.*, 1990, **79**, 470.
282 Burger, A., and Lettenbichler, A., *Pharmazie*, 1993, **48**, 262.
283 Kaye, W., *Spectrochim. Acta*, 1954, **6**, 257.
284 Osborne, B. G., Fearn, T., and Hindle, P. H., *Practical NIR Spectroscopy*, Longmans, Harlow, 2nd edn., 1993.
285 Mark, H., *Principles and Practice of Spectroscopic Calibration*, Wiley, Chichester, 1991.
286 Smith, H. J., and Carl, R. T., *Appl. Spectrosc.*, 1989, **43**, 865.
287 Ollinger, J. M., and Griffiths, P. R., *Anal. Chem.*, 1988, **60**, 2427.

288 Barnes, R. J., Dhanoa, M. S. and Lister, S. J., *Appl. Spectrosc.*, 1989, **43**, 772.
289 Aucott, L. S., Garthwaite, P. H., and Buckland, S. T., *Analyst*, 1988, **113**, 1849.
290 Miller, C. E., and Honigs, D. E., *Spectroscopy*, 1989, **4**, 44.
291 Gimet, R., and Luong, A. T., *J. Pharm. Biomed. Anal.*, 1987, **5**, 205.
292 Colthup, N. B., Daly, L. H., and Wiberley, S. E., *Introduction to Infrared and Raman Spectroscopy*, Academic, New York, 2nd edn., 1975.
293 Grasselli, J. G., and Bulkin, B. J., *Analytical Raman Spectroscopy*, Wiley, New York, 1991.
294 Hendra, P. J., Jones, C., and Warnes, J., *Fourier Transform Raman Spectroscopy*, Ellis Horwood, 1991.
295 Hirschfeld, T., and Chase, B., *Appl. Spectrosc.*, 1986, **40**, 133.
296 Bergin, F. J., and Shurvell, H. F., *Appl. Spectrosc.*, 1989, **43**, 516.
297 Messerschmidt, R. G., and Chase, B., *Appl. Spectrosc.*, 1989, **43**, 11.
298 Schrader, B., Hoffman, A., and Keller, S., *Spectrochim. Acta, Part A*, 1991, **47**, 1135.
299 Loewenschuss, A., and Moss, A., *Appl. Spectrosc.*, 1982, **36**, 183.
300 Chase, B., *Appl. Spectrosc.*, 1994, **48**, 14A.
301 Cutmore, E. A., and Skett, P. W., *Spectrochim. Acta, Part A*, 1993, **49**, 809.
302 Wright, J. D., *Molecular Crystals*, Cambridge University Press, Cambridge, 1987.
303 Cannon, C. F., *Spectrochim. Acta*, 1958, **10**, 341.
304 Schuster, P., Zundel, G., and Sandorfy, C., *The Hydrogen Bond*, North Holland, Amsterdam, 1976.
305 Paul, S. O., Schutte, C. J. H., and Hendra, P. J., *Spectrochim. Acta, Part A*,, 1990, **46**, 323.
306 Zimba, C. G., Hallmark, V. M., Swalen, J. D. and Rabolt, J. F., *Appl. Spectrosc.*, 1987, **41**, 721.
307 Waters, D. N., *Spectrochim. Acta, Part A*, 1994, **50**, 1833.
308 Deeley, C. M., Spragg, R. A., and Threlfall, T. L., *Spectrochim. Acta, Part A*, 1991, **47**, 1217.
309 Tudor, A. H., Davies, M. C., Melia, C. D., Lee, D. G., Mitchell, R. C., Hendra, P. J., and Church, S. J., *Spectrochim. Acta, Part A*, 1991, **47**, 1389.
310 Kobayashi, M., Kobayashi, T., Itoh, Y., and Sato, K., *J. Chem. Phys.*, 1984, **80**, 2897.
311 Ishii, K., Kawahara, M., Yakasaki, Y., Hibino, Y., and Nakayana, H., *J. Phys. D: Appl. Phys.*, 1993, **26**, B193.
312 Davey, R. J., Maginen, S. J., Andrews, E. J., Black, S. N., Buckley, A. M., Cotties, D., Dempsey, P., Plowman, R., Rout, J. E., Stanley, D. R., and Taylor, A., *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 1003.
313 Ip, D. P., Brenner, G. S., Stevenson, J. M., Lindenbaum, S., Douglas, A. W., Klein, S. D., and McCauley, J. A., *Int. J. Pharm.*, 1986, **28**, 183.
314 Kaneko, F., Yamazaki, K., Kobayashi, M., and Sato, K., *Spectrochim. Acta, Part A*, 1995, **50**, 1589.
315 Sawatzki, J., *Bruker Applications Note, Fourier Transform Raman Microspectroscopy*, 1990.
316 Messerschmidt, R. G., in *The Design, Sampling, Handling and Application of Infrared Microscopes*, ASTM Special Technical Publication 9494, ed. Roush, P.B., American Society for Testing and Materials, Philadelphia, 1987, p. 12.
317 Cohen, M. D., Schmidt, G. M. J., and Flavian, S., *J. Chem. Soc.*, 1964, 2041.
318 Pfeiffer, P., Braude, S., Kleber, J., Marcon, G., and Wittkop, P., *Ber. Deut. chem. Ges.*, 1915, **48**, 1777.
319 Desiraju, G. R., Paul, I. C., and Curtin, D. Y., *J. Am. Chem. Soc.*, 1977, **99**, 1594.
320 Qian, R., *Mol. Cryst. Liq. Cryst.*, 1988, **171**, 117.
321 Enokida, T., and Enashi, S., *Chem. Lett.*, 1988, 179.
322 Takano, S., Enokida, T., Kakuta, A., and Mori, Y., *Chem. Lett.*, 1984, 2073.
323 Cook, M. J., in *Advances in Spectroscopy*, ed. Clark, J. H., and Hester, R. E., 1993, **22**, 87.
324 Klebe, G., Graser, F., Haedicke, E., and Berndt, J., *Acta Cryst., Sect. B*, 1989, **45**, 69.
325 McKerrow, A. J., Buncel, E., and Kazmeier, P. M., *Can. J. Chem.*, 1993, **71**, 390.
326 Loutfy, R. O., Hor, A. M., Kazmaier, P., and Tam, M., *J. Imaging Sci.*, 1989, **33**, 151.
327 Botoschanski, M., Herbstein, F. H., and Kapon, M., *Acta Cryst., Sect. B*, 1994, **50**, 191.
328 Everett, A. J., personal communication.
329 Childers, J. W., Rohl, R., and Palmer, R. A., *Anal. Chem.*, 1986, **58**, 2629.
330 Daehnen, S., Bornowski, B., Grimm, B., Kulpe, S., Leopold D., and Naether, M., *J. Signalaufzeichnungsmater.*, 1977, **5**, 277.
331 Morel, D. L., Strogryn, E. L., Ghosh, E. K., Feng, T., Purwin, P. E., Shaw, R. F., Fushman, C., Bird, G. R., and Piechowski, A. P., *J. Phys. Chem.*, 1984, **88**, 923.
332 Becker, H.-D., Hall, S. R., Skelton, B. W., and White, A. H., *Aust. J. Chem.*, 1984, **37**, 1313.
333 Sanchez-Felix, M., personal communication.
334 Chandra, B. P., and Zink, J. I., *Phys. Rev. B, Condens. Matter*, 1980, **21**, 816.
335 Hardy, G. E., Kaska, W. C., Chandra, B. P., and Zink, J. I., *J. Am. Chem. Soc.*, 1981, **103**, 1074.
336 Lynch, L. J., Webster, D. S., and Barton, W. A., *Adv. Mag. Res.*, 1988, **12**, 285.
337 Dosseh, G., Fressigne, C., and Fuchs, A. H., *J. Phys. Chem. Solids*, 1992, **53**, 203.
338 Gavezzoti, A., and Simonetta, M., in *Organic Solid State Chemistry*, ed. Desiraju, G. R., Elsevier, Amsterdam, 1987.
339 Chirlian, L. E., and Opella, S. J., *Adv. Magn. Res.*, 1990, **14**, 183.
340 Harris, R. K., *Chem. Br.*, 1993, 601.
341 Bruker CXP *Applications Note, High Resolution NMR in Solids*, 1983.
342 Bugay, D. E., *Pharm. Res.*, 1993, **10**, 317.
343 Fletton, R. A., Lancaster, R. W., Harris, R. H., Kenwright, A. M., Parker, K. J., Waters, D. N., and Yeadon, A., *J. Chem. Soc., Perkin Trans. 2*, 1986, 1705.
344 Byrn, S. R., Pfeiffer, R. R., Stevenson, G., Grant, D. J. W., and Gleason, W. B., *Chem. Mater.*, 1994, **6**, 1148.
345 Saindon, P. J., Cauchon, N. S., Sutton, P. A., Chang, C. J., Peck, G. E., and Byron, S. R., *Pharm. Res.*, 1993, **10**, 197.
346 Suryanaryanan, R., and Wiedman, T.S., *Pharm. Res.*, 1990, **7**, 184.
347 Steiner, T., Hinrichs, W., Saenger, W., and Gigg, R., *Acta Cryst., Sect. B*, 1993, **49**, 708.
348 Opella, S. J., and Frey, H. M., *J. Am. Chem. Soc.*, 1979, **101**, 5854.
349 Byrn, S. R., Gray, G., Pfeiffer, R. R., and Frye, J., *J. Pharm. Sci.*, 1985, **74**, 565.
350 Fyfe, C. A., *Solid State NMR for Chemists*, CFC, Guelph, Ontario, 1984.
351 Etter, M., Jahn, D. A., and Urbanczyk-Lipkowska, Z., *Acta Cryst., Sect. C*, 1987, **43**, 260.
352 Smith, J. A. S., *Chem. Soc. Rev.*, 1986, **25**, 225.
353 Gourdji, M., Guibé, L., Kaplan, A., and Péneau, A., *J. Mol. Struct.*, 1983, **111**, 371.
354 Rao, C. N. R., in *Organic Solid State Reactions*, ed. Desiraju, G. R., Elsevier, Amsterdam, 1987.
355 van Driel, H. M., Wiszniewska, M., Moores, B. M., and Armstrong, R. L., *Phys. Rev.*, 1972, **6B**, 1596.
356 Dove, M. T., and Rae, A. I. M., *Faraday Discuss. Chem. Soc.*, 1980, **69**, 98.
357 Rae, A. I. M., and Dove, M. T., *J. Phys. C.*, 1983, **16**, 3233.
358 Klug, M. P., and Alexander, L. E., *X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials*, Wiley, New York, 2nd edn., 1974.
359 Takahashi, H., Takenishi, T., and Nagashima, N., *Bull. Chem. Soc. Japan*, 1962, **35**, 925.
360 Azibi, M., Draguet-Brughmans, M., Bouche, R., Tinant, B., Germain, G., Declercq, J.-P., and van Meersche, M., *J. Pharm. Sci.*, 1983, **72**, 322.
361 Forni, A., Moretti, I., Torre, G., Brueckner, S., Malpezzi, L., and DiSilvestro, G., *J. Chem. Soc., Perkin Trans. 2*, 1984, 791.
362 Fuhrup, J. H., Krull, M., and Bueldt, G., *Angew. Chem., Int. Ed. Eng.*, 1987, **26**, 698.
363 Goldberg, I., and Becker, Y., *J. Pharm. Sci.*, 1987, **76**, 255.
364 Tiers, G. V. D., *Thermochim. Acta*, 1993, **226**, 317.
365 Kuhnert-Brandstätter, M., and Lehner, G., *Sci. Pharm.*, 1984, **52**, 267.
366 Kuhnert-Brandstätter, M., and Sollinger, H. W., *Mikrochim. Acta*, 1990, **III**, 137.
367 Kuhnert-Brandstätter, M., and Vollenklee, R., *Sci. Pharm.*, 1987, **55**, 13.

368 Epple, M., and Cammenga, H. K., *Ber. Bunsenges. Phys. Chem.*, 1992, **96**, 1774.
369 Epple, M., and Cammenga, H. K., *J. Therm. Anal.*, 1992, **38**, 619.
370 Lloyd, L. F., Bruk, P., Mei-Ehe, L., Chagen, N. E., and Blow, D. G., *J. Mol. Biol.*, 1991, **217**, 19.
371 Manor, P. C., and Saenger, W., *J. Am. Chem. Soc.*, 1974, **86**, 3630.
372 Lindler, K., and Saenger, W., *Acta Cryst., Sect. B*, 1982, **38**, 1982.
373 Polishchuk, A. P., Kulishov, V. I., Antipin, M. Y., Gerr, R. G., and Struchov, Y. T., *Cryst. Struct. Commun.*, 1981, **10**, 895.
374 Polishchuk, A. P., Kulishov, V. I., Antipin, M. Y., and Struchov., Y. T., *Cryst. Struct. Commun.*, 1289.
375 Kitaigorodski, A. I., *Molecular Crystals and Molecules*, Academic Press, New York 1973, p. 36.
376 Desiraju, G. R., *Crystal Engineering*, Elsevier, Amsterdam, 1989, p. 42ff.
377 Petrouslas, V., Lemmon, R. M., and Christensen, A., *J. Chem. Phys.*, 1978, **68**, 2243.
378 Hursthouse, M., in *Encyclopaedia of Advanced Materials*, ed. Bloor, D., Brook, R. J., Flemming, M. C. and Majahu, S., Pergamon, Oxford, 1995.
379 Harding, M. M., *Chem. Br.*, 1990, 956.
380 Rizkullah, P. J., Harding, M. M., Lindsey, P. F., Aiger, A., and Bauer, A., *Acta Cryst., Sect. B*, 1990, **46**, 262.
381 King, H. E., Sirota, E. B., Shao, H., and Singer, D. M., *J. Phys. D: Appl. Phys.*, 1993, **26**, B137.
382 Sato, K., *J. Phys. D: Appl. Phys.*, 1993, **26**, B77.
383 Harmon, K. M., and Avci, G. F., *J. Mol. Struct.*, 1986, **140**, 261.
384 Marsh, R. E., *Appl. Cryst., Sect. C*, 1993, **49**, 193.
385 Aakeroy, C. B., and Seddon, K. R., *Chem. Soc. Rev.*, 1993, **22**, 397.
386 Harris, K. D. M., and Patterson, L. J., *J. Chem. Soc., Perkin Trans. 2*, 1994, 1201.
387 Lightfoot, P., Tremayne, M., Harris, K. D. M., and Bruce, P. G., *J. Chem. Soc., Chem. Commun.*, 1992, 1012.
388 Yang, S. S., and Guillory, J. K., *J. Pharm. Sci.*, 1972, **61**, 26.
389 Kitamura, S., Chang, L.-C., and Guillory, K. J., *Int. J. Pharm.*, 1984, **101**, 127.
390 Brenner, G., Roberts, F. E., Hoinowski, A., Budvari, J., Powell, B., Hinkley, D., and Schoenewaldt, E., *Angew. Chem., Int. Ed, Eng.*, 1969, **8**, 975.
391 Pfeiffer, R. R., Yang, K., and Tucker, M. A., *J. Pharm. Sci.*, 1989, **78**, 337.
392 Mynukh, Y. V., Panfilova, N. A., Petropavlov, N. N., and Uchvatova, N. S., *J. Phys. Chem. Solids*, 1975, **36**, 127.
393 Doff, D. H., Brownen, F. L., and Corrigan, O. I., *Analyst*, 1986, **111**, 179.
394 Kidd, W. C., Varlashin, P., and Li, C., *Powder Diffr.*, 1993, **8**, 180.
395 Dent Glasser, L. S., *Crystallography and its Applications*, Van Nostrand Reinhold, 1977.
396 Hall, H. E., *Solid State Physics*, Wiley, London, 1974.
397 Grindley, T. B., McKinnon, M. S., and Wasylishen, R. E., *Carbohydrate Res.*, 1990, **197**, 41.
398 Herbstein, F. H., Capon, M., Reisner, G. M., Lehmann, M. S., Kress, R. B., Shiau, W. I., Duesler, E. N., Paul, I. C., and Curtin, D. Y., *Proc. R. Soc. A*, 1985, **399**, 295.
399 Ermer, O., *Angew. Chem., Int. Ed. Eng.*, 1987, **26**, 782.
400 Bunn, C. W., *Chemical Crystallography*, Oxford University Press, 2nd edn., 1961.
401 Nyburg, S. C., *X-Ray Analysis of Organic Structures*, Academic Press, New York, 1961.
402 Giaccovazzo, G., *Fundamentals of Crystallography*, International Union of Crystallography, Oxford, 1992.
403 Dunitz, J. D., *X-ray Analysis and the Structure of Organic Molecules*, Cornell University Press, Ithica, 1979.
404 Rossi, M., and Berman, H. M., *J. Chem. Ed.*, 1988, **6**, 472.
405 *Modern Powder Diffraction*, ed. Bish, D. L., and Post, J. E., Reviews in Mineralogy, vol. 2, Mineralogical Society of America, 1989.
406 Azároff, L. V., and Buerger, M. J., *The Powder Method in X-ray Crystallography*, McGraw–Hill, New York, 1958.
407 Shafizadeh, F., and Susott, R. A., *J. Org. Chem.*, 1973, **38**, 3710.
408 Perrenot, B., and Widman, G., *Thermochim. Acta*, 1994, **234**, 31.
409 Garn, P. D., *Thermoanalytical Methods of Investigation*, Academic Press, New York, 1965, p. 21.
410 Voress, L., *Anal. Chem.*, 1994, **66**, 1035A.
411 Kopp, S., Beyer, C., Graf, E., Kubel, F., and Doelker, E., *Acta Pharm. Technol.*, 1988, **34**, 213.
412 Burger, A., and Lettenbichler, A., *Eur. J. Pharm. Biopharm.*, 1993, **39**, 64.
413 Chataing, G., and Vergnaud, J. M., *Thermochim. Acta*, 1985, **94**, 379.
414 Kuhnert-Brandstätter, M., and Seidel, D., *Mikrochim. Acta*, 1982, **I**, 243.
415 Van Dooren, A. H., and Muller, B. W., *Thermochim. Acta*, 1983, **66**, 161.
416 Kuhnert-Brandstätter, M., and Heindle, W., *Sci. Pharm.*, 1976, **44**, 18.
417 Allen, P. V., Rahn, P. D., Sarapu, A. C., and Vanderwielen, A. J., *J. Pharm. Sci.*, 1978, **67**, 1087.
418 Kuhnert-Brandstätter, M., and Proell, F., *Mikrochim. Acta*, 1983, **II**, 463.
419 Kuhnert-Brandstätter, M., Geiler, M., and Wurian, I., *Mikrochim. Acta*, 1983, **I**, 221.
420 Giron-Forest, D., Goldbrunn, C., and Piechon, P., *J. Pharm. Biomed. Anal.*, 1989, **7**, 144.
421 Kuhnert-Brandstätter, M., and Vollenklee, R., *Sci. Pharm.*, 1987, **55**, 27.
422 Haines, P., *Thermal Analysis*, Blackie, London, 1995.
423 *Thermal Analysis: an Introduction to Principles and Practice*, ed. Hodgson, A., University of York, York.
424 Takahashi, Y., *Thermochim. Acta*, 1985, **88**, 199.
425 McNaughton J. L., and Mortimer, C. T., *Differential Scanning Calorimetry*, Perkin–Elmer, Norwalk, Connecticut.
426 Westrum, E. R., and McCullough, J. P., in *Physics and Chemistry of the Organic Solid State*, ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1963, vol. 1.
427 Daniels, T., *Thermal Analysis*, Halsted Press, New York, 1973.
428 Matsunaga, J., Nambu, N., and Nagai, T., *Chem. Pharm. Bull.*, 1976, **24**, 1169.
429 Muller, B. W. W., *Pharm. Acta Helv.*, 1978, **53**, 333.
430 Taludar, M. D., Carless, J. E., and Summers, M. P., *J. Pharm. Phamacol.*, 1983, **35**, 208.
431 Matsumoto, T., Ichikawa, J., Kaneniwa, N., and Otsuka, M., *Chem. Pharm. Bull.*, 1988, **36**, 1074.
432 Forni, F., Coppi, G., Iannuccelli, V., and Cameroni, R., *J. Therm. Anal.*, 1990, **36**, 35.
433 Behme, R. J., Brooke, D., Farney, R. F., and Kensler, T. T., *J. Pharm. Sci.*, 1985, **74**, 1041.
434 Burger, A., and Ramberger, R., *Mikrochim. Acta*, 1979, **II**, 273.
435 Prasad, P. N., in *Organic Solid State Chemistry*, ed. Desiraju, G. R., Elsevier, Amsterdam, 1989.
436 Ostwald, W., *Z. Physik. Chem.*, 1897, **22**, 306.
437 Wentlandt, W. W., *Thermal Methods of Analysis*, Interscience, New York, 1964.
438 Kuhnert-Brandstätter, M., and Sollinger, H. W., *Mikrochim. Acta*, 1989, **III**, 125.
439 Sarge, S., and Cammenga, H. K., *Thermochim. Acta*, 1985, **94**, 17.
440 Barell, E. M., *Thermochim. Acta*, 1973, **5**, 377 quoted by Ford, J. L., and Timmins, P., *Pharmaceutical Thermal Analysis*, Ellis Horwood, Chichester, 1989, p. 27.
441 Harbury, L., *J. Phys. Chem.*, 1946, **50**, 190.
442 Kuhnert-Brandstätter, M., and Heindl, W., *Sci. Pharm.*, 1975, **43**, 112.
443 Kuhnert-Brandstätter, M., and Lindler, R., *Mikrochim. Acta*, 1976, **I**, 513.
444 Ubbelhohde, A. R., *The Molten State of Matter*, Wiley, Chichester, 1978, p. 317.
445 Cahn, R. W., *Nature (London)*, 1992, **356**, 108.
446 Docherty, C., and York, P., *Int. J. Pharm.*, 1988, **47**, 141.
447 Serpinet, J., *Nature (London) Phys. Sci.*, 1971, **232**, 42.
448 Serpinet, J., and Robin, J., *C.r. Acad. Sci. Paris.*, 1971, **272C**, 1765.
449 Florence, A. T., and Salole, E. G., *J. Pharm. Pharmacol.*, 1976, **28**, 637.
450 Sherwood, J. N., *The Plastically Crystalline State*, Wiley, New York, 1979.
451 Aston, J. G., in *Physics and Chemistry of the Organic Solid State*, ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1963, vol. I.

452 Adachi, K., Suga, H., and Seki, S., *Bull. Chem. Soc. Japn.*, 1970, **43**, 1916.
453 Westrum, E. R., and McCullough, J. P., in *Physics and Chemistry of the Organic Solid State*, ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1963, vol. 1, p. 83.
454 Staveley, L. A. K., *Quart. Rev.*, 1949, **3**, 65.
455 Reading, M., *Trends in Polym. Sci.*, 1993, **1**, 248.
456 Ford, J. L., and Timmins, P., *Pharmaceutical Thermal Analysis*, Ellis Horwood, Chichester, 1989.
457 Clark, G. M., *Anal. Proc.*, 1986, **23**, 393.
458 Wenzell, P. D., and Wade, A. P., *Anal. Chem.*, 1989, **61**, 2638.
459 Borka, L., *Acta Pharm. Suec.*, 1974, **11**, 413.
460 Bergman, E., Hoff, E. E., Lefiles, J. H., McKinney, L. J., and Misselbrook, J., *Eur. Pat.* 380325.
461 Pearson, J. T., and Varney, G., *J. Pharm. Pharmacol.*, 1969, **21**, 60S.
462 Mesley, R. J., and Houghton, E. E., *J. Pharm. Pharmacol.*, 1967, **19**, 295.
463 Grant, D. J. W., and Higuchi, T., *Solubility Behaviour of Organic Compounds*, Techniques in Chemistry, Vol. XXI, Wiley, New York, 1990.
464 Mader, W.J., and Grady, L. T., in *Physical Methods of Organic Chemistry*, ed. Weissberger, A., and Rossiter, B. W., Wiley, New York, 1971, vol. 1, pt. V.
465 Florence, A. T., and Attwood, D, *Physicochemical Principles of Pharmacy*, Macmillan, London, 1981.
466 Doughty, D. G., *Drug Dev. Ind. Pharm.*, 1989, **15**, 2455.
467 Windram, V. A., and Threlfall, T. L., *Anal. Proc.*, 1992, **29**, 108.
468 Abdou, H. M., *Dissolution, Bioavailablity and Bioequivalence*, Mack, Easton, 1989, p. 126.
469 Buxton, P. C., Lynch, I. R., and Roe, J. M., *Int. J. Pharm.*, 1988, **42**, 135.
470 Murthy, K. S., Turner, N. A., Nesbitt, R. U., and Fawzi, M. B., *Drug Dev. Ind. Pharm.*, 1986, **12**, 665.
471 Martínez-Oharriz, M. C., Martín, C., Goñi, M. M., Rodríguez-Espinosa, C., Tros de Ilarduya-Apadaza, M. C., and Sánchez, M., *J. Pharm. Sci.*, 1994, **83**, 174.
472 Higuchi, T., *J. Pharm. Sci.*, 1969, **56**, 200.
473 Kuhnert-Brandstätter, M., and Linsmeyer, L., *Sci. Pharm.*, 1986, **54**, 1.
474 Beall, H. D., Getz, J. J., and Sloane, K. B., *Int. J. Pharm.*, 1993, **93**, 37.
475 Albert, A., and Sargeant, E. P., *The Determination of Ionisation Constants*, Chapman and Hall, London, 3rd edn., 1984.
476 Perrin, D. D., and Dempsey, B., *Buffers for pH and Metal Ion Control*, Science Paperbacks, London, 1979.
477 Stearns, E. I., *The Practice of Absorption Spectroscopy*, Wiley, New York, 1969, p. 75.
478 Perkampus, H. H., *UV-VIS Spectroscopy and its Applications*, Springer, Berlin, 1992.
479 Burger, A., *Acta Pharm. Technol.*, 1982, **28**, 1.
480 Kitaigorodski, A. I., *Organic Chemical Crystallography*, Consultants Bureau, New York, 1961.
481 Whitesell, J. K., Davis, R. E., Saunders, L. L., Wilson, R. J., and Feagin, J. P., *J. Phys. D: Appl. Phys.*, 1993, **26**, B56.
482 Andreev, G. A., and Hartmanová, M., *Phys. Status Solidi. A*, 1989, **116**, 457.
483 Bauer, N., and Lewin, S. Z., in *Physical Methods of Organic Chemistry*, ed. Weissberger, A., and Rossiter, B. W., Wiley, New York, 1972, vol. 1, pt. IV.
484 Lowell, S., and Shields, J. E., *Powder Surface Area and Porosity*, Chapman and Hall, London, 3rd edn., 1991.
485 Dent Glasser, L. S., *Crystallography and its Applications*, Van Nostrand Reinhold, 1977, p. 111–114.
486 Orr, C., and Dallevalle, J. M., *Fine Particle Measurement*, Macmillan, New York, 1959.
487 Fukumori, Y., Fukuda, T., Yamamoto, Y., Shigitami, Y., Hanyu, Y., Takeuchi, Y., and Sato, N., *Chem. Pharm. Bull.*, 1983, **31**, 4029.
488 Carless, J. E., Moustafa, M. A., and Rapson, H. D. C., *J. Pharm. Pharmacol.*, 1966, **18**, 190S.
489 Botha, S. A., Caira, M. R., Guillory, J. K., and Lötter, A. P., *J. Pharm. Sci.*, 1988, **77**, 444.
490 Otsuka, M., and Kanemiwa, N., *Chem. Pharm. Bull.*, 1983, **31**, 1021.
491 Chapman, J. H., Page, J. E., Parker, A. C., Rogers, D., Sharp, C. J., and Staniforth, S. E., *J. Pharm. Pharmacol.*, 1968, **20**, 418.
492 Burger, A., Ratz, A. W., and Zolss, G., *Acta Pharm. Technol.*, 1988, **34**, 147.
493 Michel, G., in *Analytical Profiles*, vol. 6., ed. Florey, K., Academic Press, New York, 1977, vol. 6.
494 Kuhnert-Brandstätter, M., *Pharm. Ind.*, 1977, **39**, 377.
495 Pouchert, C. J., *Aldrich Library of Infrared Spectra*, Aldrich, Milwaukee, 3rd edn., 1981.
496 Bjaen, A. K. B., Nord, K., Furuseth, S., Agren, T., Tonneson, H. H., and Karlsen, J., *Int. J. Pharm.*, 1993, **92**, 193.
497 Chauvet, A., Masse, J., Ribet., J. P., Bigg, D., Autin, J. M., Maurel, J. L., Patoneau, J. F., and Jans, J., *J. Pharm. Sci.*, 1992, **81**, 836.
498 Williams, P. P., *Acta Cryst., Sect. B*, 1973, **29**, 1572.
499 Khankhari, R. J., Law, D., and Grant, D. J. W., *Int. J. Pharm.*, 1992, **82**, 117.
500 Harris, R. K., Say, B. J., Yeung, R. R., Fletton, R. A., and Lancaster, R. W., *Spectrochim. Acta, Part A*, 1989, **45**, 465.
501 Hendricksen, B. A., Preston, M. S., and York, P., in the press.
502 Chrzanowski, F. A., Fegley, B. J., Sisco, W. R., and Newton, M. P., *J. Pharm. Sci.*, 1984, **73**, 10.
503 Garti, N., Sarig, S., and Wellner, E., *Thermochim. Acta*, 1980, **37**, 131.
504 Hirshfeld, T., in *Fourier Transform Infrared Spectroscopy*, ed. Ferraro, J. R., and Basile, L. J., Academic Press, 1979, vol. 2.
505 Hamadah, L. M., Yeboah, S. A., Turnbull, K. A., and Griffiths, P. R., *Appl. Spectrosc.*, 1984, **38**, 486.
506 Tanninen, V. P., and Yliruusi, J., *Int. J. Pharm.*, 1992, **81**, 169.
507 Tudor, A. M., Church, S. J., Hendra, P. J., Davies, M. C., and Melia, C. D., *Pharm. Res.*, 1993, **10**, 1771.
508 Guillory, J. K., and Erb, D. M., *Pharm. Manuf.*, 1985, **2**(9), 28.
509 Lindenbaum, S., and McGraw, S. E., *Pharm. Manuf.*, 1985, **2**(1), 273.
510 Crocker, L. S., and McCauley, J. A., *J. Pharm. Sci.*, 1995, **84**, 226.
511 Pikal, M. J., Lukes, A. L., Lang, J. E., and Gaines, K., *J. Pharm. Sci.*, 1978, **67**, 676.
512 Matsuda, Y., Tatsumi, E., Chiba, E., and Miwa, Y., *J. Pharm. Sci.*, 1984, **73**, 1453.
513 Jenkins, E. W., *The Polymorphism of Elements and Compounds*, Methuen Educational, London, 1973.
514 Rosenberg, H. M., *The Solid State*, Oxford University Press, 3rd edn., 1988.
515 De Gennes, P. G., and Prost, J., *The Physics of Liquid Crystals*, Clarendon, Oxford, 1993.
516 Janner, A., and Janssen, T., *Phys. Rev. B: Condens Matter*, 1977, **13**, 643.
517 Stevens, P. W., and Goldman, A. T., *Sci. Am.*, 1991, **261**(April), 24.
518 Janot, C., Dubois, J.M., and de Boisseau, M., *Amer. J. Phys.*, 1989, **57**, 972.
519 Nelson, D. R., *Sci. Am.*, 1986, **255**(August), 32.
520 Ronchetti, M., *Philos. Mag.*, 1987, **56**, 237.
521 Goldman, A. I., and Kelton, R. F., *Rev. Mod. Phys.*, 1993, **65**, 213.
522 Holzer, J. C., and Kelton, R. F., in *Crystal-Quasicrystal Transitions*, ed. Yacaman, M. J., and Torres, M., Elsevier, Amsterdam, 1993.
523 Finney, J. L., *Stud. Phys. Theor. Chem.*, 1981, **13**, 439.
524 Mayer, E., and Pletzer, R., *NATO ASI Ser., Ser. 3*, 1985, **156**, 81.
525 Samwer, K., *Phys. Rep.*, 1988, **161**, 1.
526 Sandman, D. J., Elman, B. S., Hamill, G. P., Velazquez, C. S., and Samuelson, L. A., *Mol. Cryst. Liq. Cryst.*, 1986, **134**, 89.
527 Halebian, J. K., Koda, R. T., and Biles, J. A., *J.Pharm. Sci.*, 1971, **60**, 1485.
528 Corrigan, O. I., and Holohan, E. M., *J. Pharm. Pharmacol.*, 1984, **36**, 217.
529 Parthasathy, R., Rao, K. J., and Rao, C. N. R., *Chem. Soc. Rev.*, 1984, 361.
530 Hukins, D. W. L., *X-Ray Diffraction by Ordered and Disordered Systems*, Pergamon Press, Oxford, 1981.
531 Elliott, S. R., *Nature (London)*, 1991, **354**, 445.
532 Atalla, R. H., Gast, J. C., Sindorf, D. W., Bartuska, V. G., and Maciel, G. E., *J. Am. Chem. Soc.*, 1980, **102**, 3249.
533 Fecht, H. J., *Nature (London)*, 1992, **365**, 133.
534 Fecht, H. J., and Johnson, W. L., *Nature (London)*, 1988, **334**, 50.
535 Cahn, R. W., *Nature (London)*, 1988, **334**, 17.
536 Cahn, R. W., *Nature (London)*, 1992, **356**, 108.
537 Tallon, J. L., *Nature (London)*, 1989, **342**, 658.

538 Zarzicki, J., *Glasses and the Vitreous State,* Cambridge University Press, 1982.
539 Chauhadri, P., Giessen, B. C., and Turnbull, D., *Sci. Am.,* 1980, **242**(April), 84.
540 Kauzmann, W., *Chem. Rev.,* 1948, **43**, 219.
541 Jaeckle, J., *Rep. Prog. Phys.,* 1986, **49**, 17.
542 Randall, J. T., *The Diffraction of X-rays and Electrons by Amorphous Solids, Liquids and Gases,* Chapman and Hall, London, 1934.
543 Jaeckle, J., *Philos. Mag. B,* 1987, **56**, 113.
544 Wunderlich, B., *Thermal Analysis,* Academic, New York, 1990.
545 Sichina, W. J., *Int. Laboratory,* 1994, **March**, 20.
546 van der Plaats, G., *The Practice of Thermal Analysis,* Mettler, Greifensee, Switzerland.
547 Westrum, E. R., and McCullough, J. P., in *Physics and Chemistry of the Organic Solid State,* ed. Fox, D., Labes, M. M., and Weissberger, A., Interscience, New York, 1963, vol. 1, p. 43.
548 Kitaigorodski, A. I., *Molecular Crystals and Molecules,* Academic Press, New York, 1973, p. 18.
549 Partington, J. R., *The Properties of Solids, Advanced Treatise on Physical Chemistry, vol. III,* Oxford University Press, 1952, p. 519.
550 Saleki-Gerhardt, A., Stowell, J. G., Byrn, S. R., and Zografi, G., *J. Pharm. Sci.,* 1995, **84**, 318.
551 Burger, A., and Ratz, A. W., *Sci. Pharm.,* 1990, **58**, 69.
552 van Skoik, K. G., and Carstensen, J. T., *Int. J. Pharm.,* 1990, **58**, 185.
553 White, G. W., and Cakebread, S. H., *J. Food Technol.,* 1966, **1**, 73.
554 Talbot, G., in *Industrial Chocolate Manufacture and Use,* ed. Beckett, S.T., Blackie, London, 2nd edn., 1993.
555 Suryanarayanan, R., and Mitchell, A. G., *Int. J. Pharm.,* 1986, **32**, 213.
556 James, R. W., *The Optical Principles of the Diffraction of X-Rays,* Bell, London, 2nd edn., 1962.

Paper 5/01094B
Received February 23, 1995
Accepted July 6, 1995

Guidance for Industry

ANDAs: Pharmaceutical Solid Polymorphism

Chemistry, Manufacturing, and Controls Information

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
July 2007
OGD

Guidance for Industry

ANDAs: Pharmaceutical Solid Polymorphism

Chemistry, Manufacturing, and Controls Information

Additional copies are available from:
Office of Training and Communication
Division of Drug Information, HFD-240
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
(Tel) 301-827-4573
http://www.fda.gov/cder/guidance/index.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
July 2007
OGD

TABLE OF CONTENTS

Contains nonbinding recommendations

Guidance for Industry[1]

ANDAs: Pharmaceutical Solid Polymorphism Chemistry, Manufacturing, and Controls Information

This guidance, represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternate approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this document.

I. INTRODUCTION[2]

Chemistry, manufacturing, and controls (CMC) information must be submitted to support the approval of an abbreviated new drug application (ANDA).[3] This guidance is intended to assist applicants with the submission of ANDAs when a drug substance[4] exists in polymorphic forms.[5] Specifically, this guidance provides:

- FDA recommendations on assessing *sameness*[6] when the drug substance exists in polymorphic forms.
- Decision trees that provide recommendations on monitoring and controlling polymorphs in drug substances and/or drug products.[7]

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are

---

[1] This guidance has been prepared by the Office of Generic Drugs (OGD) in the Office of Pharmaceutical Science (OPS), Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA).

[2] Although issues relating to polymorphic forms may be relevant to new drug applications (NDAs), this guidance only addresses polymorphic forms in the context of ANDA approvals.

[3] See 21 CFR 314.94 (a)(9); see also section 505(j)(4)(A) of the Federal Food, Drug, and Cosmetic Act (the Act).

[4] For the purposes of this guidance the terms *drug substance* and *active ingredient* are used interchangeably.

[5] The terms *polymorphic forms* and *polymorphs* are synonymous and are used interchangeably in this guidance.

[6] Refer to Section IV for more information.

[7] This guidance is intended to help industry with the most common types of polymorphs. A drug substance may exist in many polymorphic forms, but some forms may be rare and not likely to form. For example, in one approved drug product, the drug substance can exist in at least twenty polymorphic forms, but in reality only a subset of polymorphic forms has the potential to develop under the process conditions used to manufacture the drug substance and drug product. Therefore, we recommend that you consider only those polymorphs that are likely to form during manufacture of the drug substance, manufacture of the drug product, or while the drug substance or drug product is in storage.

cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. DEFINITION OF TERMS: POLYMORPHIC FORMS AND POLYMORPHISM

We recommend that ANDA applicants investigate whether the drug substance in question can exist in polymorphic forms. Polymorphic forms in the context of this guidance refer to crystalline and amorphous forms as well as solvate and hydrate forms, which are described below.[8]

- Crystalline forms have different arrangements and/or conformations of the molecules in the crystal lattice.
- Amorphous forms consist of disordered arrangements of molecules that do not possess a distinguishable crystal lattice.
- Solvates are crystal forms containing either stoichiometric or nonstoichiometric amounts of a solvent.[9] If the incorporated solvent is water, the solvate is commonly known as a hydrate.

When a drug substance exists in polymorphic forms, it is said to exhibit polymorphism.

III. GENERAL PRINCIPLES OF PHARMACEUTICAL SOLID POLYMORPHISM

A. Importance of Pharmaceutical Solid Polymorphism

Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility,[10] dissolution rate, optical and mechanical properties, vapor pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability. Thus, polymorphism can affect the quality, safety, and efficacy of the drug product.

B. Characterization of Polymorphs

There are a number of methods that can be used to characterize polymorphs of a drug substance.[11] Demonstration of a nonequivalent structure by single crystal X-ray diffraction is

---

[8] Guidance for industry, Q6A *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances,* International Conference on Harmonisation (ICH), December 2000.
[9] SR Byrn, RR Pfeiffer, and JG Stowell. *Solid-State Chemistry of Drugs.* 2nd Edition, SSCI, Inc., West Lafayette, Indiana, 1999.
[10] Apparent solubility refers to the concentration of material at apparent equilibrium (supersaturation). Apparent solubility is distinct from true thermodynamic solubility, which is reached at infinite equilibrium time.
[11] H Brittain. "Methods for the characterization of polymorphs and solvates." In HG Brittain (ed.) *Polymorphism in Pharmaceutical Solids.* Marcel Dekker, Inc., New York, 1999, pp. 227-278.

currently regarded as the definitive evidence of polymorphism. X-ray powder diffraction can also be used to provide unequivocal proof of polymorphism. Other methods, including microscopy, thermal analysis (e.g., differential scanning calorimetry, thermal gravimetric analysis, and hot-stage microscopy), and spectroscopy (e.g., infrared [IR], Raman, solid-state nuclear magnetic resonance [ssNMR]) are helpful to further characterize polymorphic forms.

C. Influence of Polymorphism On Drug Substance And Drug Product

1. Influence on Solubility, Dissolution, and Bioavailability (BA) and Bioequivalence (BE)

The solid-state properties of a drug substance can have a significant influence on the apparent solubility of the drug substance. Since polymorphic forms differ in their internal solid-state structure, a drug substance that exists in various polymorphic forms can have different aqueous solubilities and dissolution rates.[12] When there are differences in the apparent solubilities of the various polymorphic forms, we recommend that you focus on the potential effect such differences can have on drug product bioavailability (BA) and bioequivalence (BE).[13]

Whether drug product BA/BE can be affected by the differences in apparent solubilities of the various polymorphic forms depends on the various physiological factors that govern the rate and extent of drug absorption including gastrointestinal motility, drug dissolution, and intestinal permeability. In this context, the Biopharmaceutics Classification System (BCS)[14, 15] provides a useful scientific framework for regulatory decisions regarding drug substance polymorphism.

For a drug whose absorption is only limited by its dissolution, large differences in the apparent solubilities of the various polymorphic forms are likely to affect BA/BE. On the other hand, for a drug whose absorption is only limited by its intestinal permeability, differences in the apparent solubilities of the various polymorphic forms are less likely to affect BA/BE. Furthermore, when the apparent solubilities of the polymorphic forms are sufficiently high and drug dissolution is rapid in relation to gastric emptying, differences in the solubilities of the polymorphic forms are unlikely to affect BA/BE.

---

[12] HG Brittain and DJW Grant. "Effect of polymorphism and solid-state solvation on solubility and dissolution rate." In HG Brittain (ed.) *Polymorphism in Pharmaceutical Solids.* Marcel Dekker, Inc., New York, 1999, pp. 279-330.

[13] Bioavailability (BA) is defined in 21 CFR 320.1(a) as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action." Bioequivalence (BE) is defined in 21 CFR 320.1(e) as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study."

[14] GL Amidon, H Lennernas, VP Shah, and JR Crison. "A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability," *Pharm. Res.* 12:413-420, 1995.

[15] LX Yu, GL Amidon, JE Polli, H Zhao, M Mehta, DP Conner, VP Shah, LJ Lesko, M-L Chen, VHL Lee, and AS Hussain. "Biopharmaceutics Classification System: The scientific basis for biowaiver extension." *Pharm. Res.* 19:921-925, 2002.

Upon demonstration of in-vivo bioequivalence between the generic drug product[16] and the reference listed drug (RLD),[17] in-vitro dissolution testing is then used to assess the lot-to-lot quality of the generic drug product. Drug product dissolution testing frequently provides a suitable means to identify and control the quality of the product from both the bioavailability and physical (stability) perspectives. In particular, inadvertent changes to the polymorphic form that may affect drug product BA/BE can often be detected by drug product dissolution testing.

2. Influence on Manufacturing of the Drug Product

Drug substance polymorphic forms can also exhibit different physical and mechanical properties, including hygroscopicity, particle shape, density, flowability, and compactibility, which in turn may affect processing of the drug substance and/or manufacturing of the drug product. Since an ANDA applicant should demonstrate that the generic drug product can be manufactured reliably using a validated process, we recommend that you pay close attention to polymorphism as it relates to pharmaceutical processing.[18]

The effect of polymorphism on pharmaceutical processing also depends on the formulation and the manufacturing process.[19] For a drug product manufactured by direct compression, the solid-state properties of the active ingredient will likely be critical to the manufacture of the drug product, particularly when it constitutes the bulk of the tablet mass. On the other hand, for a drug product manufactured by wet granulation, the solid-state properties of the active ingredient are often masked by the resultant granulation, and the solid-state properties of the active ingredient are less likely to affect the manufacture of the drug product. In the context of the effect of polymorphism on pharmaceutical processing, what is most relevant is the ability to consistently manufacture a drug product that conforms to applicable in-process controls and release specifications.

Polymorphic forms of the drug substance can undergo phase conversion when exposed to a range of manufacturing processes, such as drying, milling, micronization, wet granulation, spray-drying, and compaction. Exposure to environmental conditions such as humidity and temperature can also induce polymorph conversion. The extent of conversion generally depends on the relative stability of the polymorphs, kinetic barriers to phase conversion, and applied stress.[20] Nonetheless, phase conversion generally is not of serious concern, provided that the conversion occurs consistently, as a part of a validated manufacturing process where critical manufacturing process variables are well understood and controlled, and when drug product BA/BE has been demonstrated.

---

[16] The term *generic drug product* refers to a new drug product for which approval is sought in an ANDA submitted under section 505(j) of the Act.

[17] See 21 CFR 314.3 (b) (providing that *reference listed drug* means the listed drug identified by FDA as the drug product upon which an applicant relies in seeking approval of its abbreviated application).

[18] Section 505(j)(4)(A) provides that FDA must approve an ANDA if, among other things, the methods used in, or the facilities and controls used for, the manufacture, processing, and packing of the drug are adequate to assure and preserve its identity, strength, quality, and purity.

[19] DA Wadke, ATM Serajuddin, and H Jacobson. "Preformulation testing." In HA Lieberman, L Lachman, and JB Schwartz (eds.) *Pharmaceutical Dosage Forms: Tablets* (Vol. 1). Marcel Dekker, Inc., New York, 1989, pp. 1-73.

[20] SR Vippagunta, HG Brittain, DJW Grant. "Crystalline solids," *Adv. Drug Del. Rev.* 48:3-26, 2001.

3. Influence on Stability

Polymorphs can have different physical and chemical (reactivity) properties. The most thermodynamically stable polymorphic form of a drug substance is often chosen during development based on the minimal potential for conversion to another polymorphic form and on its greater chemical stability. However, a metastable form can be chosen for various reasons, including bioavailability enhancement. Since an ANDA applicant must demonstrate that the generic drug product exhibits adequate stability,[21] we recommend that you focus on the potential effect that a polymorphic form can have on drug product stability. Nonetheless, because drug product stability is affected by a multitude of other factors, including formulation, manufacturing process, and packaging, it is the stability of the drug product and not stability of the drug substance polymorphic form that should be the most relevant measure of drug quality.

IV. POLYMORPHISM AND *SAMENESS* IN ANDAs

Section 505(j)(2) of the Act specifies that an ANDA must contain, among other things, information to show that the active ingredient in the generic drug product is the "same as" that of the RLD. Under section 505(j)(4) of the Act, FDA must approve an ANDA unless the agency finds, among other things, that the ANDA contains insufficient information to show that the active ingredient is the same as that in the RLD. FDA regulations implementing section 505(j) of the Act provide that an ANDA is suitable for consideration and approval if the generic drug product is the "same as" the RLD. Specifically, 21 CFR 314.92(a)(1) provides that the term "same as" means, among other things, "identical in active ingredient(s)." The drug substance in a generic drug product is considered to be the same as the drug substance in the RLD if it meets the same standards for identity.[22]

When a United States Pharmacopeia (USP) monograph exists for a particular drug substance, standards for identity generally refer to the definition (e.g. chemical name, empirical formula, molecular structure, description) at the beginning of the monograph. However, FDA may prescribe additional standards that are material to the *sameness* of a drug substance.[23]

Polymorphic forms of a drug substance differ in internal solid-state structure, but not in chemical structure. In the context of *sameness* of active ingredient(s) in the preamble to the 1992 final rule, FDA specifically rejected a proposal that would have required an ANDA applicant to show that the active ingredient in its generic drug product and the active ingredient in the RLD "exhibit the same physical and chemical characteristics, that no additional residues or impurities can result from the different manufacture or synthesis process and that the stereochemistry characteristics and solid state forms of the drug have not been altered."[24] Therefore, differences in drug substance polymorphic forms do not render drug substances different active ingredients for the purposes of ANDA approvals within the meaning of the Act and FDA regulations.

---

[21] See footnote 18.
[22] See preamble to the 1992 final rule (57 FR 17958; April 28, 1992).
[23] See footnote 22.
[24] See footnote 22.

In addition to meeting the standards for identity, each ANDA applicant is required to demonstrate that, among other things, the drug product exhibits sufficient stability and is bioequivalent to the RLD.[25] While the polymorphic form can affect drug product stability and bioequivalence, these performance characteristics are also dependent on the formulation, the manufacturing process, and other physicochemical properties (e.g., particle size, moisture) of both the drug substance and formulation excipients. Using a drug substance polymorphic form that is different from that of the RLD may not preclude an ANDA applicant from formulating a generic drug product that exhibits bioequivalence and stability, and the drug substance in the generic drug product need not have the same polymorphic form as the drug substance in the RLD.

Over the years, FDA has approved a number of ANDAs in which the drug substance in the generic drug product had a different polymorphic form from the drug substance in the respective RLD (e.g., warfarin sodium, famotidine, and ranitidine). FDA also has approved some ANDAs in which the drug substance in the generic drug product differed in solvate or hydrate forms from the drug substance in the corresponding RLD (e.g., terazosin hydrochloride, ampicillin, and cefadroxil).

V. CONSIDERATIONS FOR POLYMORPHISM IN ANDAs

The decision trees shown in Attachments 1 to 3 provide ANDA applicants with a suggested process for evaluating the importance of and approaches to setting specifications for polymorphic forms in solid oral drug products and oral suspensions. Although the conceptual framework adopted by these decision trees is based primarily on the potential for polymorphic forms to affect drug product BA/BE, we recommend that you still consider the influence polymorphic forms may have on the ability to manufacture the drug product and on the stability of the drug product.

The following sections describe each of the decision trees.

A. Investigating the Importance of Setting Specifications for Polymorphs

Decision Tree 1 provides recommendations on when specifications for polymorphic form(s)[26] for the drug substance and/or the drug product may be appropriate. Polymorphs are unlikely to have a significant effect on BA/BE when all forms have the same apparent solubilities or all forms are highly soluble.

ANDA applicants are expected to have adequate knowledge about drug substance polymorphs. Information on polymorphism can come from the scientific literature, patents, compendia, other references, or in some cases, polymorph screening.

B. Setting Specifications for Polymorphs in Drug Substances

[25] See 505(j)(4) of the Act and 21 CFR 314.127.
[26] See footnote 7.

Decision Tree 2 provides an approach for setting specifications for polymorphs in the drug substance when at least one form is known to have low solubility based on the BCS. If relevant and adequate specifications for polymorphs are included in the USP, ANDA applicants may adopt these specifications for the drug substance polymorphic form. Otherwise, we recommend that a new specification for the drug substance polymorphic form be established.

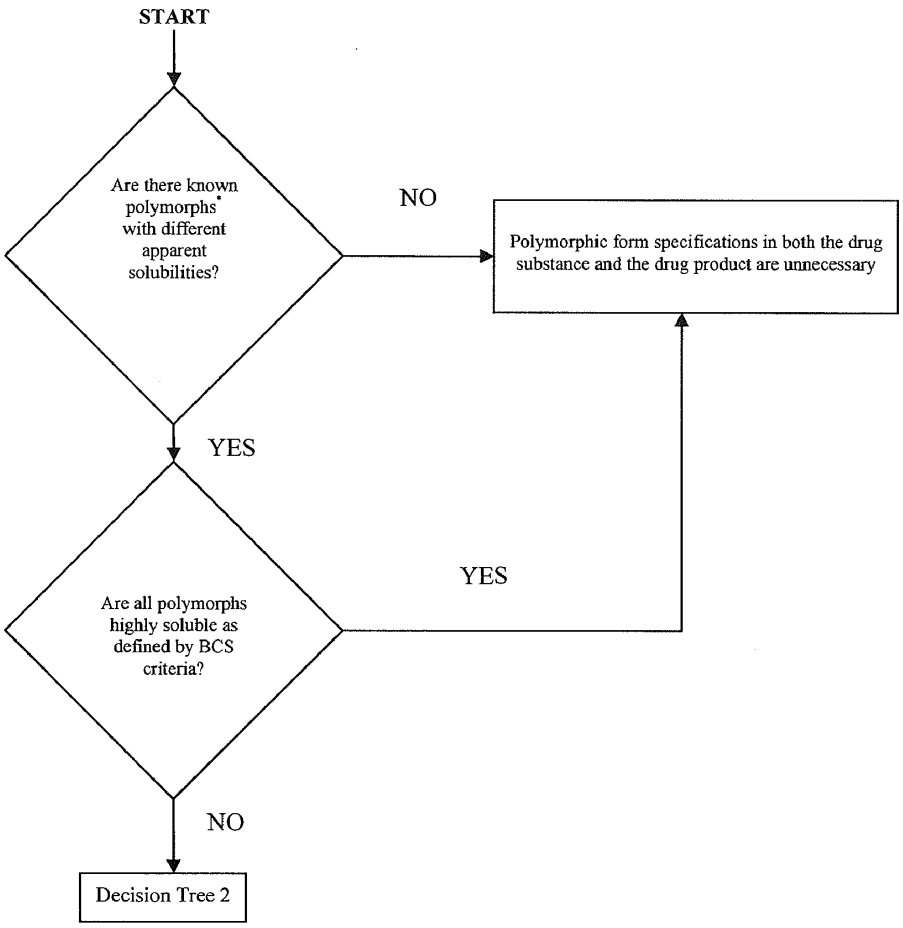
C. Investigating the Importance of Setting Specifications for Polymorphs in Drug Products

Decision Tree 3 provides an approach when considering whether to set specifications for polymorphs in the drug product. Generally, specifications for polymorphs in drug products are not necessary if the most thermodynamically stable polymorphic form is used or if the same form is used in an approved product of the same dosage form. However, since manufacturing processes can affect the polymorphic form, we recommend that you use caution if a metastable form is used.

Drug product performance testing (e.g., dissolution testing) can also generally provide adequate control of polymorph ratio changes that can influence drug product BA/BE for poorly soluble drugs. In such instances, setting specifications for polymorphs in the drug product would generally not be considered important for ensuring adequate product performance. Only in rare cases would we recommend setting specifications for polymorphic forms in drug products.

ATTACHMENT 1 – DECISION TREE 1

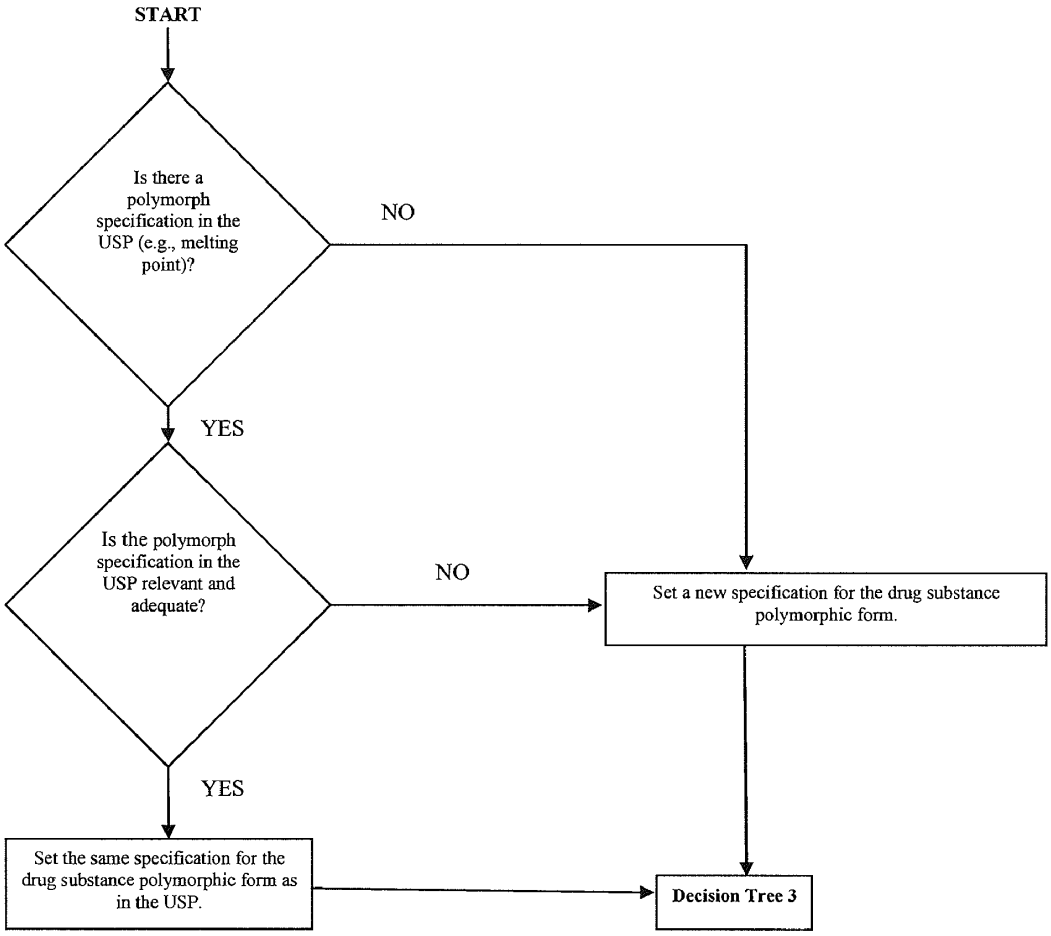
Decision Tree 1 Investigating whether to set specifications for polymorphs for solid oral and suspension dosage form products.



*We recommend that you consider only those polymorphs that are likely to form during manufacture of the drug substance, manufacture of the drug product, or while the drug substance or drug product is in storage. See footnote 7 in this guidance document.

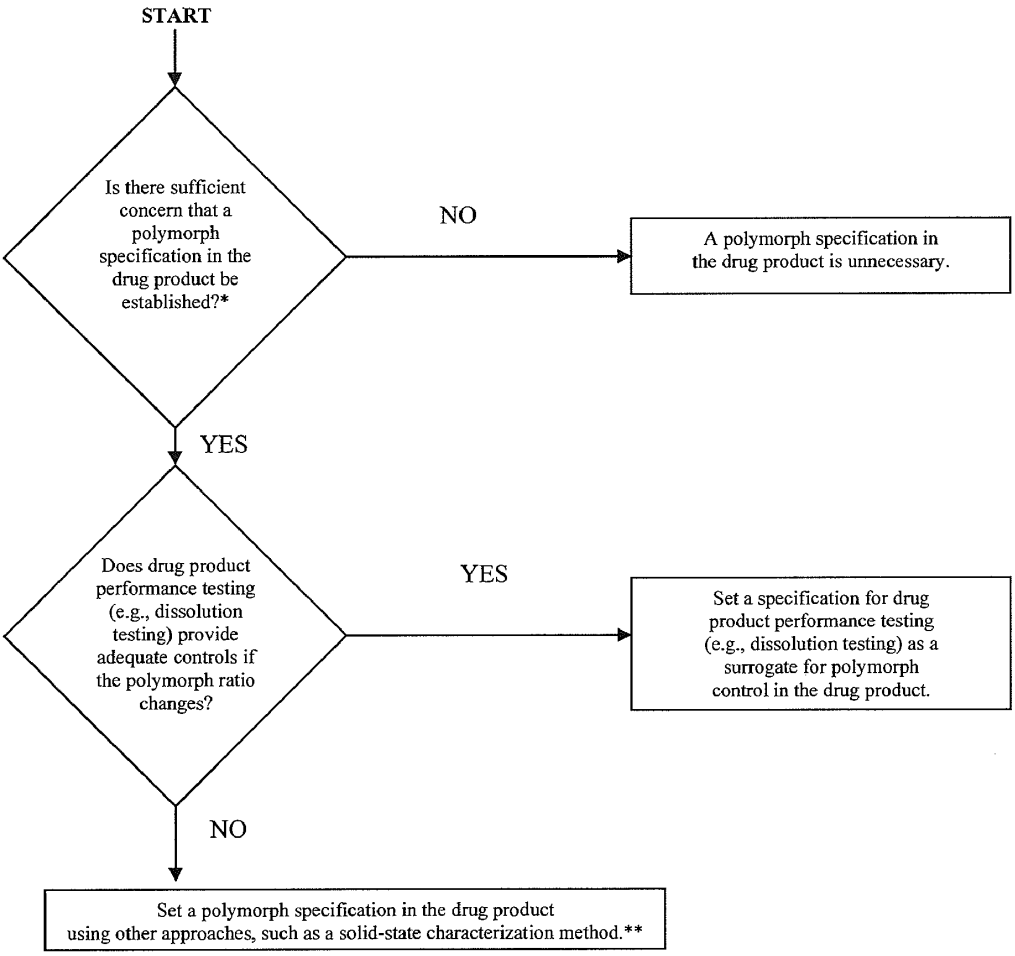
ATTACHMENT 2 – DECISION TREE 2

Decision Tree 2 Setting specifications for polymorphs in drug substances for solid oral and suspension dosage form products.



ATTACHMENT 3 – DECISION TREE 3

Decision Tree 3 Investigating whether to set specifications for polymorphs in drug products for solid oral and suspension dosage form products.



*In general, there may not be a concern if the most thermodynamically stable polymorphic form is used or the same form is used in a previously approved product of the same dosage form.

**Drug product performance testing (e.g., dissolution testing) can generally provide adequate control of polymorph ratio changes for poorly soluble drugs, which may influence drug product BA/BE. Only in rare cases would polymorphic form characterization in the drug product be recommended.

Solid-State Chemistry of Drugs

SECOND EDITION

Stephen R. Byrn
Ralph R. Pfeiffer
Joseph G. Stowell

SSCI, Inc. · West Lafayette, Indiana
www.ssci-inc.com

5

Thermal Methods of Analysis

Thermal analysis generally refers to any method involving heating the sample and measuring the change in some physical property. The most important thermal methods for the study of solid-state chemistry are **thermogravimetric analysis** (TGA), **differential scanning calorimetry** (DSC), and thermal microscopy (discussed in Section 4.4). Thermogravimetric analysis measures the change in the mass of sample as the temperature is changed. Differential scanning calorimetry involves measuring the difference between the temperature of the sample and a reference compound as the temperature of the system is changed, thus providing information on the enthalpy change of various solid-state processes. Thermal methods of analysis are important analytical tools for characterizing pharmaceutical solids. The use of TGA and DSC in conjunction with thermal microscopy (Section 4.4) can elucidate many behaviors of solids.

5.1 THERMOGRAVIMETRIC ANALYSIS (TGA)

Basically, a thermogravimetric instrument consists of a microbalance connected to a sample compartment situated in a small oven with computer-controlled temperature programming. A dry nitrogen atmosphere is most commonly used, however, other gases can be employed (the compostion and flow dynamics of the gas are important perameters.) This method measures the change in mass with temperature and is often used to study the loss of solvent of crystallization or other solid $\rightarrow$ solid + gas reactions. A typical TGA trace is shown in Figure 5.1. In studies of solid-state chemistry, TGA is usually performed in one of three modes:

1. **Isothermal mode**—the temperature is kept constant.
2. **Quasi-isothermal mode**—the sample is heated to a constant mass through a series of increasing temperatures.
3. **Dynamic mode**—the temperature is raised at a known rate, typically linear.

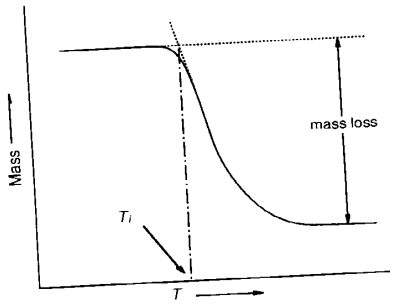


Figure 5.1 A typical TGA trace for a single-state mass loss with T_i (the transition temperature) marked. The temperature corresponding to the point at which tangents to the original baseline and to the slope of the tracing represents the transition temperature T_i.

The last approach uses high heating rates in temperature regions where no weight changes are occurring and slow rates in regions where weight changes do occur, thus avoiding transition temperature overshoot and blurring of peaks from overlapping transitions.

There are a number of factors or conditions that affect TGA curves including the heating rate, atmosphere, geometry of the sample holder (pan), particle size of the sample, nature of the reaction, treatment of the sample, thermal conductivity of the sample, and sample weight. The effect of the heating rate has been extensively studied (Wendlandt, 1974). In general, as the heating rate is increased, the apparent starting temperature of the thermal event (T_i) increases. However, this condition can sometimes be corrected by decreasing the sample size.

The atmosphere can have a dramatic effect on the TGA curve. For example, an atmosphere already containing the product gas can increase T_i or stop the reaction completely. In addition, the atmosphere can change the course of the reaction, particularly if the atmospheric gas reacts with either the products or the reactant. Knowledge of how the substance responds to changes in relative humidity (RH) is essential to proper handling of the sample before the scan is started. For these reasons, it is a prudent practice to use an atmosphere of dry nitrogen when performing a study.

Although dependent on the reaction mechanism, the particle size of the sample has a predictable effect on the TGA curve in general. The smaller the particle size, the faster the reaction and the lower the value of T_i. This is because the smaller particle sizes allow more rapid escape of the product gas. Obviously, the nature of the reaction affects T_i which will be lower for more facile reactions.

In addition, the treatment of the sample, and in particular the extent of compression of the sample, will obviously affect the T_i. For example, increased compression will increase T_i since the product gas will have less opportunity to escape.

Finally, the thermal conductivity of the sample will influence T_i. Anomalous effects may be obtained if the temperature of the sample is not uniform because of poor thermal conductivity.

The rates of reactions of the type shown in Equation 5.1 can be determined using

TGA. Obviously, isothermal TGA traces can be used to determine the rate of the reaction and the rate law governing the reaction by simply plotting weight loss versus time. These plots can then be analyzed as described in Chapter 3. Dynamic TGA has also been used to determine the rates of such gas-evolving reactions. However, in general, the kinetic data thus obtained should be substantiated by other data. Isothermal thermogravimetric analysis has been used extensively in our laboratory to study the desolvation of crystal solvates (Chapter 16).

$$A_{solid} \longrightarrow B_{solid} + C_{gas} \tag{5.1}$$

5.2 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a method which measures the difference in energy (heat flux or heat flow) between a reference (R) and a sample (S). A typical DSC sample compartment is shown in Figure 5.2.

The result of a DSC analysis is a thermogram, a plot of $\Delta T = T_s - T_r$ (temperature

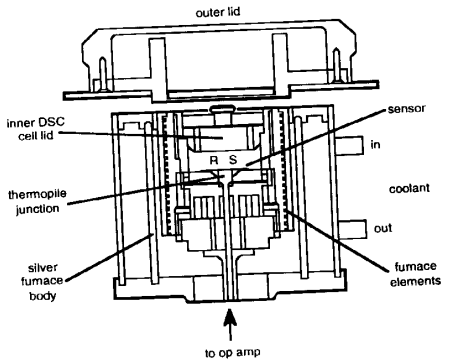


Figure 5.2 Cross section of a Cahn® DSC 4000 cell. The sample pan (S) and the reference pan (R) are positioned in the sensor (Cahn Instruments, 1996).

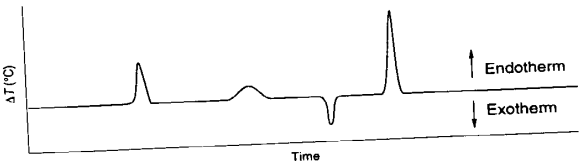


Figure 5.3 A hypothetical DSC thermogram showing the changes that might occur upon heating a sample.

difference) versus T. Figure 5.3 shows an idealized DSC trace. The endotherms represent processes in which heat is absorbed, such as solvent loss, phase transitions, or melting. The exotherms represent processes such as crystallization or chemical reactions where heat is evolved. In addition, the area under a peak is proportional to the heat change involved. Thus this method, with proper calibration, can be used to determine the enthalpies (ΔH)) of the various processes. The method can also be used as an accurate measure of the melting point and purity of the sample. In fact, the change of melting point is related to the mole fraction of impurities as given by Equation 5.2:

$$T_s = T_0 - \frac{T_0^2 R X_i}{F \Delta H_f} \tag{5.2}$$

where T_s is the sample temperature, T_0 is the melting point of the pure compound, R is the gas constant, X_i is the mole fraction of the impurity, F is the fraction of the solid melted, and ΔH_f is the enthalpy of fusion of the pure compound. According to the equation, a plot of T_s versus $1/F$ should give a straight line whose slope is proportional to X_i (Brittain *et al.*, 1991). However, the equation appears to fail when purity is less than 97%. Application of this equation is illustrated by the DSC thermograms shown in Figure 5.4.

There are a number of factors other than purity that can affect the DSC curve including heating rate, atmosphere, sample holder, particle size, and sample packing. In general, a greater heating rate will cause a shift of the peaks to higher temperatures. A decreased heating rate also usually causes endotherms and exotherms to become sharper. The shape of the sample holder and whether it is open, totally sealed, or contains a pin prick to vent gases can also affect a DSC curve. When a DSC experiment is performed in a closed pan, the resulting atmosphere within the sample holder can greatly affect the resulting DSC curve. Obviously, a tightly sealed sample holder would not allow vapor to escape, thereby changing the behavior or mechanism of a

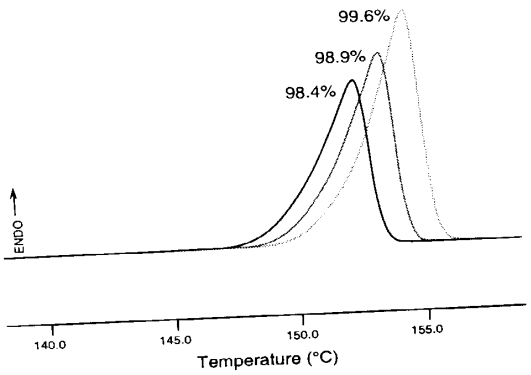


Figure 5.4 DSC thermograms of three ethoxycarbonyl-3-phenylpropyl-L-alanine samples of varying purity from different manufacturers (Giron, 1990).

desolvation processes. As with TGA, the particle size and packing of the sample has an important influence on reactions especially those of desolvation type. Any changes that affect the rate of heat transfer should also be taken into account. Thus a sample that has sublimed or melted and then recrystallized may show somewhat different DSC properties upon reheating.

Two definitions are often used to describe the relationship between the relative energies of polymorphs at different temperatures: **monotropic** and **enantiotropic**. In a monotropic system, one form is the thermodynamically stable form regardless of the temperature. In an enantiotropic system, one form is more stable below a certain (transition) temperature but another form is more stable above that temperature. Thus, high temperature recrystallization may lead to one form, whereas recrystallization at room temperature could lead to the other form. Enantiotropic systems can sometimes cause confusion and problems with crystallization. In general, to determine whether a system is enantiotropic or monotropic it would be helpful to construct an energy-termperature diagram. Burger and Ramberger (1979a–b) have constructeded two reliable rules which assist in determining whether a system is enantiotropic or monotropic using thermoanalytical results:

1. The **heat** (or **enthalpy**) **of transition rule** states that (a) if an endothermic transition is observed between the forms at some temperature it may be assumed that the two forms are related enantiotropically and (b) if an exothermic transition is observed between the forms at some temperature it may be assumed that the two forms are related monotropically.
2. The **heat** (or **enthalpy**) **of fusion rule** states that if the higher melting form has the lower heat of fusion then the two forms are related enantiotropically, otherwise they are related monotropically.

Based on this work, Grunenberg *et al.* (1996) expanded these rules with the **entropy of fusion rule** (particularly necessary for polymorphs with very different melting points but similar ethalpies of fusion) and a **heat capacity rule**. Since only about forty energy-temperature diagrams for pharmaceutical systems have been published, much more work needs to be done. In related studies, Behme and Brook (1991) calculated the heat of fusion of the lower melting of an enantiotropically related pair of polymorphs(based on the heat of transition and the heat capacities) and demonstrated the applicability of thermodynamic calculations.

DSC is also useful for studies of polymorphic mixtures. Figures 5.5 and 5.6 show the DSC scans of propyphenazone. Figure 5.5 shows the DSC scans of batches containing mixtures of Forms I and II indicating that DSC can detect as little as 5% of the higher melting form in the mixtures (Giron-Forest *et al.*, 1989). Trace A in Figure 5.6 shows pure Form I, trace B shows a mixture of Forms I and II, and trace C shows this same mixture after heating at 100°C for two days indicating that the higher melting form is converted to the lower melting form under these conditions. In a more extensive study of mixtures, (Giron, 1986) showed that DSC could be used to quantitate mixtures of polymorphs as shown in Figure 5.7. The left panel in Figure 5.7 shows the DSC thermograms of Forms I and II of a pharmaceutical; the right panel shows that DSC can be used to analyze mixtures of these two forms (Giron, 1986).

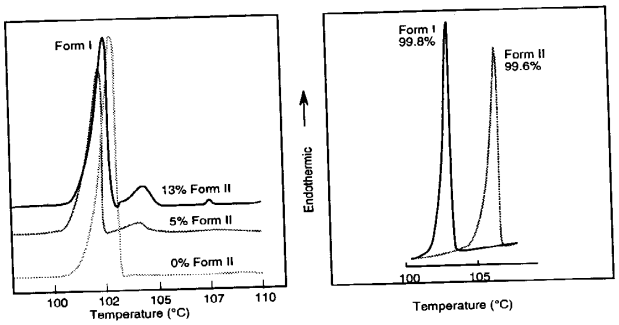


Figure 5.5 Melting behavior of batches containing propyphenazone which are mixtures of Form I with a small amount of Form II (Giron-Forest *et al.*, 1989).

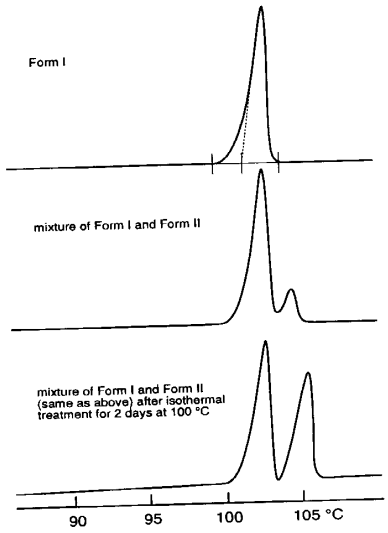


Figure 5.6 DSC scans of propyphenazone pure Form I and mixtures of Form I and Form II before and after isothermal treatment (Giron-Forest *et al.*, 1989).

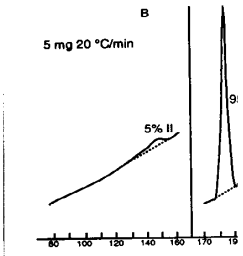
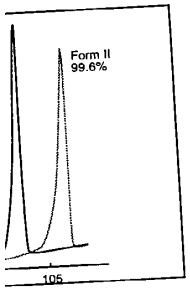


Figure 5.7 Determination of

Thermal methods have
ity (Giron, 1990). In this
varying between 10:1 and
of the heated samples is us
and the results are compar
should reflect the actual p
determine incompatibilities
the DSC thermograms of
formation; thus, a change
tive of a stability problem
One advantage of DS
thus, a study over a wid
however, will have to be
useful in the study of solic
combined with other tech
HPLC.

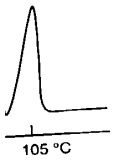
5-3 MICROCALORIMET

Microcalorimetry is a ver
given off or taken up by v
try is used, for example,
every transformation, eith
of heat, this method has si
baum and McGraw (1985
different crystal forms ha
However, the difference b
solvents should remain t
difference is the heat of tr



nperature (°C)

zone which are mixtures of Form I
1989).



d mixtures of Form I and Form II before
al., 1989).

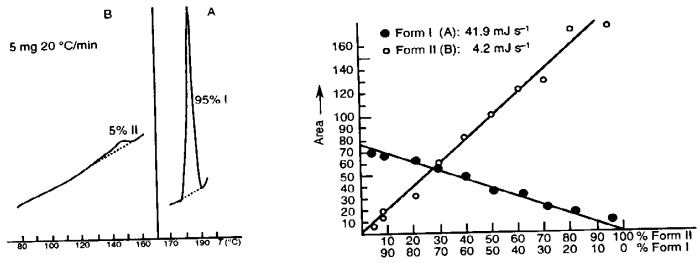


Figure 5.7 Determination of ratios of Forms I and II of a pharmaceutical (Giron, 1986).

Thermal methods have been successfully used to study drug-excipient compatibility (Giron, 1990). In this procedure, drug and excipient are intimately mixed in ratios varying between 10:1 and 1:10 and each mixture is analyzed by DSC. HPLC analysis of the heated samples is used to interpret any changes in the DSC profile of the mixture and the results are compared with those of the pure components. The ratios analyzed should reflect the actual proportions in the formulation; however, it is instructive to determine incompatibilities at other concentrations as well. It is important to note that the DSC thermograms of mixtures will show some changes simply from eutectic formation; thus, a change in DSC melting point for a drug and excipient is not indicative of a stability problem by itself.

One advantage of DSC is that the sample is subjected to different temperatures; thus, a study over a wide temperature range can be rapidly carried. Most results, however, will have to be confirmed by using other methods. Thermal methods are useful in the study of solids but the power of these methods is greatly enhanced when combined with other techniques such as X-ray powder diffraction, microscopy, and HPLC.

5.3 MICROCALORIMETRY

Microcalorimetry is a very sensitive calorimetric technique that determines the heat given off or taken up by various processes. For pharmaceutical solids, microcalorimetry is used, for example, to measure heats of solution and degradation rates. Since every transformation, either chemical or physical, occurs with evolution or absorption of heat, this method has significant potential for the study of transformations. Lindenbaum and McGraw (1985) have used microcalorimetry to study drug forms. Because different crystal forms have different structures, they have different heats of solution. However, the difference between the heats of solution of two polymorphs in different solvents should remain the same (Table 5.1) if there is no solvate formation. This difference is the heat of transition between the forms at that temperature.

Table 5.1 Heats of Solution of Sodium Sulfathiazole

Solvent	ΔH_s Form I (kJ/mol, 25 °C)	ΔH_s Form II (kJ/mol, 25 °C)	ΔH_{trans} (kJ/mol, 25 °C)
Acetone	11.94	5.144	6.798
DMF	−4.659	−11.47	6.810

Lindenbaum and McGraw, 1985.

Studies by Ip *et al.* (1986) on enalapril maleate give similar results showing that the heats of transition between the two forms determined by subtraction of the heats of solution in two different solvents are within the experimental error. With suitable calibration of known mixtures, this phenomenon can sometimes be the basis for analyzing mixtures of polymorphs or crystalline and amorphous forms of a compound. Of course these comparisons apply only to solids with the same composition (*i.e.*, when the resulting solutions are identical). Also, a hydrate and an anhydrate cannot be compared since the heat of the solution of water will be different in different solvents and thus the ΔH_{trans} will be different.

Isothermal microcalorimetry has also been used to determine the crystallinity of mixtures of amorphous and crystalline antibiotics as shown in Figure 5.8 (Thompson *et al.*, 1994). DSC could not be used since the samples decomposed prior to melting. In contrast to studies by Osawa and coworkers (1988) as well as Pikal and coworkers (1978), it was found that the heat of solution was not dependent on water content. The importance of initial water content is probably greatest when dealing with hydratable ionic species since sodium and quaternary ammonium salts have very high heats of hydration (see Figure 5.9).

Several important papers on the use of microcalorimetry for stability determinations have appeared. Hansen *et al.* (1989) studied the kinetics of decomposition of lovastatin and other HMG-CoA reductase inhibitors using **heat conduction calorimetry** (the response of the instrument is directly proportional to the rate of heat produced in the sample cell). Heat conduction calorimetry has a substantial advantage over

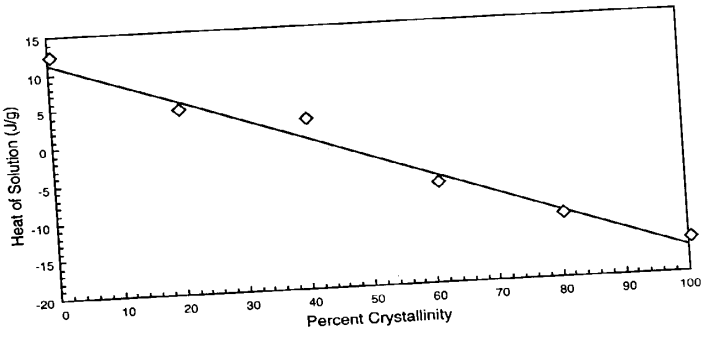


Figure 5.8 Heat of solution of antibiotic BO2669 in 0.02 *M* Na_2HPO_4 at 35 °C as a function of percent crystallinity (Thompson *et al.*, 1994).

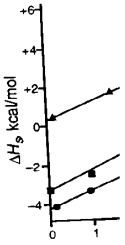


Figure 5.9 The effect of w

conventional microcalor
μW) can be detected. T
determined after only a
urement of degradation
temperature. The rate
calorimeters can also b
excipients and stabilize
rimetry to establish that
atmospheres only a sn
under oxygen atmosph
atmospheres. Further
change was about –40
oxidation. Bond ener
group would produce
tion microcalorimetry,
area of the sample has
experiments, they sh
produced under identi
oxygen than others.
that a single measuren
used to predict the tot
conduction microcalo
cases and appears to

REFERENCES

Behme, Robert J. and Da
of carbamazepine th
analysis" *J. Pharm.*

Brittain, Harry G., Susa
Ann W. Newman
963–973.

Burger, A. and R. Raml
crystals. I. Theory

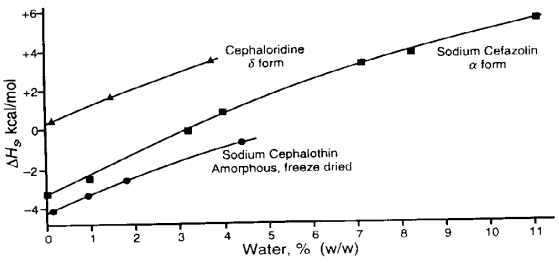


Figure 5.9 The effect of water content on the heats of solution of antibiotics (Pikal *et al.*, 1978).

conventional microcalorimetric methods in that extremely small outputs of heat (±0.1 μW) can be detected. The heat of decomposition and the kinetics of the process can be determined after only a very small percentage of reaction. This then allows the measurement of degradation of the material in the early stages of the reaction even at room temperature. The rate law and the activation energy can also be determined. These calorimeters can also be used to study freshly formulated materials and the effects of excipients and stabilizers on degradation. Hansen *et et al.* (1989) also used microcalorimetry to establish that oxygen was required for degradation of lovastatin since in inert atmospheres only a small amount of heat was produced whereas the heat produced under oxygen atmosphere was 20–90 times greater than that produced under inert atmospheres. Furthermore, they used the heat produced to estimate the enthalpy change was about –400 kJ mol^{-1} which is consistent with what one might expect for oxidation. Bond energy calculations show that reaction of oxygen with a methylene group would produce an enthalpy change of about –600 kJ mol^{-1}. Using heat conduction microcalorimetry, Hansen and coworkers were also able to show that the surface area of the sample has an effect on the rate of oxidation, as might be expected. In other experiments, they showed that there was significant lot-to-lot variation in the heat produced under identical conditions. Some lots showed much greater reactivity with oxygen than others. One of the most significant results of this study was the finding that a single measurement of the heat produced per gram of drug for each lot could be used to predict the total degradation of that lot under conventional stability testing. Heat conduction microcalorimetry has been shown to have predictive capability in some cases and appears to be an important addition to other stability studies.

REFERENCES

Behme, Robert J. and Dana Brooke (1991) "Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry analysis" *J. Pharm. Sci.* **80** 986–990.

Brittain, Harry G., Susan J. Bogdanowich, David E. Bugay, Joseph DeVincentis, Geoffrey Lewen, and Ann W. Newman (1991) "Physical characterization of pharmaceutical solids" *Pharm. Res.* **8** 963–973.

Burger, A. and R. Ramberger (1979a) "On the polymorphism of pharmaceuticals and other molecular crystals. I. Theory of thermodynamic rules" *Mikrochim. Acta* **11** 259–271.

Burger, A. and R. Ramberger (1979b) "On the polymorphism of pharmaceuticals and other molecular crystals. II. Applicability of Thermodynamic rules" *Mikrochim. Acta* **11** 273–316.

Cahn Instruments (1996) 5225 Verona Road, Bldg. 1, Madison WI 53711-4418.

Giron, D. (1986) "Applications of thermal analysis in the pharmaceutical industry" *J. Pharm. Biomed. Anal.* **4** 775–770.

Giron, Daniele (1990) "Thermal analysis in pharmaceutical routine analysis" *Acta Pharm. Jugosl.* **40** 95–157.

Giron-Forest, D., Ch. Goldbronn, and P. Piechon (1989) "Thermal analysis methods for pharmacopoeial materials" *J. Pharm. Biomed. Anal.* **7** 1421–1433.

Grunenberg, A., J.-O. Henck, and H. W. Siesler (1996) "Theoretical deviation and practical application of energy/temperature diagrams as an instrument in performulation studies of polymorphic drug substances" *Int. J. Pharm.* **129** 147–158.

Hansen, Lee D., Edwin A. Lewis, Delbert J. Eatough, Robert G. Bergstrom, and Damaris DeGraft-Johnson (1989) "Kinetics of drug decomposition by heat conduction calorimetry" *Pharm. Res.* **6** 20–27.

Ip, Dominic P., Gerald S. Brenner, James M. Stevenson, Siegfried Lindenbaum, Alan W. Douglas, S. David Klein, and James A. McCauley (1986) "High resolution spectroscopic evidence and solution calorimetry studies on the polymorphs of enalapril maleate" *Int. J. Pharm.* **28** 183–191.

Lindenbaum, Siegfried and Scott E. McGraw (1985) "The identification and characterization of polymorphism in drug solids by solution calorimetry" *Pharm. Manufacturing* 27–30.

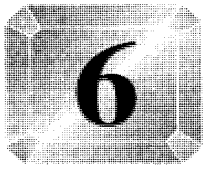
Pikal, Michael J. and Karen D. Dellerman (1989) "Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25 °C: cephalosporins in the solid and aqueous solution states" *Int. J. Pharm* **50** 233–252.

Pikal, M. J., A. L. Lukes, John E. Lang, and K. Gaines (1978) "Quantitative crystallinity determinations for β-lactam antibiotics by solution calorimetry: correlations with stability" *J. Pharm. Sci.* **67** 767–773.

Osawa, Takashi, Madhav S. Kamat, and Patrick P. DeLuca (1988) "Hygroscopicity of cefazolin sodium: application to evaluate the crystallinity of freeze-dried products" *Pharm. Res.* **5** 421–425.

Thompson, Karen C., Jerome P. Draper, Michael J. Kaufman, and Gerald S. Brenner (1994) "Characterization of the crystallinity of drugs: BO2669, a case study" *Pharm. Res.* **11** 1362–1365.

Wendlandt, Wesley W. (1974) *Thermal Methods of Analysis*, 2nd ed.; John Wiley and Sons: New York, NY; pp 9–13.



Solubilit Testing

The rate of disso important aspe and solubility same drug can obvious necessary for both pure have the proper dissoluti 1991; Banakar, 1992). dissolution testing and *USP-NF* (United States

6.1 TESTING

Dissolution tests are sp individual drugs. For e specification that 80% carbamazepine tablets m be dissolved in 60 m laboratories now measu variations. For dissolu variables (*e.g.*, time pr chosen carefully.

Dissolution tests a potential for bioequival are usually compounds disperse. Examples i digoxin, diphenylhyd quinidine, and warfarin. to ensure that the Unite

Kawakami

treprostinil

Electronic Patent Application Fee Transmittal	
Application Number:	14754932
Filing Date:	30-Jun-2015
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Filer:	Stephen Bradford Maebius/Karen Strawderman
Attorney Docket Number:	080618-1550
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**340**

Electronic Acknowledgement Receipt	
EFS ID:	27282080
Application Number:	14754932
International Application Number:	
Confirmation Number:	1865
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Karen Strawderman
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1550
Receipt Date:	21-OCT-2016
Filing Date:	30-JUN-2015
Time Stamp:	10:25:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$340
RAM confirmation Number	102116INTEFSW10264300
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		ReqReconsideration.pdf	106733 25da64a13acca8673409 4860fc9bed9d249 3ac0d	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Response After Final Action		1	1	
	Applicant Arguments/Remarks Made in an Amendment		2	3	
Warnings:					
Information:					
2	Terminal Disclaimer Filed	TerminalDisclaimer.pdf	129824 167a3ccaf4496e5f250ba3b8cf31815ba8b1 08c5	no	2
Warnings:					
Information:					
3		IDS.pdf	169245 9f14750187e32bd007f811f66eb1796f7f65 ef50	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	3	
Warnings:					
Information:					
4	Other Reference-Patent/App/Search documents	PetitionerReplyExhibits.pdf	13659957 897263441a6cca3c6e002b07d5bb97ded57 975da	no	322
Warnings:					

Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	32494 3ed16f3115c35f4f2105d598b99d29574a9f1c0f	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	14098253	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/754,932	06/30/2015	Hitesh Batra	080618-1550	1865

22428 7590 10/19/2016
Foley & Lardner LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC 20007-5109

EXAMINER	
VALENROD, YEVGENY	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
10/19/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipdocketing@foley.com

Office Action Summary	Application No. 14/754,932	Applicant(s) BATRA ET AL.	
	Examiner YEVGENY VALENROD	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *8/11/16*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1,6 and 8-14* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☒ Claim(s) *1,6 and 8-12* is/are allowed.

7)☒ Claim(s) *13-14* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Rejection of claims 1-3, 6, 8 and 9 under 35 USC102(b) as anticipated by Moriarty et al is withdrawn in view applicants' arguments, amendments and the accompanying declarations.

Rejection of claims 10-12 under 35 USC 103(a) over Moriatry in view of Phares are withdrawn in view of applicants' arguments, amendments and the accompanying declarations

Maintained Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*,

686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/forms/. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 13-14 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 24 and 26 of U.S. Patent No. 8,242,305 (‘305). Although

the claims at issue are not identical, they are not patentably distinct from each other because:

Claim 24 of '305 is directed to a process for the preparation of compound IV (treprostinil). Said method comprises alkylation od benzindene triol to prepare compound (VI) followed by hydrolyzing compound (VI) and contacting the hydrolysis product with a base. In claim 26 the contacting base is diethanolamine.

Conclusion

Claims 1, 6, 8-14 are pending

Claims 1, 6, 8-12 are allowed

Claims 13-14 are rejected

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to YEVGENY VALENROD whose telephone number is (571)272-9049. The examiner can normally be reached on mon-fri 8-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on 571-572-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YEVGENY VALENROD/
Primary Examiner, Art Unit 1672

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14754932	BATRA ET AL.
	Examiner	**Art Unit**
	YEVEGENY VALENROD	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	07/28/2015	09/10/2015	02/04/2016	10/14/2016					
	1	✓	=	✓	=					
	2	✓	=	✓	-					
	3	✓	=	✓	-					
	4	✓	-	-	-					
	5	✓	-	-	-					
	6	✓	=	✓	=					
	7	✓	-	-	-					
	8	✓	=	✓	=					
	9			✓	=					
	10			✓	=					
	11			✓	=					
	12			✓	=					
	13			✓	✓					
	14			✓	✓					

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14754932	BATRA ET AL.
	Examiner	Art Unit
	YEVEGENY VALENROD	1672

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST	10/14/2016	YV
Inventor	10/14/2016	YV

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
C07C			

	/YEVEGENY VALENROD/ Primary Examiner.Art Unit 1672

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	("8497393").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L2	1	("8242305").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L3	1	("4683330").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L4	1	("4306075").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L5	32	((Hitesh) near2 (Batra)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L6	24	((Sudersan) near2 (Tuladhar)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L7	30	((Raju) near2 (Penmasta)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L8	245	((David) near2 (Walsh)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L9	273	L5 or L6 or L7 or L8	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L10	24	L9 and treprostinil	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L11	531	c07c59/72.cpc.	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L12	870	(562/466).CCLS.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L13	1274	L11 or L12	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2016/10/14 14:12
L14	44	L13 and treprostinil	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12

EAST Search History (Prior Art)

L15	40	L14 and purity	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L16	37	L15 and HPLC	US-PGPUB; USPAT; USOCR	OR	ON	2016/10/14 14:12
L17	1	("6765117").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L18	2	wo "2005007081"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2016/10/14 14:12
L19	2	"9242350"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2016/10/14 14:12
L20	1	("8242305").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12
L21	1	("9156786").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/10/14 14:12

EAST Search History (Interference)

		<This search history is empty>				

To: ipdocketing@foley.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 22428

Oct 19, 2016 05:56:31 AM

Dear PAIR Customer:

Foley & Lardner LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC 20007-5109
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 22428 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
14754932	CTFR	10/19/2016	080618-1550

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/754,932
Filing Date:	6/30/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	1865

AMENDMENT & REQUEST FOR RECONSIDERATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This amendment is submitted in response to the outstanding, non-final Office Action mailed on February 11, 2016.

Amendments to the Claims are reflected in the listing of claims that begins on page 2 of this document.

Remarks begin on page 4 of this document.

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A pharmaceutical batch ~~comprising~~ consisting of treprostinil or a salt thereof and impurities resulting from ~~prepared by~~ (a) alkylating a benzindene triol, (b) hydrolyzing the product of step (a) to form a solution comprising treprostinil, (c) contacting the solution comprising treprostinil from step (b) with a base to form a salt of treprostinil, (d) isolating the salt of treprostinil, and (e) optionally reacting the salt of treprostinil with an acid to form treprostinil, and

~~,~~wherein the pharmaceutical batch contains at least 2.9 g of treprostinil or its salt.

2.-5. (Canceled)

6. (Currently Amended) The pharmaceutical batch of claim 1, which has been dried under vacuum.

7. (Canceled)

8. (Currently Amended) A pharmaceutical product comprising a therapeutically effective amount of treprostinil from a pharmaceutical batch as claimed in claim 1.

9. (Currently Amended) A pharmaceutical product comprising a therapeutically effective amount of a salt of treprostinil from a pharmaceutical batch as claimed in claim 1.

10. (Currently Amended) The product of claim 9, wherein the salt is the diethanolamine salt of treprostinil.

11. (Currently Amended) A method of preparing a pharmaceutical product from a ~~high purity~~ pharmaceutical batch as claimed in claim 1, comprising storing a pharmaceutical batch of a salt of treprostinil as claimed in claim 1 at ambient temperature, and preparing a pharmaceutical product from the pharmaceutical batch after storage.

12. (Previously Presented) A method as claimed in claim 11, wherein the salt of treprostinil is a diethanolamine salt.

13. (Currently Amended) A method of preparing a ~~high purity~~ pharmaceutical batch as claimed in claim 1, comprising (a) alkylating a benzindene triol, (b) hydrolyzing the product of step (a) to form a solution comprising treprostinil, (c) contacting the solution comprising treprostinil from step (b) with a base to form a salt of treprostinil, (d) isolating the salt of treprostinil, and (e) optionally reacting the salt of treprostinil with an acid to form treprostinil.

14. (Previously Presented) A method as claimed in claim 13, wherein the salt of treprostinil is a diethanolamine salt.

REMARKS

Applicants respectfully request reconsideration and allowance of the present application.

Status of Claims

Applicants have amended claim 1 to recite a "pharmaceutical" batch, "consisting of" as the transitional phrase, and "impurities" resulting from the recited steps. Conforming amendments are made to claim 13 and dependent claims. Support for these amendments can be found in the Examples 4-6 of the specification. No new matter has been added. Claims 2 and 3 are canceled. Applicants reserve the right to file one or more continuing applications directed to any subject matter omitted by the present amendment.

After the amendment, claims 1, 6, and 8-14 are pending.

Interview

Applicants thank the Examiner for the courtesy of the interview held on July 22, 2016, during which the presently-presented amendments were discussed. Applicants have followed the Examiner's suggestions for amendments and additionally address the points discussed at the Interview in the remarks below.

35 U.S.C. § 102

Claims 1-3, 6, 8, and 9 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Moriarty (2004). Applicants respectfully request reconsideration of the rejection.

Applicants filed a notification of related proceedings to bring to the Examiner's attention documents from IPR2016-00006, which involves parent U.S. Patent No. 8,497,393. Certain information is redacted in those documents due to confidentiality. Documents provided in that notification include the Patent Owner's Response and expert declarations from Dr. Williams and Ruffolo. These documents address the subject matter of the '393 patent claims although certain information is relevant to the present claims as explained herein.

Claim 1 recites steps (a)-(e), which read on a commercial process used by the assignee of the present application. Prior to the current commercial process, the assignee used a process

based on Moriarty 2004. Because the assignee used both processes, the assignee had the opportunity to analyze the resulting products as reflected in certificates of analysis. In the IPR, Dr. Williams and Dr. Ruffolo used these certificates of analysis to explain that a pharmaceutical batch produced according to steps (a)-(e) of claim 1 is different from the product produced by the process described in Moriarty 2004. Williams Dec. at ¶¶94-99; Ruffolo Dec. at ¶¶66-72. Specifically, the processes result in products having different impurity profiles, and in fact, the pharmaceutical batch of claim 1 has higher average purity. Patent Owner's Response at Section III.C.

The differences are not merely academic, but critical to the successful manufacture of a clinical product. FDA uses both overall purity and levels of individual impurities ("purity specification") as a basis to regulate the manufacturing of pharmaceuticals. Batches that fall outside of the purity specification cannot be sold or used to treat patients. As noted in the Patent Owner's IPR Response, the differences between claim 1's pharmaceutical batch and a product produced according to the process of Moriarty were significant enough to result in FDA's acceptance of a new purity specification for the commercial product, thus proving that the products are not the same in the eyes of the FDA. Patent Owner's Response at Section III.C. Furthermore, this change constitutes a "major" change according to the classification system for manufacturing changes used by FDA. Ruffolo Dec. at ¶¶70- 72. Clearly, the pharmaceutical batch of claim 1 differs from the product resulting from Moriarty's synthesis.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 102(b) is requested.

35 U.S.C. § 103

Claims 10-12 stand rejected under 35 U.S.C. § 103(a) as obvious over Moriarty (2004) in view of Phares (WO 2005/007081 A2). Applicants respectfully request reconsideration of the rejection.

The rejection cites Phares for showing that it would have been obvious to form a diethanolamine salt using Moriarty's treprostinil. However, the differences in the resulting products, as explained above, would not have been expected based on the prior art. In particular, it would not have been obvious to use the salt formation step of Phares to decrease amounts of

stereoisomer impurities of treprostinil, which are acidic rather than neutral or basic. Williams Dec. at ¶102. When subject to salt-forming conditions, one of ordinary skill in the art would expect that any undesired stereoisomer of treprostinil would be included in the final salt product because the stereoisomer would also be converted to the corresponding salt under such salt-forming conditions. One of ordinary skill in the art would have had no reasonable expectation of success in removing any undesired treprostinil stereoisomer impurities by salt formation and subsequent regeneration of the free acid.

In addition, FDA's decision to adopt a new purity specification for the resulting product further establishes unobviousness of the presently claimed invention. Indeed, as noted above, the specification change is classified as a "major" change according to the FDA's classification system for manufacturing changes. *See Knoll Pharm. Co., Inc. v. Teva. Pharm. USA, Inc.*, 367 F.3d 1381, 1385 (Fed. Cir. 2004) (explaining that while FDA approval is not determinative of nonobviousness, it can be relevant in evaluating the objective indicia of nonobviousness). As noted in Dr. Ruffolo's Declaration, even small changes in impurity are important to FDA: "Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of safety to patients." Ruffolo Dec. at ¶36. This is due to the fact that even trace amounts of impurities can sometime pose serious health concerns.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 103(a) is requested.

Double Patenting

Claims 13-14 have been rejected for non-statutory double patenting as unpatentable over claims 24 and 26 of US Patent No. 8,242,305. Applicants will address the rejection by filing a terminal disclaimer if still necessary after the above amendments upon confirming that the present claims are otherwise in condition for allowance.

Concluding Remarks

Applicants believe that the application is in condition for allowance. Favorable reconsideration is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance prosecution.

The Commissioner is hereby authorized to charge any additional fees that may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing or a credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. § 1.136 and authorize payment of any such extension fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date Aug. 11, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/754,932
Filing Date:	6/30/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	1865

PETITION FOR EXTENSION OF TIME

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant hereby petitions the Commissioner under 37 C.F.R. §1.136(a) for a three-month extension of time for response in the above-identified application for the period required to make the attached response timely.

The extension fee for response within the third month is $1,400.00.

The above-identified fees of $1,400.00 are being paid by credit card via EFS-Web.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to

Deposit Account No. 19-0741.

Respectfully submitted,

Date Aug. 11, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

Electronic Patent Application Fee Transmittal	
Application Number:	14754932
Filing Date:	30-Jun-2015
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Filer:	Stephen Bradford Maebius/Mary Jo Boyce
Attorney Docket Number:	080618-1550
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
		Total in USD ($)		**1400**

Electronic Acknowledgement Receipt	
EFS ID:	26612937
Application Number:	14754932
International Application Number:	
Confirmation Number:	1865
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Mary Jo Boyce
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1550
Receipt Date:	11-AUG-2016
Filing Date:	30-JUN-2015
Time Stamp:	12:48:24
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1400
RAM confirmation Number	12335
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response.pdf	139386 f085cceff502cc993b4fb25a5df0a8bb344009bd	yes	7
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	3	
	Applicant Arguments/Remarks Made in an Amendment		4	7	
Warnings:					
Information:					
2	Extension of Time	Petition.pdf	96884 23a89f2e7ce89f7a352065a866cd677f9a53acda	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	31180 a3c43360a0c85cf05cec66c3e7177d7dcc9e0d69	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	267450		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/754,932	Filing Date 06/30/2015	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	08/11/2016	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 9	Minus	** 20	=	X $ =	
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
TERRANCE LAWRENCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/754,932	06/30/2015	Hitesh Batra	080618-1550	1865

22428 7590 07/27/2016
Foley & Lardner LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC 20007-5109

EXAMINER	
VALENROD, YEVGENY	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
07/27/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipdocketing@foley.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	14/754,932	BATRA ET AL.	
	Examiner	Art Unit	
	YEVGENY VALENROD	1672	

All participants (applicant, applicant's representative, PTO personnel):

(1) *YEVGENY VALENROD*. (3)_____.

(2) *Stephen Maebius*. (4)_____.

Date of Interview: *22 July 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☒102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1*.

Identification of prior art discussed: *Moriarty et al.*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

A proposed amendment to Claim 1, wherein the phrase "batch comprising" would be replaced with the phrase "pharmaceutical batch consisting of" was discussed. Examiner expressed concern with the "consisting of language" excluding the impurities that are present in the product as a result of the preparatory steps. It was agreed that along with the reply Applicants will submit a declaration that would outline the difference in the impurities of the instant product compared with the product of Moriarty. In addition an amendment to claim 1 that would also generically include the process derived impurities into the "consisting of" grouping of product components. No agreement regarding patentability was reached. .

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/YEVGENY VALENROD/ Primary Examiner, Art Unit 1672	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

To: ipdocketing@foley.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 22428

Jul 27, 2016 05:33:58 AM

Dear PAIR Customer:

Foley & Lardner LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC 20007-5109
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 22428 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
14754932	INTV.SUM.APP	07/27/2016	080618-1550

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/754932
Filing Date:	6/30/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	1865

NOTIFICATION OF RELATED PROCEEDINGS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant hereby updates the Office concerning the status of a related proceeding styled *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, which involves the issued parent of the above-captioned patent application. Other documents from the above-identified Inter Partes Review (IPR) were submitted in the present application with an Information Disclosure Statement filed on December 8, 2015, and a Notification of Related Proceedings filed on February 29, 2016, for the Examiner's consideration. The purpose of this notice is to provide a copy of Patent Owner's Response to Petition and public exhibits filed on July 6 and 13, 2016, and the public Decision

Instituting the IPR from the IPR proceeding. Certain information is redacted and certain exhibits are not provided due to their filing under seal in the IPR proceeding.

Respectfully submitted,

Date JUL 19 2016

By _____

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

Trials@uspto.gov
571-272-7822

Paper No. 28
Entered: May 12, 2016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,
Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,
Patent Owner.

Case IPR2016-00006
Patent 8,497,393 B2

Before LORA M. GREEN, JONI Y. CHANG, and JACQUELINE T. HARLOW, *Administrative Patent Judges.*

HARLOW, *Administrative Patent Judge.*

DECISION
Redacted Institution of *Inter Partes* Review
37 C.F.R. § 42.108

I. INTRODUCTION

Petitioner, SteadyMed LTD ("SteadyMed"), filed a Petition requesting an *inter partes* review of claims 1–22 of U.S. Patent No. 8,497,393 B2 (Ex. 1001, "the '393 patent"). Paper 1 ("Pet."). Patent Owner, United Therapeutics Corporation ("UTC"), filed a Preliminary Response on January 14, 2016. Paper 10[1] ("Prelim. Resp."). We have jurisdiction under 35 U.S.C. § 314, which provides that an *inter partes* review may not be instituted unless the information presented in the petition "shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition."

For the reasons set forth below, we institute an *inter partes* review of claims 1–22 of the '393 patent.

A. Related Matters

The '393 patent is asserted in: *United Therapeutics Corp. v. Sandoz, Inc.*, No. 14-cv-05499 (D.N.J.); *United Therapeutics Corp. v. Teva Pharmaceuticals U.S.A., Inc.*, No. 14-cv-05498 (D.N.J.); and *United Therapeutics Corp. v. Watson Laboratories, Inc.*, No. 15-cv-05723 (D.N.J). Pet. 1. SteadyMed is not party to the above identified litigations. *Id.*

[1] Paper 10 is the Unredacted Preliminary Response. Paper 8, filed concurrently with Paper 10, is a redacted version of the Preliminary Response.

B. The '393 Patent

The '393 patent, titled "Process to Prepare Treprostinil, the Active Ingredient in Remodulin®," issued July 30, 2013, from U.S. Patent Application No. 13/548,446 ("the '446 application") (Ex. 1002), filed July 13, 2012. Ex. 1001, [54], [45], [21], [22]. The '446 application is a continuation of U.S. Patent Application No. 12/334,731 ("the '731 application") (Ex. 1002), filed on December 15, 2008, now issued as U.S. Patent No. 8,242,305 ("the '305 patent"). Ex. 1001, [63]. The '393 patent claims priority to U.S. Provisional Patent Application No. 61/014,232 (Ex. 2008), filed December 17, 2007. Ex. 1001, [60].

The '393 patent recites 22 product-by-process claims for prostacyclin derivatives, including treprostinil.[2] *Id.* at 17:51–21:16; Pet. 5; Prelim. Resp. 3. The process disclosed by the '393 patent takes advantage of carbon treatment and salt formation steps to remove impurities, eliminating the need for purification by column chromatography. *Id.* at 17:29–32; *see also id.* at 5:41–45 ("purification by column chromatography is eliminated . . . . [T]he salt formation is a much easier operation than column chromatography.").

---

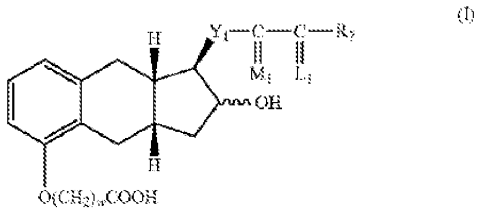
[2] The '305 patent, which issued from the parent to the application for the '393 patent, recites claims to a process for the preparation of prostacyclin derivatives comprising steps similar to those set forth in the product-by-process claims of the '393 patent. *Compare* Ex. 1001, 17:51–21:16, *with* Ex. 2007, 17:39–24:3.

The process for forming prostacyclin derivatives described in the '393 patent includes four steps: (a) alkylating a prostacyclin derivative to form an alkylated prostacyclin derivative; (b) hydrolyzing the alkylated prostacyclin derivative with a base to form a prostacyclin acid; (c) contacting the prostacyclin acid with a base to form a prostacyclin carboxylate salt; and (d) optionally reacting the prostacyclin carboxylate salt formed in (c) with an acid to form the desired compound, or pharmaceutically acceptable salt thereof. *Id.* at 1:65–3:19.

C. Illustrative Claim

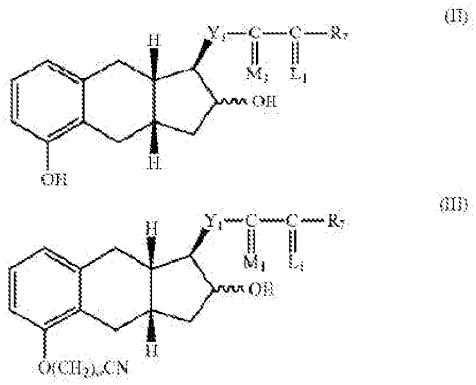
Each of the challenged claims is a product-by-process claim. Of the challenged claims, claims 1 and 9 are independent. Claim 1, reproduced below, is illustrative of the claimed subject matter.

1. A product comprising a compound of formula I



or a pharmaceutically acceptable salt thereof, wherein said product is prepared by a process comprising

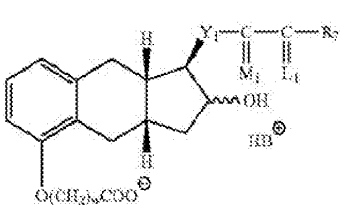
a) alkylating a compound of structure II with an alkylating agent to produce a compound of formula III,



wherein [recitation of Markush groups for the specified structures],

b) hydrolyzing the product of formula III of step (a) with a base,

c) contacting the product of step (h)[3] with a base B to form a salt of formula I_s.



and

d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I.

---

[3] We note that the reference to "step (h)," rather than "step (b)," in claim 1 is an apparent typographical error. *See* Ex. 1001, 3:66–67 ("(c) contacting the product of step (b) with a base B to for a salt of formula IV_s"); *see also* Pet. 25; Ex. 1009 ¶ 51.

Ex. 1001, 17:51–19:29. Claim 9 is drawn to a product comprising a specific treprostinil compound within the genus set forth in claim 1, and made by the process recited in claim 1. *Id.* at 19:48–20:46.

D. Prior Art Relied Upon

SteadyMed relies upon the following prior art references (Pet. 4–6):

Phares	WO 2005/007081 A2	Jan. 27, 2005	(Ex. 1005)
Kawakami	JP 56-122328A	Sept. 25, 1981	(Ex. 1006[4])

Moriarty et al., *The Intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzindene Prostacyclins: Synthesis of UT-15 (Treprostinil)*, 69 J. Org. Chem. 1890–1902 (2004) ("Moriarty") (Ex. 1004); and

Seyhan N. Eğe, ORGANIC CHEMISTRY 543–547 (2d ed. 1989) ("Eğe") (Ex. 1008).

E. Asserted Grounds of Unpatentability

SteadyMed asserts the following grounds of unpatentability (Pet. 3–4):

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 102(b)	Phares
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Phares or Kawakami
6, 10, 15, 21, and 22	§ 103(a)	Moriarty, Phares, Kawakami, and Eğe

[4] SteadyMed submitted a certified English translation of Kawakami as Ex. 1007. As discussed in Part II.F below, UTC argues the admissibility of this translation.

II. ANALYSIS

A. 35 U.S.C. § 325(d)

UTC urges the exercise of our discretion under 35 U.S.C. § 325(d) to deny some or all of the grounds of unpatentability presented by SteadyMed because the same, or substantially similar issues were addressed during prosecution. Prelim. Resp. 25–26. UTC states that the Patent Office considered Moriarty alone, and in combination with Phares, during prosecution of the '393 patent. *Id.* at 8–10, 26. UTC also reports that Phares was considered alone, and in combination with Moriarty, during prosecution of U.S. Patent Application No. 13/910,583 ("the '583 application") (Ex. 2010) filed June 5, 2013, which is a continuation of the '446 application. *Id.* at 11–14.

Regarding the patentability of claims 6, 15, 21, and 22, in particular, UTC asserts that Eğe "is nothing more than a first-year organic chemistry textbook," and that SteadyMed "relies on nothing more than conclusory statements in three paragraphs of the [Declaration of Jeffery D. Winkler]" to support its unpatentability arguments. *Id.* at 26. UTC therefore contends that SteadyMed "has provided no evidence of probative value that is any different than what was already before the Patent Office during prosecution." *Id.* at 26–27.

Although it is within our discretion to "reject the petition or request because, the same or substantially the same prior art or arguments previously were presented to the Office" pursuant to 35 U.S.C. § 325(d), we decline to do so here.

We note that during prosecution of the '446 application, which issued as the '393 patent, the Examiner rejected the claims as anticipated by Moriarty, but subsequently withdrew that rejection, without elaboration, in response to a declaration filed by David A. Walsh ("Walsh Declaration") (Ex. 1002, 346–350), one of the named inventors of the '393 patent, and the Executive Vice President of Chemical Research and Development at UTC. Ex. 1002, 344, 346–360. Although Phares is listed as a cited reference on the face of the '393 patent (Ex. 1001, [56]), we observe that the Examiner neither relied on, nor otherwise discussed Phares during prosecution of the '446 application (Ex. 1002, 295–296, 327–330, 359). In addition, neither Eğe nor Kawakami was considered during prosecution of the '446 application. *Id.* at 235–359. The grounds of unpatentability asserted in the instant Petition likewise differ from the rejections entered by the Examiner during prosecution of the '731 application, the parent to the '446 application. *See* Ex. 1002, 122–124.

Moreover, as discussed in detail in Part II.B below, the Declaration of Jeffrey D. Winkler ("Winkler Declaration") (Ex. 1009), submitted in support of SteadyMed's Petition, calls into question Dr. Walsh's conclusion that treprostinil prepared according to the process claimed in the '393 patent is "physically different from treprostinil prepared according to the process of 'Moriarty'" (Ex. 1002, 347 (¶ 6)). Ex. 1009 ¶¶ 63–71. In addition, as set forth in Part II.F, we disagree with UTC's characterization of Dr. Winkler's testimony as conclusory. *See, e.g.,* Ex. 1009 ¶¶ 80–90.

We, therefore, decline to exercise our discretion to deny the Petition pursuant to 35 U.S.C. § 325(d). *See Nestle USA, Inc. v. Steuben Foods, Inc.*, Case IPR2014-01235, slip op. at 7 (PTAB Dec. 22, 2014) (Paper 12) ("[W]e conclude that Petitioner's arguments regarding the unpatentability of claims 18–20, which include arguments relating to Biewendt and a combination of references previously not considered and supported by a declaration previously not considered, are persuasive. . ."); *Merial Ltd., v. Virbac*, Case IPR2014-01279, slip op. at 9 (PTAB Jan. 22, 2015) (Paper 13) (noting the different burdens of proof and evidentiary standards applicable to *ex parte* examination and *inter partes review* proceedings).

B. Claim Construction

In an *inter partes* review, claim terms in an unexpired patent are given their broadest reasonable interpretation in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); *see also In re Cuozzo Speed Techs., LLC*, 793 F.3d 1268, 1278–79 (Fed. Cir. 2015) ("Congress implicitly approved the broadest reasonable interpretation standard in enacting the AIA," and "the standard was properly adopted by PTO regulation."), *cert. granted sub nom. Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 890 (2016) (mem.). Under this standard, we may take into account definitions or other explanations provided in the written description of the specification. *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997). Any special definition for a claim term must be set forth in the specification with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d

1475, 1480 (Fed. Cir. 1994). Only those terms that are in controversy need be construed, and only to the extent necessary to resolve the controversy. *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999).

"Product" / "A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof"

Independent claims 1 and 9 recite the phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof . . . ." Ex. 1001, 19:48–20:46. In addition, each challenged dependent claim recites the term "product." *Id.* at 17:51–21:16. Because the parties advance similar arguments pertaining to the construction of these terms, we address these terms together.

SteadyMed asserts that the phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof" should be interpreted to mean "a chemical composition that includes, but is not limited to, a compound of Formula I, or a pharmaceutically acceptable salt thereof, and that may also include other non-mentioned substances (including impurities), additives, or carriers, without limitation as to the types or relative amounts thereof." Pet. 11. SteadyMed contends that because independent claims 1 and 9 recite "[a] product comprising," the claim term "product" should be construed to include "the treprostinil compound along with other substances (including impurities)," i.e., a "chemical composition." *Id.* at 11.

UTC counters that "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof" should be interpreted as "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof." Prelim. Resp. 21. As an initial matter, UTC notes that SteadyMed's proposed construction refers only to Formula I, and asserts that SteadyMed "inexplicably read[s] Formula IV out of the term entirely." *Id.* at 22.

UTC further argues that the claims and Specification of the '393 patent use "product" to refer to a substance resulting from a chemical reaction. *Id.* at 17. UTC also contends that the prosecution history for the '393 patent supports its proposed construction because "during prosecution, the Patent Owner and Examiner explicitly discussed the 'product' of the claims as a real world substance that results from employing a specific chemical process, as differentiated from the substance obtained from employing a different chemical process." *Id.* at 18–19. UTC points to chemistry textbooks as buttressing its position that a skilled artisan would understand the claim term "product" as referring to "a substance resulting from a chemical reaction." *Id.* at 19. UTC further reasons that "the 'product' claimed in a product-by-process claim is necessarily a substance that results from the process specified in that claim" (*id.*), and that SteadyMed's proposed construction "contradicts this inherent limitation of the claims" (*id.* at 22).

On this record, and for purposes of this decision, we interpret the phrase "[a] product comprising a compound [of/having] formula [I/IV] or a

pharmaceutically acceptable salt thereof," to mean "a product including, but not limited to, a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof."

The claim term "product," as it is used in the '393 patent, does not require construction because the claimed "product" is defined by the limitations recited in the challenged claims. This is evidenced by independent claims 1 and 9, which recite "[a] product comprising . . . ," and go on to define the essential elements of the claimed product. The transitional term "'comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim." *Genentech, Inc. v. Chiron Corp.*, 112 F.3d 495, 501 (Fed. Cir. 1997); *see also* Ex. 1001, 4:23–25 (defining "comprising" as "including, but not limited to"). Thus, the open-ended structure of the challenged claims forecloses limitation of the term "product" beyond that achieved by the recited claim elements.

Indeed, neither UTC nor SteadyMed identifies any disclosure in the '393 patent or its prosecution history that necessitates a contrary understanding of the term "product." For example, the portions of the Specification to which UTC points comport with an understanding of "product" as being defined only by the recited claim elements. *See* Ex. 1001, 5:45–46, 7:16–20, 17:37–40. Furthermore, far from disavowing or otherwise limiting claim scope, the portions of the prosecution history identified by UTC are consistent with an understanding that the claimed "product" is defined solely by the recited claim elements. *See* Ex. 1002,

315, 328–329, 346–350. We similarly are unpersuaded that the chemistry textbook glossaries to which UTC points (Exs. 2011, 2012, 2014) provide a basis for narrowly interpreting "product" to require that the product result from a chemical reaction.

Regarding the larger claim phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof," as explained above, we determine that the embedded claim term "comprising" means "including, but not limited to." *See Genentech*, 112 F.3d at 501; *see also* Ex. 1001, 4:23–25. Accordingly, we reject UTC's proposal that claims 1 and 9 be read to require a product "*constituted primarily of* formula I/IV or a pharmaceutically acceptable salt thereof." Prelim. Resp. 21 (emphasis added).

"[A/the] process comprising"

SteadyMed argues that the claim phrase "[a/the] process comprising," which appears in independent claims 1 and 9, should be interpreted as "a process that includes, but is not limited to, the recited process steps, and may include, without limitation, any other non-recited steps." Pet. 12. UTC counters that this claim phrase should be construed to mean "a/the process including but not limited to." Prelim. Resp. 23–24. For the reasons set forth above, we agree with UTC that these claim phases should be interpreted to mean "a/the process including, but not limited to."

Product-by-Process Claims

Each of the challenged claims is a product-by-process claim. Ex. 1001, 17:51–21:16; Pet. 5; Prelim. Resp. 3. The general rule when determining patentability of a product-by-process claim is to "focus . . . on the product and not on the process of making it." *Amgen, Inc. v. Hoffman-La Roche Ltd.*, 580 F.3d 1340, 1369 (Fed. Cir. 2009). This general rule embodies the long-standing principle that "an old product is not patentable even if it is made by a new process." *Id.* at 1370. An exception applies when process steps recited in the claim impart "structural and functional differences" to the claimed product. *Greenliant Sys., Inc. v. Xicor LLC*, 692 F.3d 1261, 1267–1268 (Fed. Cir. 2012). If the exception applies, the structural and functional differences conveyed by the recited process steps "'are relevant as evidence of no anticipation' although they 'are not explicitly part of the claim.'" *Id.* at 1268 (citing *Amgen*, 580 F.3d at 1370).

SteadyMed contends that the challenged claims do not yield a treprostinil product having structural or functional differences as compared to treprostinil products produced by prior art methods. Pet. 19–22. Specifically, SteadyMed asserts that the Walsh Declaration, relied on by UTC during prosecution as evidencing differences in the treprostinil products of the '393 patent and Moriarty, fails to demonstrate any functional or structural differences between the instantly claimed and prior art treprostinil products. *Id.* SteadyMed relies on the Winkler Declaration (Ex. 1009) to support its position. *Id.*

UTC acknowledges that "at the time of the '393 patent, there existed at least three prior art methods" for making treprostinil. Prelim. Resp. 33. Relying on the Walsh Declaration, UTC asserts that the process steps recited in independent claims 1 and 9 are entitled to patentable weight because they yield a "physically different and improved final product with significantly reduced overall impurities and a distinct and unexpected impurity profile" as compared to treprostinil produced using prior art methods. *Id.* at 3.

The Walsh Declaration compares the impurity profile of treprostinil free acid "prepared according to the process of 'Moriarty'" to the impurity profiles of treprostinil free acid and treprostinil diethanolamine "prepared according to the process specified in claim 1 or [9]" of the '393 patent.[5] Ex. 1002, 347–348 (¶ 6). Dr. Walsh concludes that the treprostinil free acid and treprostinil diethanolamine prepared according to the process of claims 1 and 9 is physically different from the treprostinil diethanolamine prepared according to the process of Moriarty "at least because neither of [the '393 patent products] contains a detectable amount of any of benzindene triol, treprostinil methyl ester, 1AU90 treprostinil stereoisomer and 2AU90 treprostinil stereoisomer, each of which were present in detectable amounts in treprostinil produced according to the process of 'Moriarty'." *Id.* at 349 (¶ 8). In addition, Dr. Walsh provides "data obtained from representative Certificates of Analysis" indicating that treprostinil free acid "prepared

[5] Issued claim 9 of the '393 patent is identified as claim 10 in the Walsh Declaration, and other documents in the prosecution history in the '393 patent.

according to 'Moriarty'" is 99.4% pure, while the treprostinil free acid and treprostinil diethanolamine "prepared according to the process specified in claim 1 or [9]" are 99.8% pure and 99.9% pure, respectively. *Id.* at 347–348 (¶ 6).

SteadyMed disputes Dr. Walsh's contention that there are physical differences between the treprostinil products of the '393 patent and prior art. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71. As an initial matter, SteadyMed points out that the 99.7% treprostinil purity reported by Moriarty (Ex. 1004, 13) is higher than the 99.5% purity recited in claims 2 and 10 of the '393 patent, the only challenged claims that recite a purity level. Pet. 20; *see also* Ex. 1009 ¶ 65. In addition, Dr. Winkler testifies that the limited sample set, consisting of "*only two specific batches* of treprostinil" (Ex. 1009 ¶ 66), and absence of any disclosure concerning the reaction conditions, reagents, and solvents used in carrying out the process of claims 1 and 9 of the '393 patent (*id.* ¶ 67), undermine the veracity of Dr. Walsh's conclusion regarding the purity of these products. *Id.* ¶¶ 66–67. SteadyMed also observes that the statement in the Specification of the '393 patent that in one embodiment the purity of treprostinil is "at least 90.0%, 95.0%, 99.0%, 99.5%" (Ex. 1001, 8:66–67), supports the conclusion that the 99.8% purity purportedly achieved by Dr. Walsh "is based on a particular set of process steps that are not claimed and which must have been found after the filing date." Pet. 20.

Dr. Winkler additionally testifies that the alleged differences in purity between the treprostinil batches described by Dr. Walsh are attributable to

experimental error. *Id.* ¶¶ 68–70. Dr. Winkler testifies that "the literature on [High Performance Liquid Chromatography's ("HPLC's")] precision indicates that the 'RSD' or 'relative standard deviation' for a typical instrument is about 1%. (Ex. 1017.)." *Id.* ¶ 70. Dr. Winkler further observes that "[i]n the present case, we can estimate the precision of the equipment the inventors actually used, since the inventors found that Example 4's Batch 1 had an HPLC Assay of 100.4%, which is obviously greater than the 100% value theoretically achievable. (Ex. 1001, col. 13, lines 50-65)." *Id.* Dr. Winkler, thus, concludes that "[t]his deviation between experimental and theoretical shows that the instrument can have variations of at least 0.4%, which is greater than the differences in purity that the inventors offered to support their contention regarding greater purity over the prior art." *Id.* On this record, we credit Dr. Winkler's testimony, as it is consistent with the disclosures of the prior art and the disclosure of the '393 patent itself.

UTC does not challenge SteadyMed's arguments concerning the shortcomings of the Walsh Declaration. Rather, UTC points to correspondence with, and reports submitted to, the Food and Drug Administration ("FDA") relating to the acceptance of a supplemental new drug application for treprostinil. Prelim. Resp. 36–38. UTC contends that these reports show that "the purity of the treprostinil improved close to 100%" for treprostinil prepared as described in claims 1 and 9 of the '393 patent as opposed to the prior process implemented by UTC. Prelim. Resp. 38; *see also* Ex. 2006, 3–4.

On the record before us, and for purposes of this decision, we conclude that the process steps recited in the challenged claims do not impart structural or functional differences to the claimed product.

As an initial matter, we observe that the challenged product-by-process claims are drawn to "[a] product comprising a compound" of either formula I or formula IV, or a pharmaceutically acceptable salt of the recited formula. Ex. 1001, 17:51–19:29, 19:48–20:46). "'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim." *Genentech*, 112 F.3d at 501. Thus, a product comprising a particular compound must contain that compound, but may additionally include other substances, such as impurities. On this record, therefore, it is unclear how claims 1, 3–9, and 11–22, which claim a product comprising a particular compound, but do not recite limitations concerning the purity profile of that product, could be restricted to a product including the claimed compound, but also having a particular purity profile. In addition, although claims 2 and 10 require a purity of at least 99.5% (Ex. 1001, 19:29–30, 20:47–48), these claims similarly are drawn to a product comprising a compound, and do not specify the type of impurities that may be present in the compound or restrict the amount of any particular impurity that may be present, so long as the product remains at least 99.5% pure.

Furthermore, the evidence presently before us, including UTC's own testing results, suggests that inter-batch variability in impurity profiles,

experimental error in impurity measuring equipment, and variations in reagents, solvents, and reaction conditions, rather than the instantly recited process steps, account for any purported improvements in purity reported by UTC. We observe that UTC offers no explanation for the variation between the 99.7% purity reported by Moriarty, and the 99.4% purity Dr. Walsh obtained for treprostinil purportedly prepared according to the process described by Moriarty. Neither does UTC offer reasoning for crediting Dr. Walsh's results over those reported by Moriarty himself. Similarly, UTC neglects Dr. Winker's assessment of the experimental error present, but unaccounted for, in the impurity measurements reported in the Walsh Declaration, and fails to account for the absence of any disclosure regarding the experimental protocols followed by Dr. Walsh, such as the reaction conditions, or the solvents or reagents used, in synthesizing treprostinil according to Moriarty or the '393 patent.

Moreover, the Process Optimization Report (Ex. 2005) proffered by UTC supports the conclusion that the process steps recited in the '393 patent do not produce a treprostinil product that differs, either structurally or functionally, from that produced using prior art methods.

The Process Optimization Report discloses the impurity analyses for five batches of treprostinil identified by UTC as having been prepared using the process recited in the '393 patent. Ex. 2005, 4–6; *see also* Prelim. Resp. 36 ("Ex. 2005 is a Process Optimization Report that provides results

for batches resulting from step (d) of claims 1 and 10 in the '393 patent,[6] which was performed on specific batches of the diethanolamine salt intermediate produced by steps (a)-(c) [redacted]."). The Process Optimization Report states that the purity of these batches, as determined by HPLC analysis, ranged from [redacted] to [redacted].[7] Ex. 2005, 6. Additionally, the Process Optimization Report indicates that each of the following impurities were detected by HPLC analysis in one or more of the above referenced treprostinil batches: [redacted]. *Id.*

We also observe that although UTC sought, and obtained from the FDA, modification of the specification for the HPLC assay for treprostinil to require a purity range of [redacted], rather than [redacted] Ex. 2006, 3–4, 6; Ex. 2003. Notably, UTC's specification for treprostinil produced according to the '393 patent permits

---

[6] We note that UTC likely intended to reference independent claim 9 of the '393 patent, rather than dependent claim 10; however our analysis is equally applicable to claim 9 or claim 10.

[7] The reported batch purity values were [redacted], for an average purity of [redacted]. Ex. 2005, 6.

each of the following impurities: [redacted]. Ex. 2006, 6. The analysis of treprostinil purportedly prepared according to the process of Moriarty, set forth in the Walsh Declaration, reveals that each of the impurities detected in Moriarty treprostinil was present in an amount [redacted]. *Compare* Ex.1002, 347, *with* Ex. 2006, 6.

Accordingly, on the record before us, and for purposes of this decision, we conclude that the process steps recited in the challenged claims of '393 patent do not impart structural or functional differences to the claimed product as compared to prior art processes, and therefore, that these process steps do not patentably limit the claimed product. We note, however, that the factual dispute between the parties concerning the existence of any structural or functional differences between treprostinil products produced according to the process recited in the '393 patent and prior art processes, as well as arguments addressing our concerns regarding the relevance of the impurity profile of a product obtained by the recited process to the patentability of claims drawn to a product *comprising* a compound, are appropriate for further development at trial.

C. Principles of Law

To establish anticipation, each and every element in a claim, arranged as recited in the claim, must be found in a single prior art reference. *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008). "A reference anticipates a claim if it discloses the claimed invention 'such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention.'" *In re Graves*, 69 F.3d 1147, 1152 (Fed. Cir. 1995) (emphasis omitted) (quoting *In re LeGrice*, 301 F.2d 929, 936 (CCPA 1962)).

A patent claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

> When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the

> same way, using the technique is obvious unless its actual application is beyond his or her skill. *Sakraida* [*v. Ag Pro, Inc.*, 425 U.S. 273 (1976)] and *Anderson's–Black Rock* [*v. Pavement Salvage Co.*, 396 U.S. 57 (1969)] are illustrative—a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions.

KSR, 550 U.S. at 417.

The level of ordinary skill in the art is reflected by the prior art of record. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001); *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995); *In re Oelrich*, 579 F.2d 86, 91 (CCPA 1978).

D. Anticipation Grounds of Unpatentability Based on Phares

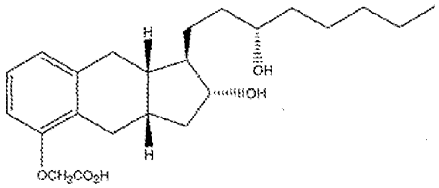
SteadyMed asserts that claims 1–5, 7–9, 11–14, and 16–20 are unpatentable under § 102(b) as anticipated by Phares. Pet. 22–37. Claims 2–5, 7, 8, and 19 depend directly from claim 1, and claims 11–14, 16–18, and 20 depend, directly or indirectly, from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how Phares discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC counters that the treprostinil product of Phares is physically different from that produced by the process disclosed in the '393 patent, and, therefore, that the process steps disclosed in the claims of the '393 patent are limiting for purposes of the patentability determination. Prelim. Resp. 33–36. UTC also argues that SteadyMed improperly engages in picking and choosing among distinct embodiments in Phares to piece together an

anticipation argument as to the recited process steps. *Id.* at 29–31. UTC further asserts that explicit disclosure of certain claimed process steps is absent from SteadyMed's anticipation analysis, and that SteadyMed fails to show that those limitations are inherently disclosed by Phares. *Id.* at 31–36.

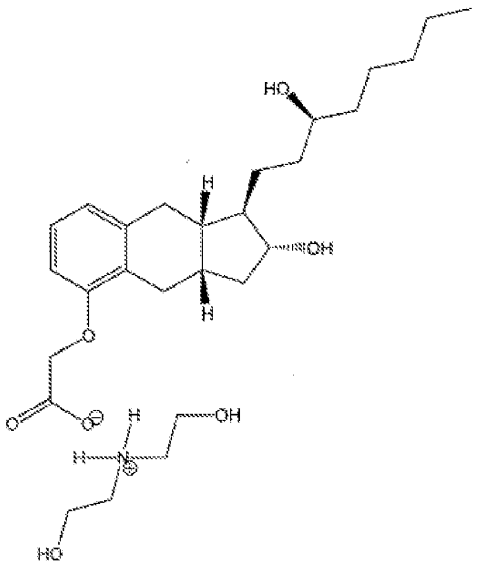
Phares

Phares describes "compounds and methods for inducing prostacyclin-like effects in a subject or patient," including treprostinil and derivatives thereof. Ex. 1005, 10. The chemical structure of treprostinil disclosed by Phares, on page 10 of Exhibit 1005, is reproduced below:



Id. Phares explains that "[t]reprostinil is a chemically stable analog of prostacyclin, and as such is a potent vasodilator and inhibitor of platelet aggregation." *Id.*

Phares further discloses that "[a] preferred embodiment of the present invention is the diethanolamine salt of treprostinil. . . . A particularly preferred embodiment of the present invention is form B of treprostinil diethanolamine." *Id.* at 11. The structure of the diethanolamine salt of treprostinil described by Phares, on page 99 of Exhibit 1005, is reproduced below:

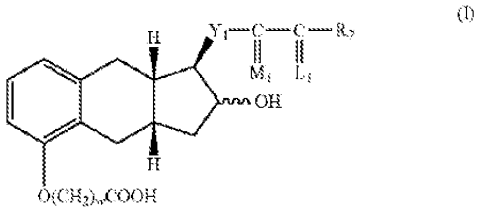


Id. at 99 (claim 49). Phares reports that form B of the diethanolamine salt of treprostinil "appears to be a crystalline material which melts at 107°C." *Id.* at 91.

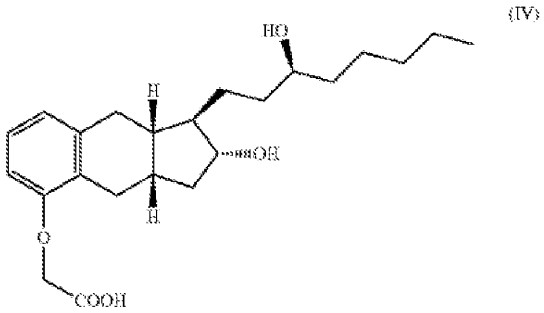
Phares describes the synthesis of (-)-treprostinil, the enantiomer of treprostinil. Ex. 1005, 41–42. Phares explains that "[e]nantiomers of these compounds . . . can be synthesized using reagents and synthons of enantiomeric chirality of the above reagents." *Id.* at 41. In particular, Phares teaches that "the enantiomer of the commercial drug (+)-Treprostinil was synthesized using the stereoselective intramolecular Pauson Khand reaction as a key step and Mitsunobu inversion of the side-chain hydroxyl group." *Id.* at 42. Phares discloses the following reaction procedure: "i. $ClCH_2CN$, K_2CO_3. ii, KOH, CH_3OH, reflux. 83 % (2 steps)." *Id.*

A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof

Claim 1 of the '393 patent recites "[a] product comprising a compound of formula I



or a pharmaceutically acceptable salt thereof," and sets forth a series of process steps for obtaining the claimed product. Claim 9 recites "[a] product comprising a compound having formula IV

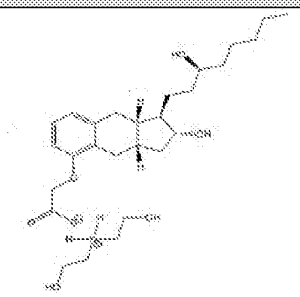
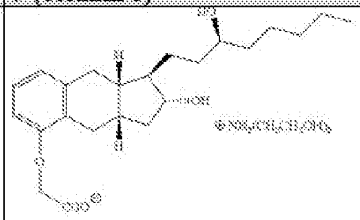


or a pharmaceutically acceptable salt thereof," and includes the same process steps for obtaining the claimed product as recited in claim 1. Claim 9 is identical to claim 1, except that it is drawn to a product comprising the specific treprostinil compound, a species of the genus of claim 1. Accordingly, we address these claims together.

SteadyMed contends that "Phares discloses in its Claim 49 the identical, pharmaceutically acceptable treprostinil diethanolamine salt" claimed in the '393 patent. Pet. 26; *see also* Ex. 1005, 24, 85–93, 99

(claim 49); Ex. 1009 ¶¶ 50–53. In support of SteadyMed's position, Dr. Winkler testifies that "[o]ther than a change in formatting, the two structures [for treprostinil diethanolamine salt] from Phares and the '393 Patent are identical." Ex. 1009 ¶ 53.

Paragraph 52 of the Winkler Declaration depicts a side-by-side comparison of the chemical structures disclosed in claim 49 of Phares, and column 8, lines 50–63 of the '393 patent, reproduced below:

Phares (Ex. 1005), Claim 49	'393 Patent (Ex. 1001), Claims 1 & 9 (column 8)
	

Id. ¶ 52. As shown in the figure from paragraph 52 of the Winkler Declaration, the treprostinil diethanolamine salt disclosed by Phares is structurally identical to that disclosed in the '393 patent.

As set forth in Part II.B above, SteadyMed, relying on the Winkler Declaration, further asserts that the process disclosed in claims 1 and 9 of the '393 patent does not result in a treprostinil product that is physically different or unique from treprostinil produced by prior art methods. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71. In support of this position, Dr. Winkler testifies that "[i]n both the '393 Patent and Phares (Ex. 1005), treprostinil diethanolamine salt Form B is made . . . . Phares further discloses a melting point of 107° C (Ex. 1005, p. 91 & Fig. 21) for the Form B salt."

Ex. 1009 ¶ 59; *see also* Ex. 1005, 90–93; Pet. 27. Dr. Winkler also testifies that Phares discloses the same procedure as is claimed in the '393 patent, but describes this procedure in reference to the synthesis of the enantiomer of treprostinil. Ex. 1009 ¶¶ 55–57; Ex. 1005, 41–42; Pet. 25–26. Dr. Winkler thus concludes that in "making the most stable crystal form (Form B) and preparing a product that melts at a higher temperature higher than that described in the '393 Patent, Phares necessarily discloses a salt of at least equal purity to the salt in the '393 Patent." Ex. 1009 ¶ 62; *see also id.* ¶ 60 (citing Ex. 1018, 6); Pet. 27–28.

SteadyMed also contends that Phares anticipates the process steps recited in claim 1. Pet. 24–28; Ex. 1005, 24, 41–42, 85–93, 99 (claim 49); Ex. 1009 ¶¶ 44–71.

UTC does not dispute Phares' disclosure of a treprostinil product; rather, as previewed in relation to its claim construction arguments above, UTC contends that the treprostinil product of Phares is "physically different" from that claimed in the '393 patent, and, therefore, not anticipatory. Prelim. Resp. 33–36. UTC argues that as Phares does not disclose which treprostinil starting material is used, it "cannot inherently anticipate the final treprostinil product of the '393 patent because each method would result in a distinct impurity profile." Prelim. Resp. 34. Referring to the Walsh Declaration, UTC further asserts that "even if the Moriarty treprostinil was used for Phares, Petitioner has failed to provide any evidence that the final Phares treprostinil product would necessarily be the same as the products claimed in the '393 patent." *Id.* UTC also asserts that SteadyMed's reliance

on the melting point of the treprostinil product of Phares as a proxy for purity is misplaced because "melting point does not disclose any specific impurity level and instead may demonstrate a different form, or polymorph, of treprostinil diethanolamine altogether." *Id.* at 35.

UTC additionally argues that Phares does not disclose the same process for generating treprostinil as recited in claims 1 and 9, and that SteadyMed improperly "cobbles together disclosure from four disparate portions of Phares covering multiple distinct embodiments" to arrive at the claimed invention. Prelim. Resp. 27. Further, UTC asserts that even if SteadyMed were permitted to pick and choose steps from various embodiments of Phares, SteadyMed nevertheless must rely on inherency to prove anticipation because "Phares lacks express disclosure of certain claim elements." *Id.* at 28.

The present record supports SteadyMed's contention that the treprostinil diethanolamine salt taught by Phares is identical in structure to the pharmaceutically acceptable treprostinil diethanolamine salt recited in claims 1 and 9. Pet. 24; *see also* Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶¶ 52–53. Dr. Winkler testifies that the process for producing treprostinil disclosed by Phares yields the same form (Form B) of treprostinil diethanolamine salt as the process of the '393 patent, and that the treprostinil diethanolamine salt of Phares is at least equal in purity to the treprostinil product of the '393 patent. Ex. 1009 ¶¶ 59–62. Dr. Winkler further testifies that Phares discloses the same process for synthesizing treprostinil as the

'393 patent. Ex. 1009 ¶¶ 55–57, 62; Ex. 1005, 41–42; Pet. 25–26. On this record, we credit Dr. Winkler's testimony.

We are not persuaded by UTC's arguments concerning the possibility that treprostinil produced according to Phares might have a different impurity profile than that produced according to the process disclosed in the '393 patent. First, for the reasons set forth in Part II.B above, it is unclear on this record how the use of the transitional phrase "comprising" excludes any impurities that may possibly be produced by the process of Phares. In addition, the present record supports a finding that the impurity profiles for treprostinil diethanolamine salt prepared as described by Phares and that prepared according to the '393 patent are the same. As explained above, Dr. Winkler's testimony regarding the form and melting point of Phares' treprostinil product, is consistent with the conclusion that the products of Phares and the '393 patent are the same.

Furthermore, we note that, as explained in Parts II.A and II.B above, the inter-batch variability in treprostinil impurity profiles, experimental error inherent in impurity measurements, and the variety and extent of impurities permitted in UTC's specification for the manufacture of treprostinil according to the process of the '393 patent, which remained unchanged when UTC migrated from a prior art process to the process of the '393 patent, support the conclusion that the process steps recited in claims 1 and 9 of the '393 patent do not impart any structural or functional differences over prior art treprostinil products.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that Phares teaches the treprostinil diethanolamine salt product recited in claims 1 and 9. Because we determine, on the record before us, and for purposes of this decision, that the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product and are therefore not limiting, we do not address the parties' contentions concerning Phares' anticipation of the recited process steps.

Conclusion

UTC has not raised any additional arguments with regard to the dependent claims other than those addressed above. We have reviewed SteadyMed's evidence, arguments, and claim charts, and conclude that SteadyMed has sufficiently demonstrated that the dependent claims are also anticipated by Phares. Thus, for the foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 1–5, 7–9, 11–14, and 16–20 are anticipated by Phares.

E. Obviousness Grounds of Unpatentability Based on Moriarty and Phares

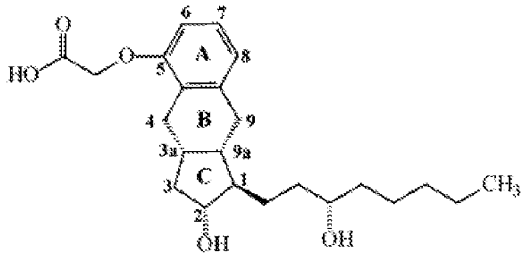
SteadyMed asserts that claims 1–5, 7–9, 11–14, and 16–20 are unpatentable under § 103(a) as obvious in view of Moriarty and Phares. Pet. 37–52. Claims 2–5, 7, 8, and 19 depend directly from claim 1, and claims 11–14, 16–18, and 20 depend, directly or indirectly, from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how

the combination of Moriarty and Phares discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC counters that "Phares fails to disclose the synthetic route or purity of the claimed treprostinil product. Moriarty adds nothing to cure these deficiencies." Prelim. Resp. 43. UTC asserts that the process described in the '393 patent "unexpectedly reduced the impurity level in the claimed treprostinil product even more" than Moriarty, and reiterates its position that treprostinil produced according to the process of the '393 patent has "a superior purity profile compared to the prior art." *Id.* at 44.

Moriarty

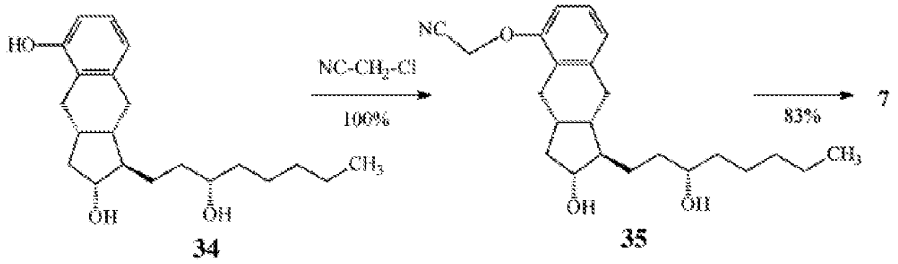
Moriarty describes the synthesis of treprostinil "via the stereoselective intramolecular Pauson-Khand cyclization." Ex. 1004, 1. Formula 7 of Moriarty is reproduced below:



7

Id. at 3. Formula 7 of Moriarty depicts the chemical structure of treprostinil. *Id.*

An excerpt of Scheme 4 of Moriarty is reproduced below:



Id. at 6. The excerpted portion of Scheme 4 of Moriarty illustrates the alkylation Formula 34 to yield Formula 35, and subsequent hydrolysis of Formula 35 with a base (followed by acidification) to yield Formula 7, treprostinil. Ex. 1004, 6, 13.

A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof

SteadyMed contends that Moriarty and Phares respectively disclose treprostinil acid and treprostinil diethanolamine salt, as recited in claims 1 and 9 of the '393 patent. Pet. 22–23, 24, 33, 39, 48; *see also* Ex. 1004, 6, 13; Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶¶ 74, 76. Furthermore, Dr. Winkler testifies that the combination of Moriarty and Phares "discloses the same process steps and same product of the '393 Patent. For the same reasons discussed above regarding Phares, the purity of the combinations would be of at least equal purity to that claimed in the '393 Patent." Ex. 1009 ¶ 76.

SteadyMed asserts that Moriarty discloses steps (a) and (b) of claims 1 and 9, and that Phares discloses step (c) of these claims. Pet. 43; *see also* Ex. 1004, 6, 13; Ex. 1005, 24; Ex. 1009 ¶ 74. Dr. Winkler testifies

that a relevant skilled artisan would have recognized that the treprostinil acid produced in Moriarty could be purified by contacting it with a base as described by Phares. Ex. 1009 ¶ 74. In addition, as discussed in Part II.D above, Dr. Winkler testifies that Phares "details the same Claim 1 and 9 steps (a) or (b) as were used to make treprostinil in the '117 Patent and Moriarty reference, but applies them to make (-)-treprostinil, the enantiomer of (+)- treprostinil (Ex. 1005, p. 42)." *Id.* ¶55. Dr. Winkler further testifies that a relevant skilled artisan would have had "more than a reasonable expectation of success that the reaction of treprostinil with diethanolamine would be successful" because "Phares (Ex. 1005, p. 24, p. 99, Claim 49) performed the same reaction and it was successful." Ex. 1009 ¶ 80.

UTC reasserts the arguments described above concerning the purity of treprostinil produced according to the process disclosed in the '393 patent. UTC acknowledges that Moriarty itself was an improvement over the prior art, but contends that "the '393 patent unexpectedly reduced the impurity level in the claimed treprostinil product even more." Prelim. Resp. 44. Specifically, UTC contends that "performing step (c) on a product that resulted from steps (a) and (b) provided a product with reduced impurities." *Id.* UTC also reiterates its arguments concerning the Walsh Declaration, and highlights the purported differences in the impurity profile of treprostinil produced according to Moriarty compared to that produced according to the '393 patent.

The present record supports SteadyMed's contention that the treprostinil diethanolamine salt disclosed by the combination of Moriarty

and Phares is identical in structure to the pharmaceutically acceptable treprostinil diethanolamine salt recited in claims 1 and 9. Pet. 41–42; *see also* Ex. 1004, 6, 13; Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶ 76.

First, as explained in Part II.B above, the present record does not support the conclusion that claims drawn to "[a] product comprising a compound . . ." can be distinguished from prior art products on the basis of differences in the impurity profiles of those products.

Moreover, as explained in detail in Parts II.A, II.B, and II.D above, we determine that the present record supports the contention that the treprostinil product of Moriarty and Phares is the same as that produced according to the steps recited in claims 1 and 9 of '393 patent.

As discussed in Part II.B, the Walsh Declaration fails to disclose the protocols followed in producing the Moriarty and '393 patent treprostinil samples analyzed, and fails to account for the experimental error in Dr. Walsh's impurity measurements. In addition, the inter-batch variability in the types and amounts of impurities observed in treprostinil prepared according to the '393 patent, and the fact that the treprostinil Dr. Walsh prepared according to Moriarty satisfies the FDA purity specification for treprostinil prepared per the '393 patent, lends further support to the conclusion that no structural or functional differences exist between treprostinil produced according to Moriarty, and that produced according to the'393 patent.

Similarly, as discussed in Part II.D, the present record supports a finding that the impurity profile of treprostinil diethanolamine salt prepared

as described by Moriarty in combination with Phares is the same as that prepared according to the '393 patent. Dr. Winkler's testimony regarding the form and melting point of Phares' treprostinil product (Ex. 1009 ¶¶ 59–60, 62), as well as his testimony regarding the disclosure by Phares of the same synthesis process as described by Moriarty (Ex. 1009 ¶¶ 55–57), is consistent with the conclusion that treprostinil diethanolamine generated by reacting Formula 7 of Moriarty with a base, as disclosed by Phares, to form a salt of Formula 7 would result in a treprostinil diethanolamine salt of at least equal purity to that disclosed in the '393 patent.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Moriarty and Phares renders obvious the treprostinil diethanolamine salt product recited in claims 1 and 9. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product and are therefore not limiting, we need not address the parties' contentions concerning the obviousness of the recited process steps.

Conclusion

UTC has not raised any additional arguments with regard to the dependent claims other than those addressed above. We have reviewed SteadyMed's evidence, arguments, and claim charts, and conclude that SteadyMed has sufficiently demonstrated that the dependent claims are also rendered obvious by the combination of Moriarty and Phares. Thus, for the

foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 1–5, 7–9, 11–14, and 16–20 are obvious in view of Moriarty and Phares.

F. Obviousness Grounds of Unpatentability Based on Moriarty, Phares, Kawakami, and Eğe

SteadyMed asserts that claims 6, 10, 15, 21, and 22 are unpatentable under § 103(a) as obvious in view of Moriarty, Phares or Kawakami, and Eğe. Pet. 37–52. Although SteadyMed nominally identifies this ground of unpatentability as being over "Moriarty (Ex. 1004) with Phares (Ex. 1005) or Kawakami (Exs. 1006 & 1007) and in further combination with Ege (Ex. 1008)" (Pet. 53 (emphasis omitted), as discussed below, SteadyMed explicitly relies on Kawakami in arguing unpatentability in view of Moriarty, Phares, and Eğe. Accordingly, we understand SteadyMed's stated ground of unpatentability as relying on the combination of Moriarty, Phares, Kawakami, and Eğe. Claims 6, 21, and 22 depend, directly or indirectly, from claim 1, and claims 10 and 15 depend directly from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how the combination of Moriarty, Eğe, Phares, and Kawakami discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC contends that Kawakami should not be considered as evidence of unpatentability because the declaration certifying the accuracy of the translation is deficient. Prelim. Resp. 38–39. UTC also asserts that Eğe is merely a generic introductory chemistry text, and irrelevant to the

'393 patent. *Id.* at 47. UTC further argues that SteadyMed has not identified a rationale for, or expectation of success in, combining either Moriarty, Phares, and Eğe, or Moriarty, Kawakami, and Eğe. *Id.* In addition, UTC contends that SteadyMed improperly asserts that the cited combination would inherently result in the claimed product. *Id.* at 54.

Kawakami

Kawakami describes "a crystalline dicyclohexylamine salt of a methanoprostacyclin derivative, a manufacturing method thereof, and a purifying method thereof." Ex. 1007, 3. Kawakami discloses obtaining a dicyclohexylamine salt by "mixing a methanoprostacyclin derivative [I] . . . with dicyclohexylamine in an appropriate solvent." Ex. 1007, 5–6. Kawakami explains that "[t]he dicyclohexylamine salt of the methanoprostacyclin derivative [I] thus obtained generally has fairly high purity, and the purity can be further improved by recrystallization as needed with the use of an appropriate solvent." *Id.* at 6.

Kawakami further teaches that "[t]he dicyclohexylamine salt obtained by the present invention can be easily reverted to a free methanoprostacyclin derivative [I] by conventional methods, and the resulting methanoprostacyclin derivative exhibits excellent crystallinity compared with substances not purified according to the present invention." *Id.*

Eğe

Eğe is an organic chemistry textbook. Ex. 1008, 1. Eğe discloses:

> Carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction

> with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds.

Id. at 8 (reference omitted).

Compliance with 37 C.F.R. § 42.63(b)

Kawakami is a Japanese patent application. Ex. 1006. SteadyMed submitted an English translation of Kawakami (Ex. 1007), as well as an affidavit certifying that translation (Ex. 1011) with its Petition.

UTC nevertheless contends that Kawakami should not be considered as evidence of unpatentability because the President of the translation service, rather than the individual who prepared the translation, executed the certification affidavit. Prelim. Resp. 38–39. UTC asserts that certification affidavit is objectionable because the affiant lacks personal knowledge of the relevant facts, the accuracy of the translation cannot be determined, and the translator is shielded from cross-examination. *Id.* at 39.

In view of the record before us, and for purposes of this decision, we decline UTC's invitation to disregard Kawakami. No credible prejudice to UTC has been called to our attention, and none is apparent. An English translation of Kawakami was available to UTC in time to prepare its Preliminary Response.[8] Furthermore, UTC has not identified any error in

[8] It does not appear that UTC has served objections on SteadyMed concerning the adequacy of the English translation of Kawakami or the certifying affidavit.

the translation that would call into question its authenticity. Regarding UTC's contention that the accuracy of the translation cannot be determined absent a certification affidavit from the translator himself, we note that the commission of an independent translation would confirm the veracity of the translation submitted by SteadyMed. We also observe that even if the individual personally responsible for generating the English translation of Kawakami had submitted a certification affidavit, UTC would not have had the opportunity to cross-examine him prior to the submission of its Preliminary Response.

Accordingly, on the record before us, and for purposes of this decision, we decline UTC's request that we disregard Kawakami. We observe, however, that the adequacy of the Kawakami translation and certification affidavit may be subject to further challenge during trial.[9]

Rationale to Combine Prior Art Teachings

Building on the rationale for combining Moriarty and Phares discussed in Part II.E above, SteadyMed contends that a relevant skilled

---

[9] Pursuant to 37 C.F.R. § 42.64(b)(1), "[a]ny objection to evidence submitted during a preliminary proceeding must be served within ten business days of the institution of the trial. . . . The objection must identify the grounds for the objection with sufficient particularity to allow correction in the form of supplemental evidence." "The party relying on evidence to which an objection is timely served may respond to the objection by serving supplemental evidence within ten business days of service of the objection." 37 C.F.R. § 42.64(b)(2). Furthermore, "[a] motion to exclude evidence must be filed to preserve any objection. . . . The motion may be filed without prior authorization from the Board." 37 C.F.R. § 42.64(c)

artisan would add further purification steps from Kawakami and Eğe because Kawakami "discloses that the dicyclohexylamine salt of a methanoprostacyclin derivative 'can be easily reverted to the free methanoprostacyclin derivative by *conventional methods*,'" and that the "fairly high purity" of the salt obtained "can be further improved by recrystallization as needed with the use of an appropriate solvent." Pet. 53; *see also* Ex. 1007, 6; Ex. 1009 ¶ 83. Dr. Winkler testifies that, as evidenced by Eğe, a relevant skilled artisan "would understand that one such conventional method for converting the dicyclohexylamine salt of a methanoprostacyclin derivative to the free methanoprostacyclin derivative, or converting the treprostinil diethanolamine salt to treprostinil (*i.e.*, the free acid) is by treating the salt with a strong acid such as HCl or H_2SO_4." Ex. 1009 ¶ 84; *see also* Pet. 53–54.

Dr. Winkler elaborates on this rationale for combining the cited references, testifying that a relevant skilled artisan

> would want to form the treprostinil diethanolamine salt, purify it, and then convert it back to its free form (*i.e.*, treprostinil) in order to obtain excellent crystallinity and increased purity. And Ege (Ex. 1008, p. 8) teaches that one such method for obtaining the free form of treprostinil or any carboxylic acid would be by treatment of the carboxylate salt with a strong acid.

Ex. 1009 ¶ 88; *see also* Ex. 1008, 8; Pet. 54.

UTC does not address the combination of Moriarty, Eğe, Phares, and Kawakami. Instead, UTC addresses Moriarty, Eğe, and Phares as one combination, and Moriarty, Eğe, and Kawakami as an alternative combination. Prelim. Resp. 46–47.

As an initial matter, UTC asserts that Eğe is irrelevant to the '393 patent because it does not discuss prostacyclin derivatives or pharmaceutical synthesis. *Id.* at 47. UTC argues that Eğe in fact "would teach away or discourage the use of salt formation for purifying a mixture of compounds that includes other carboxylic-acid containing compounds as impurities." *Id.* at 48.

Regarding the combination of Moriarty, Eğe, and Phares, UTC contends that "even though Phares discloses forming a salt from treprostinil free acid, and Ege generally discusses that carboxylate salt formation was known in the art, there would have been no motivation or expectation of success in using these teachings on the already-formed free acid disclosed in Moriarty." Prelim. Resp. 50. Pertaining to the combination of Moriarty, Eğe, and Kawakami, UTC asserts that SteadyMed "fails to establish that a [relevant skilled artisan] would reasonably expect the teachings of Kawakami to extend to the products in Moriarty." *Id.* at 52.

UTC also argues that Dr. Winkler's testimony regarding the reasons a relevant skilled artisan would want to form treprostinil diethanolamine salt, and treat it with a strong acid to convert it back to its free form (treprostinil) is improperly conclusory. *Id.* at 50, 52.

On the record before us, and for purposes of this decision, we agree that SteadyMed has sufficiently demonstrated that a relevant skilled artisan would have had reason to include the carboxylate salt formation and regeneration of the neutral carboxylic acid with the syntheses of Moriarty and Phares based on the teachings of Kawakami and Eğe.

We recognize, but do not find persuasive, UTC's position that Eğe is irrelevant to the synthesis of prostacyclin derivatives, and that it teaches away from the use of salt formation for purifying a mixture of compounds that includes other carboxylic-acid containing compounds as impurities. First, we observe that SteadyMed relies on Eğe not for any teachings specific to prostacyclin derivative synthesis, but rather, to support the contention that the addition of a strong acid to a carboxylate salt to regenerate the neutral carboxylic acid is a conventional purification technique in organic chemistry. Pet. 53–55; Ex. 1009 ¶¶ 86, 88. In particular, Dr. Winkler testifies that the "addition of a strong acid to a carboxylate salt to regenerate the neutral carboxylic acid is a common reaction in organic chemistry and this process is well within the skill of one of ordinary skill in the art (indeed, a process that I teach to my organic chemistry students)" (Ex. 1009 ¶ 85), and that Eğe, an introductory organic chemistry text, "discloses that sodium benzoate (i.e., a carboxylate salt) can be converted back to benzoic acid (i.e., a carboxylic acid) by treatment with the acid HCl" (*id.* ¶ 86). On this record, we credit Dr. Winkler's testimony, as it is consistent with the prior art.

Second, we note that even crediting UTC's position that the use of salt formation would not be effective for purifying treprostinil from its stereoisomers (Prelim. Resp. 47–48), the present record suggests that it would be effective for removing other impurities (Pet. 53–55; Ex. 1009 ¶¶ 86, 88). Moreover, as explained below, the present record, including Kawakami, indicates that treprostinil diethanolamine salt formation followed

by regeneration of treprostinil using a strong acid is an effective purification step. Pet. 53–55; *see also* Ex. 1007, 6; Ex. 1008, 8; Ex. 1009 ¶¶ 82–90.

Additionally, we agree with SteadyMed that a relevant skilled artisan would have had reason to combine Moriarty, Phares, Kawakami, and Eğe. Pet. 53–55; Ex. 1009 ¶¶ 82–90. For example, Dr. Winkler testifies that a relevant skilled artisan would want to include a carboxylate salt formation and regeneration of the neutral carboxylic acid as described by Eğe with the syntheses of Moriarty and Phares because Kawakami teaches that "'the dicyclohexylamine salt obtained by the present invention can be easily reverted to a free methanoprostacyclin derivative [I] by conventional methods, and the resulting methanoprostacyclin derivative exhibits excellent crystallinity compared with substances not purified according to the present invention.'" Ex. 1009 ¶ 86; *see also* Ex. 1007, 6; Pet. 53–55. Dr. Winkler additionally testifies that a skilled artisan would be motivated to form treprostinil diethanolamine salt, and treat it with a strong acid to "obtain excellent crystallinity and increased purity" of the final treprostinil product (Ex. 1009 ¶ 88), and that a skilled artisan would have a reasonable expectation of success in performing such reaction because it is "a common reaction in organic chemistry and this process is well within the skill of one of ordinary skill in the art" (*id.* ¶ 90).

On this record, we credit Dr. Winkler's testimony, as it is consistent with the prior art. Moreover, we disagree with UTC that Dr. Winkler's testimony is improperly conclusory. Rather, as illustrated by the excerpts of his testimony referenced above, Dr. Winkler supports his opinions with

reference to the cited art, as well as his experience as a chemist and chemistry professor.

Accordingly, on the record before us, we agree that SteadyMed has sufficiently demonstrated that one of ordinary skill in the art would have included the carboxylate salt formation and regeneration of the neutral carboxylic acid of Eğe with the syntheses of Moriarty and Phares based on Kawakami's disclosure that the conversion of salts of prostacyclin derivatives to their free forms by conventional methods increases purity of the final product. *See KSR*, 550 U.S. at 417 ("[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.").

Claims 6, 15, and 21

Claims 6, 15, and 21 each recite the product of either claim 1 or claim 9, subject to additional process steps. For example, claim 6 recites "[t]he product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4." Ex. 1001, 19:39–40. Claim 15 similarly recites "[t]he product of claim 9, wherein the acid in step (d) is HCl." *Id.* at 20:59–60. Claim 21 simply recites "[t]he product of claim 1, wherein step (d) is performed." *Id.* at 21:13.

The present record supports SteadyMed's contention that claims 6, 15, and 21 would have been obvious in view of Moriarty, Eğe, Phares, and

Kawakami. Pet. 53–56; Ex. 1009 ¶¶ 82–90. For example, Dr. Winkler testifies that

> the combination of Moriarty (Ex. 1004) and Phares (Ex. 1005) (or Kawakami, Exs. 1006 & 1007) and Ege (Ex. 1008) would disclose . . . treprostinil of at least equal purity to that claimed in the '393 Patent, since the combination of these references discloses the same product and same process of Claims 1 and 9.

Ex. 1009 ¶ 89; *see also* Pet. 54. In addition, as explained above, Dr. Winkler testifies that a skilled artisan would have made the cited combination, with an expectation of success, in order to obtain a treprostinil product of improved purity. Ex. 1009 ¶¶ 88–90; Pet. 54–55. On this record, we credit Dr. Winkler's testimony.

UTC does not offer evidence or argument to suggest that the additional process steps recited in claims 6, 15, and 21 impart structural or functional differences to the claimed product beyond that discussed above in Parts II.B, II.D, and II.E. Rather, UTC contends that SteadyMed has not asserted that the products of claims 6, 15, and 21 would have been obvious in view of the cited art. Prelim. Resp. 54. UTC frames SteadyMed's position as an argument that the recited process steps would have been obvious, and would have inherently resulted in the claimed product. *Id.*

We do not find UTC's contentions persuasive. We observe that claims 6, 15, and 21 differ from their respective independent claims only in that they require the performance of optional step (d) from claims 1 and 9, and in the case of claims 6 and 15, specify the acid to be used in carrying out that process step. Ex. 1001, 19:39–40, 20:59–60. As set forth in detail in Parts II.A, II.B, II.D, and II.E, on the record before us, and for purposes of

this decision, we conclude that the process steps recited in the challenged claims, including step (d), do not impart structural or functional differences over prior art treprostinil products.

Furthermore, we disagree with UTC's characterization of SteadyMed's obviousness argument. We note, for example, that under the general rule for the interpretation of product-by-process claims, which we determine applies here, the products of claims 1, 6, and 21 are interpreted to be the same, namely, the product of claim 1. Likewise, the same analysis applies for the products of claims 9 and 15.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the treprostinil products of claims 6, 15, and 21. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 6, 15, and 21 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps.

Claim 10

Claim 10 recites "[t]he product of claim 9, wherein the purity of product of step (d) is at least 99.5%." Ex. 1001, 20:47–48. The present record supports SteadyMed's contention that claim 10 is obvious in view of Moriarty, Eğe, Phares, and Kawakami. Pet. 55–56; *see also* Ex. 1009 ¶¶ 82–90. As detailed in Parts II.B, II.D, and II.E, the present record supports SteadyMed's position that Moriarty discloses treprostinil free acid having a

purity of 99.7% (Pet. 20; *see also* Ex. 1004, 13; Ex. 1009 ¶ 65), and Phares discloses treprostinil diethanolamine salt of the same form and at least the same purity as that claimed in the '393 patent (Pet. 27–28; Ex. 1005, 88–93; Ex. 1009 ¶¶ 59–62). The present record further supports SteadyMed's contention that even if Dr. Walsh's impurity measurements are credited, the 0.1% difference between the purity of the sample prepared according to Moriarty, and claim 10 is within the expected level experimental error for impurity measurements, and the degree of inter-batch variability in impurity content is such that Dr. Walsh's results are insufficient to support a conclusion of nonobviousness. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71.

UTC does not offer evidence or argument to suggest that the additional process step recited in claim 10 imparts structural or functional differences to the claimed product beyond that discussed above in Parts II.A, II.B, II.D, and II.E. Neither does UTC present any additional argument regarding the recited purity requirement beyond those already addressed above. UTC does reassert its position, discussed with regard to claims 6, 15, and 21, that SteadyMed has not asserted that the product of claim 10 would have been obvious in view of the cited art. Prelim. Resp. 54. For the reasons set forth above, however, we do not find this contention persuasive.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the treprostinil product of claim 10. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claim 10

do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps at this time.

Claim 22

Claim 22 recites "[t]he product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d)." Ex. 1001, 21:14–16. The present record supports SteadyMed's contention that claim 22 is obvious in view of Moriarty, Eğe, Phares, and Kawakami. Pet. 56–57; *see also* Ex. 1009 ¶¶ 82–90. As discussed above in Parts II.D and II.E, the present record supports SteadyMed's position that the cited combination renders obvious a pharmaceutically acceptable treprostinil salt.

UTC does not offer evidence or argument to suggest that the additional process step recited in claim 22 imparts structural or functional differences to the claimed product beyond that discussed above in Parts II.A, II.B, II.D, and II.E. Neither does UTC present any additional argument regarding the recited purity requirement beyond those already addressed above. UTC does reassert its position, discussed with regard to claims 6, 15, and 21, that SteadyMed has not asserted that the product of claim 22 would have been obvious in view of the cited art. Prelim. Resp. 54. For the reasons set forth above, however, we do not find this contention persuasive

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the

treprostinil products of claim 22. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 22 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps at this time.

Conclusion

For the foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 6, 10, 15, 21, and 22 are obvious in view of Moriarty, Eğe, Phares, and Kawakami.

G. Secondary Considerations of Non-Obviousness

UTC contends that objective indicia of non-obviousness, such as purported evidence of long-felt but unmet need, unexpected results, commercial success, and copying support the patentability of the challenged claims of the '393 patent. Prelim. Resp. 55–58.

We conclude that the evidence of secondary considerations currently of record is not sufficient, at this point in the proceeding, to support UTC's contention. As an initial matter, we observe that "secondary considerations are better considered in the context of a trial when the ultimate determination of obviousness is made." *Crocs, Inc. v. Polliwalks, Inc.*, Case IPR2014-00424, slip op. 16 (PTAB Aug. 20, 2014) (Paper 8). In addition, we note that UTC's contentions regarding long-felt need and unexpected results are predicated on UTC's claim that treprostinil made according to the process described in the '393 patent has fewer impurities than treprostinil produced by other methods. However, as explained in Parts II.B, II.D, and

II.E above, the present record does not support that contention. We also observe that UTC does not offer evidence of a nexus between the claimed invention and its commercial success. For example, UTC does not offer evidence concerning its relative share of the market for treprostinil products, or demonstrating that its revenues or market share increased after it began manufacturing treprostinil according to the process described in the '393 patent. Finally, we note that the mere existence of litigation concerning the '393 patent alone is insufficient to establish copying. *See Iron Grip Barbell Co. v. USA Sports, Inc.*, 392 F.3d 1317, 1325 (Fed. Cir. 2004) ("Not every competing product that arguably fails within the scope of a patent is evidence of copying. Otherwise every infringement suit would automatically confirm the nonobviousness of the patent.").

H. Other Asserted Grounds of Unpatentability

SteadyMed also asserts the following ground of unpatentability:

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Kawakami

In light of the grounds specifically discussed above, on the basis of which we institute review, we exercise our discretion and decline to consider these other grounds asserted in the Petition. *See* 37 C.F.R. § 42.108(a). We observe that SteadyMed presents the above ground of unpatentability and the obviousness of claims 1–5, 7–9, 11–14, and 16–20 in view of Moriarty and Phares, a ground on which we institute review, in the alternative.

III. CONCLUSION

For the foregoing reasons, we determine that the information presented in the Petition establishes that there is a reasonable likelihood that SteadyMed would prevail in challenging claims 1–22 of the '393 patent. At this juncture, we have not made a final determination with respect to the patentability of the challenged claims, nor with respect to claim construction.

IV. ORDER

For the foregoing reasons, it is

ORDERED that pursuant to 35 U.S.C. § 314(a), an *inter partes* review is hereby instituted for the following grounds of unpatentability:

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 102(b)	Phares
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Phares
6, 10, 15, 21, and 22	§ 103(a)	Moriarty, Phares, Kawakami, and Eğe

FURTHER ORDERED that no other ground of unpatentability asserted in the Petition is authorized for this *inter partes* review; and

FURTHER ORDERED that pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4, notice is hereby given of the institution of a trial; the trial will commence on the entry date of this decision.

PETITIONER:

Stuart E. Pollack
Lisa A. Haile
DLA Piper LLP
stuart.pollack@dlapiper.com
lisa.haile@dlapiper.com
steadymed-ipr@dlapiper.com

PATENT OWNER:

Stephen B. Maebius
George Quillin
FOLEY & LARDNER LLP
smaebius@foley.com
gquillin@foley.com

Shaun R. Snader
UNITED THERAPEUTICS CORP.
ssnader@unither.com

Douglas Carsten
Richard Torczon
Robert Delafield
WILSON, SONSINI, GOODRICH & ROSATI
dcarsten@wsgr.com
rtorczon@wsgr.com
bdelafield@wsgr.com

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
Patent 8,497,393

Patent Owner Response to Petition

4814-0612-4340.3

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page(s)

Federal Cases

Federal Statutes

Regulations

Other Authorities

I. INTRODUCTION

United Therapeutics Corporation ("UTC") submits this Response in accordance with 35 U.S.C. § 316(a)(8) and 37 C.F.R. § 42.120, responding to the instituted grounds of the Petition for *Inter Partes* Review filed by SteadyMed Ltd. ("SteadyMed") challenging claims 1-22 of U.S. Patent No. 8,497,393 ("the '393 patent"). The Declaration of Dr. Williams ("Ex. 2020") and of Dr. Ruffolo ("Ex. 2022") are filed herewith in support of the Response (Ex. 2020 and Ex. 2022, respectively). The Board should conclude that SteadyMed has failed to prove by a preponderance of the evidence that the instituted claims are unpatentable, as required under 35 U.S.C. § 316(e).

II. SUMMARY OF THE ARGUMENT

SteadyMed's anticipation and obviousness arguments are flawed for two fundamental reasons. First, SteadyMed's arguments rely on Moriarty (Moriarty *et al.*, J. Org. Chem. 2004, 1890-1902; Ex. 1004) and Phares (International Publication No. WO 2005/007081; Ex. 1005), but neither reference discloses the same highly pure treprostinil or treprostinil diethanolamine product claimed by the '393 patent when properly construed, let alone the same synthesis recited in the instituted claims. In fact, the Office considered both references during prosecution of the '393 patent, and the Office construed the claims of the '393 patent in a way that distinguished the product of the '393 patent specifically from the Moriarty

product. Moreover, a person of ordinary skill in the art ("POSA") would not look to either Eğe (Seyhan N. Eğe, Organic Chemistry 543-547 (2d ed. 1989) (Ex. 1008) or Kawakami (JP 56-122328A) (Ex. 1007) as neither reference is relevant to further purification of the complex treprostinil carboxylic acid structure that is at issue in the '393 patent, and a POSA would have no reasonable expectation of success in combining these references with either Moriarty or Phares.

Second, SteadyMed's anticipation and obviousness arguments are flawed because they misunderstand, both the error associated with such measurements and the difference between "assay purity" against a standard and measurements of purity that directly measure the level of impurities. As explained in the Williams and Ruffolo Declarations, this misunderstanding resulted in Petitioner's incorrect assertion that there are inconsistencies between the purity values recited in the '393 specification, the Walsh Declaration, and the Moriarty prior art. Ex. 2020 at ¶¶88-89; Ex. 2022 at ¶¶73-74. Dr. Williams notes that the '393 patent itself expressly refers to assay purity values as "HPLC (assay)" values whenever it uses such measurements, as opposed to other purity values based on measuring amount of impurities. Ex. 2020 at ¶89. Dr. Ruffolo further explains that FDA drug approval system rests on precise measurements of individual impurities that make up a purity "specification" for a drug, which can be reliably determined within the detection limits of HPLC measurements. Ex. 2022 at ¶¶32-35 and 44-50. Dr.

Ruffolo also specifically notes that it is routine to have assay purity values above 100% because it is a relative value measurement. Ex. 2022 at ¶53.

SteadyMed's purported expert, Dr. Winkler, confirmed this misunderstanding. Dr. Winkler acknowledged at his deposition that FDA's purity specification of less than [redacted]% for the impurity [redacted] indicates that precise measurements of impurities are possible: "I would think that the error in the measurement for [redacted] would be, should be less than [redacted] percent." Ex. 2051 at 64:7-9. Dr. Winkler further acknowledged that he did not know how the treprostinil purity specification adopted by FDA could change from [redacted]% to [redacted]% and stated that he viewed purity levels above 100% as errors: "I think the thing that I am able to conclude from the data that is on page 6 of this, of this letter [Ex. 2006] is that the error in the HPLC assay could be as high as [redacted] percent in the first column and by my analysis could be as high as [redacted] percent in the second column." Ex. 2051 at 86:15-21; 24-25; 87:2-9. As Dr. Williams explained, Dr. Winkler's conclusions on this point appear "to arise from Dr. Winkler's fundamental misunderstanding of how assay purity values are calculated." Ex. 2020 at ¶¶90-92; *see also* Ex. 2022 at ¶¶74. Moreover, Dr. Winkler admitted he did not know what the actual error was associated with the measurements submitted in the Walsh declaration. Ex. 2051 at 62:16-25; 63:2-14. Because Dr. Winkler does not understand the basic differences in types of purity measurements and their related

errors that are used in the '393 patent, discussed in the Walsh Declaration, and which form the basis for FDA's regulation of drug product manufacturing, his declaration should not be credited.

Moreover, the Williams Declaration establishes that there are measurable structural differences between the average impurity profiles of the Moriarty product and the claimed product based on data obtained from 175 batches. Ex. 2020 ¶¶94-99, Appendices A-B; see also Ex. 2005, Ex. 2036, Ex. 2037, Ex. 2052, Ex. 2053. The average impurity profiles show that Moriarty process and the '393 process produce two physically distinct products that contain different total and specific impurities. *Id.* Specifically, the claimed product essentially lacks certain impurities found in the Moriarty product, such as [redacted], and [redacted]. Ex.2020 at ¶¶96-97. The claimed product also contains much smaller amounts of other impurities that are found in the Moriarty product, such as [redacted], [redacted]. *Id.* at ¶96.

Furthermore, based on the same 175 batches, the average purity of the '393 product is [redacted] greater than the average purity of the Moriarty product, thereby corroborating that the Moriarty process and the '393 process produces two physically distinct products that contain measurable and significant structural differences. *Id.* at ¶98.

Finally, the initial claim construction of the preamble "a product… comprising" urged by SteadyMed and adopted by the Board would violate the canon that patent claims may not be construed to encompass material that was clearly disavowed in order to obtain allowance of claims. Even under the broadest reasonable interpretation standard, the Board has found in its own cases that the prosecution history may limit the plain meaning of a limitation in a claim, which otherwise is presumed to apply. The '393 claims were allowed after submission of the Walsh Declaration, which established the differences between the '393 products and the Moriarty product. This disavowal of the Moriarty subject matter is further reinforced by additional intrinsic evidence. The '393 patent includes a side-by-side comparison in Example 6 to show the difference between the Moriarty product and the '393 product and repeatedly references higher purity and different impurity profile compared to Moriarty. In the face of this disavowal, it is improper to construe "a product …comprising" to allow the impurities "without limitation," as such a construction would encompass the impurity profile of Moriarty.

In addition, the Williams Declaration explains why Phares cannot anticipate the claimed products because of the particular conditions used to prepare the Phares product for polymorph screening and because of the uncertain provenance of starting treprostinil used to make the diethanolamine salt.

As to instituted grounds 2 and 3, Dr. Williams also explains why the references in the instituted obviousness grounds would not have been combined in the asserted manner due to lack of motivation and the failure of the references to provide an expectation of success for achieving the purity level and impurity profile of the '393 patent in the specific case of treprostinil. Kawakami teaches away from the selection of diethanolamine, the salt specifically claimed in claims 14 and 18. Lastly, secondary considerations of long-felt need and unexpected results would rebut any case of obviousness as to grounds 2 and 3.

In view of the foregoing, SteadyMed has not met its burden of proving the unpatentability of claims 1-22 by a preponderance of the evidence, as required under 35 U.S.C. § 316(e).

III. STRUCTURAL/FUNCTIONAL DIFFERENCES OF THE CLAIMED PRODUCTS OVER THE CITED ART

The combined Declarations of Dr. Williams and Dr. Ruffolo establish that the '393 product has a different impurity profile than the Moriarty product, and in fact, that the '393 product has higher average purity. These differences matter. FDA uses both overall purity and levels of individual impurities ("purity specification") as a basis to regulate the manufacturing of pharmaceuticals. Batches that fall outside of the purity specification cannot be sold or used to treat

patients. Thus, differences in purity and impurity profile are not merely academic, but critical to the successful manufacture of a clinical product.

A. The Importance of Purity in Pharmaceuticals

As noted by the '393 patent itself, "because Treprostinil, and other prostacyclin derivatives are of great importance from a medicinal point of view, a need exists for an efficient process to synthesize these compounds on a large scale suitable for commercial production." Ex. 1001, col. 1:57-61. The invention therefore "provides for a process that is more economical, safer, faster, greener, easier to operate, and provides higher purity." *Id.*, col. 5:47-50. As the treprostinil product is a drug product subject to the rules of FDA, the reduction of impurities is of great importance in the drug. Drug purity is defined by FDA as "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product." See, Ex. 2022 at ¶33; see also 21 C.F.R. §600.3 (r) (2015). The purity of a drug is of such importance to FDA that the purity level of a drug substance must appear in the drug product specification, which is a collection of data about the drug required by FDA. *See*, Ex. 2022 at ¶¶32-34. "Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of safety to patients." *Id.* at ¶36. This is due to

the fact that even trace amounts of impurities can sometime pose serious health concerns.

For example, the drug penicillin is one of the best known and extensively studied examples of trace impurities that can cause serious, life-threatening adverse events. *Id.* at ¶62. While penicillin is safe and effective for most people, it can cause serious allergic reactions resulting in anaphylaxis and death. *Id.* Because the amount of trace impurity of penicillin needed to cause an allergic reaction is so low, FDA has mandated the production of penicillin active pharmaceutical ingredient (API) and finished product to be made in buildings entirely separate from buildings that manufacture other APIs or finished drug product. *Id.*, *see also* FDA Guidance for Industry, Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination, (2013) (Ex. 2047) at 1-6. The same is true for the drug cephalosporin. Ex. 2022 at ¶63; *see also* Ex. 2047 at 1-6.

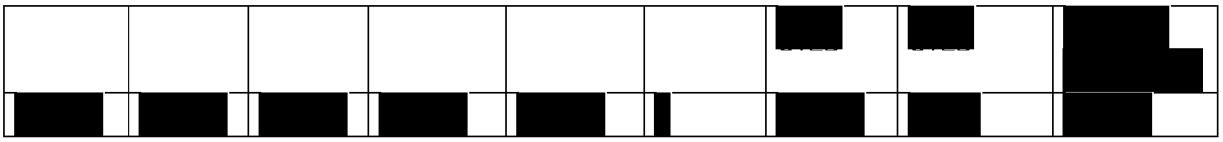
Additionally, human insulin is another example. For many years, human insulin was derived from pig pancreases, but then it became possible to produce human insulin in the bacteria *E. coli* using large bioreactors. Ex. 2022 at ¶64. Even though the human insulin derived from *E. coli* was highly pure, it contained very small trace amounts of *E. coli*, a very dangerous bacteria causing reactions (directly from the trace amounts of bacteria, and not due to infection) in some people even in trace amounts. *Id.* As a result, the product needed to be even more

highly purified to further minimize or eliminate the trace bacterial contaminants. *Id.* These examples highlight the importance of drug purity in pharmaceutical formulations and the potential risks to patients between two products that differ in their impurity profile and purity. By having a different impurity profile and overall purity, two products are structurally and functionally different.

B. The '393 Product Has A Different Impurity Profile and a Higher Purity Than Moriarty

As detailed in Dr. Williams' Declaration and supporting exhibits, comparing the average impurity profiles for the '393 product and the Moriarty product using data obtained from over 175 batches reveals measurable structural differences, as the two processes produce physically different products which contain different total and specific amounts of impurities. Ex. 2020 ¶¶94-99 and Appendices A-B; *see also* Ex. 2005, Ex. 2036, Ex. 2037, Ex. 2052, Ex. 2053. The batch reports show that the Moriarty product and the claimed product exhibit different impurity profiles and that the claimed product has a higher average purity than Moriarty's product. *Id.*

Moriarty Process Impurities (Average Percent Detected)								
1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substance**
0.0473	0.0407	0.2545	0.1646	0.1025	0.0405	0.0889	0.1028	**0.9545**
'393 patent Process Impurities (Average Percent Detected)								
[redacted]								



In total, the '393 product has [redacted] times fewer impurities than the Moriarty product.[1] Ex. 2020 ¶¶94-95. Additionally, certain specific impurities found in the prior art Moriarty product are essentially eliminated in the '393 product, as the '393 product does not contain detectable amounts of the impurity [redacted], and none of the commercial batches of the '393 product contain detectable amounts of [redacted] or [redacted]. Ex. 2020 ¶¶94, 96-97. Other impurities, including [redacted], [redacted], [redacted], and [redacted], are also greatly reduced in the '393 product as compared to the Moriarty product, while the level of the [redacted] impurity is slightly increased in the '393 product. Ex. 2020 ¶96. These substantial differences between the impurity profiles of the '393 product and the Moriarty product constitute structural differences between the claimed product and the prior art.

Furthermore, the average purity based on data from over 175 batches is higher for the '393 product than that of Moriarty. As shown above, the average purity of a Moriarty batch was 99.05% while the average purity of a '393 batch

[1] Moriarty Total Related Substances: 0.9545; '393 patent Process Total Related Substances: [redacted]

was [redacted]. Ex. 2020 ¶¶94-99. This is a marked improvement in overall purity. Moreover, the purity analyzed in these batches – the total related substances – is exactly the same type of analysis Dr. Walsh referred to in his declaration when referring to purity of the '393 patent process versus that of the Moriarty process. Thus, this analysis is consistent with how the inventor interpreted the purity of the '393 patent. And this analysis also persuaded the Office to allow the claims.

The Institution Decision cited to the Walsh Declaration for revealing "that each of the impurities detected in [the tested batch of] Moriarty treprostinil was present in an amount below that identified as acceptable in UTC's own specification for treprostinil produced according to the process disclosed in the '393 patent." Paper 12 at 20-21. First, the above data shows that the average amount of each impurity and the average purity is different between Moriarty treprostinil and the '393 product. Second, whether an isolated batch of Moriarty treprostinil does or does not satisfy the new FDA purity specification is not relevant to patentability. The question for patentability is whether or not a given batch of *starting* Moriarty treprostinil (steps a and b of the '393 independent claims) will be physically changed when step (c) is performed *on that batch*. The above averages show that it does change, as do the large scale synthesis examples 4-6 in the '393 patent. While Moriarty treprostinil may show inter-batch variation in overall purity and impurity profiles, the data of record establishes that

performing step (c) *on a given starting batch* of Moriarty treprostinil will lead to a higher purity and a different impurity profile in the end product. Petitioner has not established that any specific batch of Moriarty treprostinil is not physically changed by performing step (c), and all the evidence suggests that it is.

C. The Differences In Impurity Profile And Average Purity Between The '393 Product And Moriarty Are Functionally Important

The higher purity of the claimed product resulted in FDA approving a new assay purity for the treprostinil drug as noted in the January 2009 letter submitted to FDA by UTC. Ex. 2006 at 4-6; Ex. 2022 at ¶¶66-68; Ex. 2020 at ¶91. Furthermore, this change constitutes a "major" change according to the classification system for manufacturing changes used by FDA. Ex. 2022 at ¶¶70-72. FDA requires continuous testing of pharmaceutical batches to ensure that they fall within the established purity specification. Ex. 2022 at ¶¶32-40. If a given batch falls outside the established purity specification, then it will be rejected by FDA and cannot be sold for patient use. *Id.* at ¶32. FDA is so concerned about purity of pharmaceuticals that it requires companies to test for very tiny amounts of individual known impurities carried over into the final product based on the manufacturing process. *Id.* at ¶¶32-40. Thus, the change in the '393 product is commercially important and has real-world value.

IV. CLAIM CONSTRUCTION

In the Decision on Institution (Paper 28), the preliminary claim construction construes "[a] product comprising a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof" and "product" in an unreasonably broad manner. The Board is not bound by that preliminary construction based on an incomplete record. *See e.g., The Scotts Co., LLC v. Encap, LLC*, IPR2013-00110, Paper 79 (PTAB June 24, 2014) (overturning preliminary claim construction in final written opinion) (Ex. 2024). On the fuller record now available to it, the Board should adopt UTC's construction of the disputed terms.

A. Intrinsic Evidence Can Override The Presumption That "Comprising" Creates An "Open" Claim Construction

The claims at issue in an IPR must be given their broadest reasonable interpretation (BRI) in light of the specification, but the Board must still interpret claim terms according to established principles. The transition phrase "comprising" is only *presumed* to be an "open" phrase. *Crystal Semiconductor Corp. v. TriTech Microelectronics Int'l, Inc.*, 246 F.3d 1336, 1348 (Fed. Cir. 2001) ("In the parlance of patent law, the transition 'comprising' creates a presumption that the recited elements are only a part of the device, that the claim does not exclude additional, unrecited elements."). "While it is true that, as a general rule, the words of a patent claim are to be given their plain, ordinary and accustomed

meaning to one of ordinary skill in the relevant art, *Toro Co. v. White Consol. Indus., Inc.*, 199 F.3d 1295, 1299 (Fed. Cir. 1999), a court must nevertheless examine the remaining intrinsic evidence to determine whether the patentee has set forth an explicit definition of a term contrary to its ordinary meaning, has disclaimed subject matter, or has otherwise limited the scope of the claims." *Day Intern., Inc. v. Reeves Brothers, Inc.*, 260 F.3d 1343, 1349 (Fed. Cir. 2001).

The intrinsic record, both the specification and the prosecution history, must be reviewed to determine if there are limits to terms in the claims that would otherwise be given their presumptive plain meanings. Prosecution history "limits the interpretation of claims so as to exclude any interpretation that may have been disclaimed or disavowed during prosecution in order to obtain claim allowance." *Standard Oil Co. v. American Cyanamid Co.*, 774 F.2d 448, 452 (Fed. Cir. 1985). Similarly, the specification may contain repeated statements distinguishing the prior art that limit the claims. *SafeTCare Mfg., Inc. v.Tele-Made, Inc.*, 497 F.3d 1262, 1269-70 (Fed. Cir. 2007) (finding disclaimer where the specification repeatedly indicated that the invention operated by "pushing (as opposed to pulling) forces," and then characterized the "pushing forces" as "an important feature of the present invention").

Under the BRI standard, the Board should take into account both the specification and the prosecution history because the patent examiner and the

applicant have already worked together to determine the scope of the claimed invention. *See In re Buszard*, 504 F.3d 1364, 1366-67 (Fed. Cir. 2007) ("The patent examiner and the applicant, in the give and take of rejection and response, work toward defining the metes and bounds of the invention to be patented."); *In re Zletz*, 893 F.2d 319, 321 (Fed. Cir. 1989) ("When the applicant states the meaning that the claim terms are intended to have, the claims are examined with that meaning, in order to achieve a complete exploration of the applicant's invention and its relation to the prior art.").

The Board has followed these principles of claim construction in other IPR proceedings. *See, e.g.*, *The Scotts Co., LLC v. Encap, LLC*, IPR2013-00110, Ex. 2024 at 14-16. In *Scotts*, the Board changed its preliminary claim construction of "being in a solid state at time of coating" because the Board found that the patent owner had disavowed claim scope during prosecution in order to overcome a specific prior art reference. Ex. 2024 at 15. The Board relied on statements made in Examiner Interview Summaries which confirmed that claim amendments and arguments presented overcame the prior art. *Id.*; *see also* Prosecution History of U.S. Patent No. 6,209,259 (Ex. 2025). As another example, the Board recently construed a phrase to exclude trace amounts of a substance based on statements made during prosecution distinguishing prior art containing trace amounts of the substance. *Daicel Corp. v. Celanese Int'l Corp.*, IPR2015-00171, Paper 86 at 41

(PTAB June 23, 2016). Thus, the BRI cannot be divorced from the intrinsic evidence, including the prosecution history. Such a construction is not reasonable.

B. The Distinct Impurity Profile And Higher Purity Of the '393 Patent Product Were Clearly Considered Part of the Claimed Product During Prosecution

As explained during prosecution,"[e]ach of treprostinil as the free acid and treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 . . . is physically different from treprostinil prepared according to the process of 'Moriarty' due to differences in their impurity profiles." Ex. 1002 at 344. In fact, the Examiner required UTC to provide evidence in declaration form showing that the product of claims 1 and 10 was different than Moriarty's product. *Id.* at 328. In response, UTC filed the Walsh Declaration, which demonstrated that the claimed product had a different impurity profile and higher purity than Moriarty's product. *Id.* at 347-349. It was upon these statements and evidence that Moriarty was overcome, and shortly thereafter the Examiner issued a Notice of Allowance. *Id.* at 354-360.

In addition, the '393 specification repeatedly refers to the differences of the '393 product compared to Moriarty. The entirety of Example 6 in the '393 specification is a large scale, side-by-side comparison between Moriarty and the '393 product, which shows a purity of 99.0% for Moriarty and 99.9% for the '393 product. Ex. 1001, 17:step 53. At the end of this example, the '393 specification

further states that "impurities carried over from intermediate steps (i.e., alkylation of triol and hydrolysis of benzindene nitrile) are removed during the carbon treatment and salt formation step" (Ex. 1001, 17:29-32), which are the same differences (higher purity and different impurity profile) that UTC relied upon in the Walsh Declaration during prosecution as noted above.

These statements by UTC demonstrate that the claimed "product" must have an impurity profile conferred by its process steps. *See Purdue Pharma L.P. v. Endo Pharms. Ins.*, 438 F.3d 1123, 1136 (Fed. Cir. 2006); *see also Atofina v. Great Lakes Chem. Corp.*, 441 F.3d 991, 997 (Fed. Cir. 2006) (statements made during prosecution history that distinguished the claimed invention from the prior art constituted a prosecution disclaimer); *see also United Therapeutics Corp. v. Sandoz, Inc.*, 2014 WL 4259153, *54-56 (D.N.J. Aug 29, 2014) (finding compounds made by different processes resulted in different impurity profiles meaning they were structurally different).

D. The Plain Meaning Of "Product" In The Context Of The '393 Product-By-Process Claims Requires The Characteristics Conferred By The Process Steps Be Present

The term "product" in the context of the '393 patent should be construed as "a substance resulting from a chemical reaction." This is consistent with the '393 patent itself (Ex. 1001 at col. 3, lines 3, 4, 65, and 66; col. 5, line 45; col. 6, lines 65 and 66; and col. 7, line 17), as well as the understanding of a POSA and the

generally accepted definition in chemistry. Ex. 2020 at ¶¶60-62. Additionally, Dr. Williams and Dr. Winkler both use the term product to refer to the result of a chemical reaction in their own work. Id. at ¶¶63-65; *see also* Ex. 2031 at 155:2-11 ("the product of a chemical reaction would be essentially all of the substances that result from the treatment of a particular reactant with a particular set of reagents."). To construe the term "product" as "a chemical composition" is too broad and improperly disregards a significant portion of the intrinsic record. As described above, a product is the result of a chemical reaction and has its own impurity profile depending upon how it is made. "A chemical composition" could be anything and is in no way limiting to what the term "product" actually means. Ex. 2020 at ¶¶66-68.

V. GROUND 1: PHARES FAILS TO EXPLICITLY OR INHERENTLY DISCLOSE EACH AND EVERY LIMITATION OF CLAIMS 1-5, 7-9, 11-14 OR 16-20

The Board instituted Ground 1 based on the conclusion that Phares teaches the treprostinil diethanolamine salt product recited in claims 1 and 9, and that the recited process steps of the claims do not impart structural or functional differences over Phares' treprostinil diethanolamine salt. As discussed below, SteadyMed has failed to establish anticipation based on Phares.

A. SteadyMed Cannot Pick and Choose From Unrelated Portions of Phares to Establish Anticipation

In attempting to show anticipation, SteadyMed cites four different portions of Phares, Ex. 1005, as teaching the combined elements of claims 1 and 9. However, SteadyMed selectively ignores other portions in the Phares disclosure that suggest the four disparate portions of Phares should not be cobbled together to a single allegedly anticipatory embodiment. Petition at 22-24 and 33-34.

The portions of Phares cited by SteadyMed each relate to distinct subject matter, and Phares provides no description that would lead to the combination of these separate disclosures. Ex. 2020 at ¶¶79-84. Phares' only disclosure of steps (a) and (b) is directed to the enantiomer (-)-treprostinil, which are not the same as the synthesis for treprostinil. Ex. 2020 at ¶¶79-81. In fact, the intermediate products disclosed in the enantiomer synthesis as well as several reagents are different than the synthesis of treprostinil. *Id.* at ¶81. In contrast, Phares' separate alleged disclosure of step (c) is silent as to how the starting treprostinil acid was prepared. Ex. 1005 at 85. Thus, there is no reason set forth in Phares to combine the single teaching of steps (a) and (b) directed to one enantiomer with the other teachings of step (c), which are all directed to the other enantiomer. Ex. 2020 at ¶¶79-81.

Despite the alleged disclosure in Phares' that enantiomers of the disclosed compounds can be prepared using the proper chiral reagents, Phares itself teaches that treprostinil can be prepared in other ways that do not include steps (a) and (b), including the processes disclosed in US Patent Nos. 4,306,075 (Ex. 2032) and 5,153,222 (Ex. 2033). Ex. 1005 at 11; Ex. 2020 at ¶78. Thus, a POSA would reasonably conclude that the diethanolamine salts of Phares were prepared based on other disclosed methods that do not require steps (a) and (b). Ex. 2020 at ¶78. If the diethanolamine salts of Phares were prepared differently than the recited process steps, nothing in Phares establishes that the diethanolamine salts are necessarily the claimed product.

B. The Proper Construction of a "product comprising a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof" Precludes A Finding That Phares Anticipates the Present Claims

The Board's institution of Ground 1 was partly based on its preliminary finding that "comprising" does not exclude impurities that may possibly be produced by the process of Phares and that the impurity profile of Phares' diethanolamine salt is identical to that of the claimed product. *See* Paper 12 at 30. However, such a finding does not take into consideration the reasonable construction of "product comprising a compound [of/having] formula [I/IV] or a

pharmaceutically acceptable salt thereof," which is set forth in this Response and supported by the record now before the Board.

As discussed above in Section IV, both the specification and the prosecution history of the '393 patent distinguish the claimed product from prior art treprostinil products based on its higher purity and different impurity profile, which is achieved through the recited process steps. Thus, to prevail on Ground 1, SteadyMed must show that the Phares' diethanolamine salt necessarily possesses an impurity profile that is distinct from that of the Moriarty product and with higher purity.

Steadymed simply assumes that the diethanolamine salt discussed by Dr. Winkler is prepared from Moriarty treprostinil and does not acknowledge that the source of treprostinil would impact both the overall purity and impurity profile of the resulting salt. As exemplified in the '393 patent, the claimed process provides an improved treprostinil product due to its superior purity. As evidenced by the Williams Declaration and the batch record data, the claimed product has an average purity of [redacted] and a distinct impurity profile from Moriarty's product. Ex. 2020 at ¶¶94-99. Importantly, SteadyMed has failed to show that, at a minimum, the Phares' diethanolamine salt possesses an impurity profile that is distinct from that of the Moriarty product and contains fewer overall impurities than the Moriarty product. Nor has SteadyMed shown that the Phares'

diethanolamine salt has a higher purity than the Moriarty product. Indeed, SteadyMed's only argument regarding the purity of Phares' diethanolamine salt is based on the theory that the higher melting point of Phares' diethanolamine salt necessarily means that it must be at least equal in purity to that of the exemplified batches in the '393 patent. *See* Petition at 27-28. However, for the reasons noted below, that is an incorrect conclusion based on the evidence now in the record.

C. The Higher Melting Point of Phares' Diethanolamine Salt Does Not Necessarily Mean That it is of Higher Purity Than the Diethanolamine Salts of the '393 Patent

The Board relied on incorrect statements in the Winkler Declaration alleging that Phares' diethanolamine salt must be more or at least equally pure as the claimed product solely because the former has a higher melting point. Paper 12 at 28-29. However, melting point is just one factor in assessing a compound's purity and is not necessarily a reliable metric of purity. This is especially applicable to Phares because only one melting point value was obtained in a sample for a polymorph screen. A POSA would not rely upon a single melting point value, absent any other impurity information, to determine the purity of a substance made under unspecified conditions. Ex. 2020 ¶76. Indeed, the "higher" melting point of Phares' diethanolamine salt could be indicative of the inclusion of impurities or the result of the use of different solvent systems for the crystal forms. *Id.* Accordingly,

the purity of a compound cannot be assessed based solely on its melting point value.

Moreover, even if the melting point could be relied upon, the data cited by Dr. Winkler does not indicate a product of high purity. To the contrary, Fig. 21 of Phares "shows a broad melting peak with a range of close to 10 degrees which is indicative of a lower purity substance." Ex. 2020 ¶76; *see also*, Marti, E., *Purity determination by differential scanning calorimetry*, Thermochimica Acta, 5(1972) 173-220 at 214 ("The melting of diphenyl is extremely sharp because of the purity level; on the other hand, the melting region of phenacetin-benzamide is rather broad.") (Ex. 2031).

Additionally, Phares discloses several different conditions for preparing Polymorph A of the diethanolamine salt and that Polymorph A is required to make Polymorph B. Ex. 2020 at ¶73. The '393 patent does not indicate that making Polymorph A first is required. *Id.* Phares also indicates many conditions used to make Polymorph A and Polymorph B, but it is not clear what conditions were specifically used for the sample analyzed in Figure 21 that Dr. Winkler relies upon. *Id.* at ¶¶73-74. It is well known that the use of different solvent systems in forming different crystal forms can have a significant effect on the melting point of a substance, as well as other characteristics, including purity, and a higher melting point does not always mean a higher purity. *Id.* at ¶¶75-76; *see also* R. Adhiyaman,

et.al., *Crystal modification of dipyridamole using different solvents and crystallization conditions*, Int'l J. Pharm.321 (2006) 27-34 at 33 ("Adhiyaman") ("In conclusion, it can be said that the crystallization conditions and medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves.") (Ex. 2030).

Dr. Williams, therefore, has concluded that "[i]t is known in the art that sample size, rate of heating, the recrystallization solvent(s) used, and the conditions under which the crystalline sample was obtained can significantly affect the DSC data. Dr. Winkler's conclusion based on this single vague and incompletely described DSC data is not scientifically sound." *Id.* at ¶76.

Thus, nothing in Phares establishes that the disclosed diethanolamine salt is at least of equal purity to the diethanolamine salts of the '393 patent. With respect to claim 2 of the '393 patent specifically, nothing in Phares discloses a purity of at least 99.5%. Ex. 2020 at ¶82. For this additional reason, Phares cannot anticipate claim 2.

D. Phares Fails To Disclose the Claimed Process for Making Treprostinil or Any Purity or Impurity Profile for Treprostinil Diethanolamine

SteadyMed has failed to establish that Phares' diethanolamine salt (Form B) is the claimed product.

First, as Dr. Williams notes, the samples of treprostinil diethanolamine disclosed in Phares were "made for a polymorph screen, not large scale batches." Ex. 2020 ¶73. Accordingly, "the samples of polymorph B described in Phares are prepared in a completely different way under different conditions than those described in the '393 patent." Ex. 2020 ¶75. Specifically, Phares discloses first preparing polymorph A by any one of a variety of methods and then preparing polymorph B from some sample of polymorph A. In contrast, the '393 patent makes no mention of first forming polymorph A. Ex. 2020 ¶¶73-74. Additionally, Phares describes reaction conditions for making the polymorph samples that are not described anywhere in the '393 patent. *Id.* In particular, the reaction conditions disclosed for the sample of polymorph B characterized by Phares, heated slurries of form A in 1,4-dioxane and toluene, are not described anywhere in the '393 patent. *Id.* It is well-known that the use of different reaction conditions, including different solvents, can significantly affect the characteristics of a given crystal form. Ex. 2020 ¶75. As a result, the diethanolamine salt disclosed in Phares cannot be directly compared to the diethanolamine salt disclosed in the '393 patent.

Second, the Williams Declaration clearly establishes that the claimed product has an average purity of [redacted], thus giving it a superior purity and distinct impurity profile over that of the prior art treprostinil products. Ex. 2020 ¶¶94-99. The purity of the claimed product provides a structural difference from the prior art

treprostinil, as evidenced by the differences in the average impurity profiles for the Moriarty product and the '393 product. *Id.*, Ex. 2036, Ex. 2037. Indeed, the higher purity of the claimed product resulted in FDA approving a new purity specification for the treprostinil drug as noted in the January 2009 letter submitted to FDA by UTC. Ex. 2006 at 4-6; Ex. 2022 at ¶¶70-72; Ex. 2020 at ¶91.

The impurity profile of the *starting* treprostinil acid used to prepare the Phares diethanolamine salt is crucial to assess whether the diethanolamine salt is the same as the claimed product, *i.e.*, whether the impurity profile of the diethanolamine salt in Phares is identical to that of the claimed product. Ex. 2020 ¶¶76-78. However, nowhere does Phares disclose the process of preparing the treprostinil acid used to prepare the diethanolamine salt. As acknowledged in both Phares and the '393 patent, several different processes can produce treprostinil acid. *See, e.g.*, Ex. 1005 at 11; *see also,* Ex. 2020 ¶78. Each known process can produce a treprostinil acid with a unique impurity profile. Ex. 2020 ¶78. Because Phares does not disclose the process of preparing the starting treprostinil acid for the diethanolamine salt, the impurity profile of the diethanolamine salt cannot be established. Without knowing the impurity profile and level of purity of Phares' diethanolamine salt, SteadyMed cannot show that it is necessarily identical to the claimed product or has equal purity to the claimed product.

Consequently, SteadyMed has not carried its burden on Ground 1.

VI. GROUND 2: MORIARTY AND PHARES FAIL TO RENDER OBVIOUS CLAIMS 1-5, 7-9, 11-14, OR 16-20

Moriarty does not teach salt formation and regeneration of the free acid. SteadyMed attempts to cure this deficiency in Moriarty by citing Phares for allegedly teaching step (c). However, Moriarty teaches three distinct methods of preparing the treprostinil free acid. Nothing in Moriarty directs a POSA to select one specific process over the three disclosed for purposes of further modification by adding a salt formation step. Furthermore, SteadyMed fails to recognize that the performance of step (c) after steps (a) and (b) unexpectedly results in a product with an improved average purity over that of the prior art. Indeed, the Williams Declaration demonstrates that, out of 122 samples, the claimed product has an average purity of greater than [redacted]. Ex. 2020 at ¶¶94-95 and Appendices A-B.

As discussed above, the claimed product is structurally different from Moriarty's product because the claimed product has a distinct impurity profile, including a marked reduction in several specific impurities, and a higher average purity relative to Moriarty's product. Ex. 2020 at ¶¶94-99 and Appendices A-B. This evidence shows that, in the recited combination, performing step (c) in conjunction with steps (a) and (b) of the present claims produces a treprostinil product that is significantly improved over that of the prior art. Ex. 2020 at ¶¶48-49, 70.

Moreover, Moriarty's product cannot render obvious the claimed product because during prosecution of the '393 patent, UTC overcame a rejection based upon Moriarty by providing evidence of representative sample impurity profiles, showing the physical difference between the product of the '393 patent and the Moriarty product. Ex. 1002 at p. 347. Phares does not cure this deficiency because, as noted above, nothing in Phares establishes that the diethanolamine salt either 1) has an impurity profile similar to the claimed product or 2) has an overall purity at least equal to the claimed product.

In particular, it would not have been obvious to use the salt formation step of Phares to decrease amounts of at least [redacted] and [redacted], which are stereoisomers of treprostinil, and accordingly, are acidic rather than neutral or basic. Ex. 2020 at ¶102. Thus, when subject to salt-forming conditions, a POSA would expect that any undesired stereoisomer of treprostinil would be included in the final salt product because the stereoisomer would also be converted to the corresponding salt under such salt-forming conditions. A POSA has no reasonable expectation of success in removing any undesired treprostinil stereoisomer impurities by salt formation and subsequent regeneration of the free acid. *Id.* Instead, a POSA would expect the salt formation and subsequent regeneration to produce a final product with the same initial amount of stereoisomer impurities before the salt formation step. *Id.* Yet these impurities are each detected in only a single optimization batch

of the '393 product, and in none of the commercial batches. Even taking these optimization batches into consideration, this represents a greater than 100-fold reduction as compared to the Moriarty product. *Id.* at ¶¶94-96.

Additionally, as described above, there is no basis for comparing the "purity" in Moriarty with the purity described in the Walsh Declaration. *Id.* at ¶88. Walsh's Declaration makes clear that purity in terms of the '393 patent is assessed by looking to the total related substances of a batch. *Id.* at ¶¶88-89. The Moriarty reference, while not specifying a reference standard, does refer to a comparison to an authentic sample. *Id.* As a result, it is not clear what method was used to determine the purity in Moriarty and therefore a direct comparison of the value reported in Moriarty cannot be made to the '393 patent.

Moreover, Dr. Winkler fundamentally misunderstands the error associated with various purity measurements used in the Walsh Declaration, the '393 patent, the prior art, and FDA. Dr. Winkler states in his declaration that:

> even a difference of 0.4% as discussed below, between the claimed processes of the '393 Patent and the prior art, such as Moriarty (Ex. 1004), would be attributable to experimental error, and thus the claimed degree of purity under the claimed processes of the '393 Patent presents no distinction from the prior art.
>
> Ex. 1009 at ¶69.

He goes on to state that "HPLC's precision indicates that the 'RSD' or 'relative standard deviation' for a typical instrument is about 1%." *Id.* at ¶70.

This is wrong for several reasons. First, during his deposition, Dr. Winkler admitted he did not know what the actual error in the measurement was for the data submitted in the Walsh Declaration during prosecution of the '393 patent. Ex. 2051 at 62:16-25; 63:2-14.[2] While he did not know the error associated with the measurements made in the data submitted with the Walsh Declaration, he did admit that "the error in the measurement for the [redacted] [treprostinil impurity] would be, should be less than .1 percent," and in general, "[t]he error should be less than the maximum number reported, that's correct, for the measurement of the materials described here." Ex. 2051 at 63:25-64:4; 64:7-16. By his own admission, the error associated with the measurement of impurities in treprostinil batch records such as those submitted in Walsh's Declaration are therefore far less than the alleged error of 1% or 0.4% he stated in his declaration.

[2] Indeed, Dr Winkler admitted he was not familiar with FDA guidelines regarding impurity profiles for a drug, did not know what is required in order to change a drug specification, and was not familiar with published guidances from FDA regarding changes to new drug applications or abbreviated new drug applications. Ex. 2051 at 19:3-24.

In contrast, FDA requires that impurity determinations must be measured at or below 0.05% for drugs such as treprostinil. *See*, Ex. 2022 at ¶47; Ex. 2020 at ¶92. As Dr. Ruffolo explains, impurities in drug substances such as treprostinil that are administered in dosages less than 2 grams per day require that impurities be reported if they are present at a level less than or equal to 0.05%. *See, e.g.*, Ex. 2022 at ¶¶44-47; *see also* ICH Impurities in New Drug Substances Q3A(R2) monograph at 5-11 (Ex. 2038). "As a result of these thresholds, by definition, the limit of detection for impurities (and therefore total related substances) must be at least as low as 0.05%." Ex. 2022 at ¶50.

Furthermore, the '393 patent is directed to an improved and more pure treprostinil product. *See, e.g.*, Ex. 1001, 17:27-40. Given that Moriarty discloses the use of column chromatography for purification, a POSA would not be motivated to create the salt form in Phares, as Phares does not disclose any benefit or increased purity as a result of using the diethanolamine salt. Ex. 2020 at ¶101. "In fact, Phares does not allege that the diethanolamine salt is superior in any way to the treprostinil product of Moriarty and instead identifies other earlier treprostinil disclosures as a means to create the treprostinil used to form the diethanolamine salt." *Id.* A POSA would not have a reasonable expectation of success by using salt formation as a purification step separate from or in addition to the column chromatography of Moriarty, as Phares does not disclose any alleged

benefit to forming the salt and a POSA would have no expectation that only certain acidic and neutral impurities would be reduced or completely eliminated while others remained. *Id.* at ¶102. Thus, the combination of Moriarty and Phares cannot render obvious claims 1-5, 7-9, 11-14, or 16-20.

Similarly, as described above, there is no basis to compare the purity disclosed in Moriarty to the measurements obtained in the '393 patent or those obtained by Dr. Walsh in his declaration, and therefore, claim 2 would also not be rendered obvious by the combination of Phares and Moriarty for this additional reason. *Id.* at ¶103.

Claims 8 and 16 also require the additional limitation that the formula (VI) compound of step (a) is not purified. In fact, the '393 patent specifically distinguishes this limitation over the prior art. Ex. 1001, Example 6. Moriarty expressly discloses that the compound of formula (VI) from step (a) is purified. Ex. 2020 at ¶104. Phares does not disclose any synthesis for treprostinil and, even in the abbreviated synthesis of the enantiomer, no details of purification are disclosed. *Id.* Thus, claims 8 and 16 are not rendered obvious by the combination of Phares and Moriarty for this additional reason. Process advantages should be considered as secondary considerations to rebut obviousness, even if the process steps or advantages are not considered in the initial determination of whether there is *prima*

facie obviousness (where the products are compared regardless of how they are made).

Consequently, SteadyMed has not carried its burden on Ground 2.

VII. GROUND 3: MORIARTY, PHARES, KAWAKAMI, AND EĞE FAIL TO RENDER OBVIOUS CLAIMS 6, 10, 15, 21, AND 22

A. The Product of Claims 6, 15, and 21 Are Different Than the Prior Art Treprostinil Products

The Board concluded that the process steps of claims 6, 15, and 21, including step (d), do not impart structural or functional differences over prior art treprostinil products. Paper 12 at 46-47.

Based on the evidentiary record now before the Board, and in view of the reasons set forth in Section III, above, the free acid substance formed by step (d) of claims 6, 10, 15, 21 and 22 is structurally different from the prior art treprostinil products in Phares and Moriarty. The evidentiary record shows that the free acid substance of claims 6, 10, 15, 21 and 22 contains a distinct impurity profile and a higher average purity over the treprostinil free acid of Moriarty, and thus is structurally different. Further, Phares' diethanolamine salt of treprostinil is structurally and functionally distinct from the free acid substance formed by step (d) of claims 6, 15 and 21.

1. The '393 Patent Product is Structurally and Functionally Distinct from Moriarty's Product

As explained in the Williams Declaration and discussed above, the free acid substances of claims 6, 10, 15, 21 and 22 are structurally distinct from Moriarty's product because the formation of the salt in step (c) leads to a product that has a distinct and improved impurity profile. *See* Sections III, VI, *supra*. Additionally, the average purity of the product of claim 21 is about [redacted] greater than that of Moriarty. Ex. 2020 ¶¶94-99 and Appendices A-B. Indeed, as evidenced by Dr. Ruffolo's Declaration, a [redacted] difference in average purity for a highly potent drug, such as treprostinil is a very significant difference. *See, e.g.*, Ex. 2022 at ¶70.

B. There Is No Motivation For A POSA To Combine Moriarty and Phares with Eğe and Kawakami

In the Institution Decision, the Board determined "on the record before us, and for purposes of institution, that the process steps recited in claims 6, 15, and 21 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps." Paper 12 at 47. However, the fuller record now indicates that the claimed treprostinil product is structurally and/or functionally different from Moriarty's treprostinil free acid and Phares' treprostinil diethanolamine salt. Thus, the recited process steps must now be considered.

Similarly, the board credited Dr. Winkler's opinion regarding the combination of Kawakami and Eğe with Moriarty and Phares. Paper at 42. Dr. Winkler, however, too easily dismisses the complexity and difficulty associated with further purifying a drug substance as complex as treprostinil. Dr. Winkler attempts to portray the chemistry involved in the '393 patent as "nothing more than basic organic chemistry techniques – in my view 'organic chemistry 101'" in an effort to minimize the significant invention of the '393 patent. Ex.1009 at ¶3. Yet, Dr. Winkler contradicts himself by defining a POSA as having "a master's degree or Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively a person of ordinary skill would include a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." *Id.* at ¶14. Indeed, Dr. Winkler goes on to testify that to understand the science and chemistry of the patent, you would need that level of skill in the art. Ex. 2051 at 29:12-16. As a result, a POSA would not look to an undergraduate textbook like Eğe, for example, to figure out improved purification techniques for a complex drug substance such as treprostinil.

1. There Is No Motivation to Follow the Carboxylate Salt Formation With Regeneration of the Carboxylic Acid

The Board credited Dr. Winkler's opinion regarding the combination of Kawakami and Eğe with Moriarty and Phares. Paper 12 at 42. Dr. Winkler,

however, too easily dismisses the complexity and difficulty associated with further purifying a drug substance as complex as treprostinil. After first referencing "organic chemistry 101" to minimize the significance of the '393 patent (Ex. 1009 at ¶3), Dr. Winkler contradicts himself by defining a POSA as having "a master's degree or Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively a person of ordinary skill would include a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." *Id.* at ¶14. At his deposition, Dr. Winkler conceded that, to understand the science and chemistry of the '393 patent, you would need this higher level of skill in the art. Ex. 2051 at 29:12-16. As a result, a POSA would not look to an undergraduate textbook like Eğe, for example, to figure out improved purification techniques for a complex drug substance such as treprostinil.

As explained previously, the claimed free-acid compounds, including treprostinil, produced by the processes of claims 6, 10, 15, and 21 provide a new product that induced FDA to adopt a new purity standard for treprostinil free acid due to the excellent purity of the final product. Furthermore, UTC demonstrated that treprostinil free acid made by the claimed methods provides a compound that lacks or reduces the levels of the impurities found in the free acid treprostinil of the Moriarty process.

Neither Phares nor Eğe provide a reason that a POSA would include a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step. *See* Petition, p. 54. Phares merely discloses forming a salt from treprostinil free acid of undisclosed origin. *See* Section V.E., *supra*. There is no suggestion that this salt should then be converted *back* to the free acid (*e.g.*, there is no suggestion of using the salt formation as a purification method). "Given that the purification techniques disclosed in Moriarty include chromatography and recrystallization after many years of research to optimize the process of making treprostinil, a POSA would not have been motivated to use a salt purification technique disclosed in an undergraduate chemistry textbook. More importantly, a POSA would not have had a reasonable expectation of success in further purifying the treprostinil product of Moriarty by using such a technique. To the extent a POSA was motivated to further purify treprostinil, a POSA would have focused on the known impurities and investigated methods of removing those." Ex. 2020 at ¶106. Indeed, stereoisomers were known impurities in treprostinil. *Id.* Eğe, however, simply discloses that "carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds." *Id.* at ¶107.

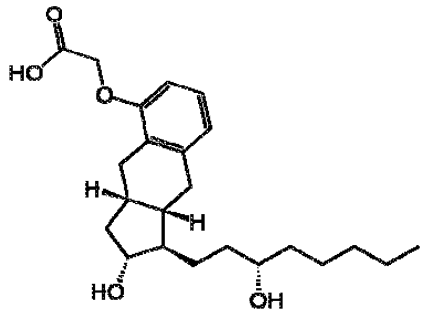
Indeed, the only example given in Eğe is of benzoic acid – a very simple aromatic acid that is quite different from the structure of treprostinil, as it has no chiral centers and therefore no stereoisomeric impurities. *Id.* at ¶108. Given that Eğe only predicts the removal of neutral and basic compounds by a salt purification step followed by acidification and only describes a simple non-chiral carboxylic acid, a POSA would have no motivation to look to Eğe for purification and no reasonable expectation of success given that many of the impurities in treprostinil are acidic stereoisomers. *Id.* at ¶¶108-109.

As discussed above, the average impurities found in samples of the Moriarty product include three different stereoisomers of treprostinil free acid. Eğe suggests that a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step would not remove these compounds from the product. Thus, a POSA would have understood Moriarty, Phares, and Eğe to suggest simply making the treprostinil free acid product of Moriarty, and not undergoing the additional time and expense of a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step because Eğe actually teaches away from the usefulness of this step when impurities include acidic stereoisomers are present because a POSA would have to ignore Eğe's teaching that these types of impurities could not be removed by carboxylate salt formation. *See* Ex. 2020 ¶¶107-109; *see also United States v. Adams*, 383 U.S. 39, 42-43 (1966).

The Institution Decision cites *KSR* for the proposition that "a technique has been used to improve one device, and a POSA would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill." Paper 12 at 45. However, the simple application of this proposition regarding devices (a predictable art) should not be applied to an unpredictable field, such as the chemical arts, without truly examining whether the technique would improve *similar compounds* in the *same way*. *See, e.g., In re Fisher*, 427 F.2d 833, 839 (C.C.P.A., 1970)(contrasting "predictable factors, such as mechanical or electrical elements" from "unpredictable factors, such as most chemical reactions"); *see also, Ortho-McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364 (Fed. Cir. 2008).

For example, Kawakami teaches purification of a methanoprostacyclin derivative by forming the dicyclohexyl amine salt and then regenerating the free acid to achieve a "fairly high" purity. Analogizing to the language cited from *KSR*, a POSA must have recognized that the "technique" of salt formation followed by regeneration of the free acid would improve *similar compounds* in the *same way*.

However, as can be seen by the below comparison, the structures of treprostinil and the methanoprostacyclin derivative of Kawakami are structurally very different – they are not *similar compounds/devices*.



Treprostinil

methanoprostacyclin compound in Kawakami

First, the methanoprostocyclin compound in Kawakami is a-two fused-ring structure, while treprostinil is a three-fused-ring structure. Ex. 2020 at ¶112. Second, Kawakami does not actually disclose a purification method for separating diastereomers, but instead one for separating E and Z isomers. Ex. 2020 ¶¶112-113.

Indeed, Kawakami teaches that the starting material does not vary at each chiral center other than the alkene double bond. *Id.* In other words, Kawakami discloses a mixture of two compounds: (1) the E-isomer of a stereoisomerically pure compound and (2) the Z-isomer of a stereoisomerically pure compound. *Id.* at ¶113. Treprostinil contains no mixture of E and Z isomers because it does not contain a carbon-carbon double bond that is capable of forming E and Z isomers. Indeed, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of a much more complex compound with

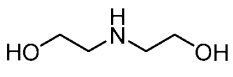
multiple chiral centers such as treprostinil could be isolated from entirely different impurities and then converted back to the free acid form. *Id.*

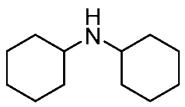
Thus, the purification of treprostinil from its stereoisomers and related impurities is quite different from the purification of the methanoprostacyclin derivative from its structural isomer – the compositions are not improved in the *same way*.

As a result of these differences, "a POSA would not have looked to Kawakami (or Eğe) if they were looking for additional purification techniques for treprostinil because neither reference discloses how to remove stereoisomeric impurities." *Id* at ¶112.

2. Kawakami Would Have Motivated One of Ordinary Skill In The Art To Select A Dicyclohexyl Amine Salt, Teaching Away From The Diethanolamine Salt of Claims 14 and 18

Not only are there structural differences between treprostinil and the "methanoprostacyclin compound" in Kawakami, but the counter-ion used to prepare the salt is structurally different. *Id.* at ¶114. Specifically, Kawakami teaches preparing the dicyclohexyl amine salt, whereas particular claims of the '393 patent require use of the diethanolamine salt.





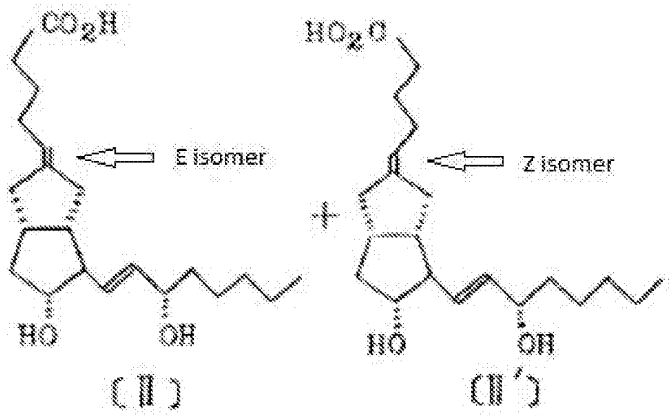
Diethanolamine **dicyclohexyl amine**

Because Kawakami uses a different salt to remove a different sort of impurity from a different structure, a POSA would have no reason to combine the teachings of Kawakami with Moriarty and Phares in the particular manner of the asserted grounds in the Petition, or a reasonable expectation of success of achieving a more pure treprostinil product by such a combination. Ex. 2020 ¶114. For this reason, claims 14 and 18 are separately patentable.

3. Kawakami Does Not Provide A Reasonable Expectation Of Success That Treprostinil Products Could Be Further Purified Because Different Impurities Are Targeted

The purification of treprostinil from its stereoisomers and related impurities is quite different from the purification of the methanoprostacyclin derivative from its structural isomer, and thus, Kawakami provides no reasonable expectation of success. Ex. 2020 ¶¶112-114

To illustrate this point further, Kawakami is directed to purifying E- and Z-isomers of methanoprostacyclin compound from one another. In order for the E- and Z-isomers to exist, the "prostacyclin compound" must have an alkene. For example, Kawakami discusses separating a mixture of the following compounds:



Treprostinil, on the other hand, contains no mixture of E/Z isomers. In fact, it cannot because it does not contain an alkene capable of E/Z isomerization. SteadyMed has failed to provide a factual basis as to how or why the separation of E/Z isomers of an alkene would provide a motivation to combine or reasonable expectation of success in a compound not containing an alkene capable of E/Z isomerization, such as treprostinil. As explained in the Williams Declaration, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of an entirely different compound, such as treprostinil, could be isolated from entirely different impurities, such as stereoisomers and related impurities. Ex. 2020 ¶¶112-114.

Furthermore, the Kawakami reference would have provided no motivation or rationale to attempt to remove the trace impurities of the Moriarty treprostinil free acid through the process of salt formation followed by conversion back to the

free acid. Indeed, Kawakami was concerned with isolating a particular isomer from a 7:2 E/Z isomeric mixture. Ex. 1007 at 4. In other words, the composition in Kawakami contained, at most, a purity of 77.8% prior to the salt formation step. Kawakami provides a crude purification of the desired E-isomer through a particular salt formation, and suggests that not all impurities were removed by formation of a salt and conversion back to the free acid. *Id.* at 5 ("purity can be further improved by recrystallization"). Nothing in the reference suggests that a substance as pure as the Moriarty treprostinil free acid (a substance with about 99.4% assay purity) – a substance that had already been "further improved" by recrystallization (*see* Ex. 1004 at 13, right column) – would be improved by formation of a salt and conversion back to the free acid. Ex. 2020 ¶¶113-114.

Thus, even if formation of a salt and conversion back to the free acid was known in the art, it would not have rendered the present claims obvious without some motivation and expectation of success in its use on the Moriarty treprostinil free acid. To put it another way, there would have been no reason to incur additional time and expense to form a salt of the valuable, relatively pure Moriarty treprostinil free acid only to then convert it back to the free acid, even though the addition would have been technologically possible. *In re Omeprazole Patent Litigation,* 536 F.3d 1361 (Fed. Cir. 2008).

4. Any "Close" Structural Similarity of the Moriarty Free Acid Does Not Render the Claims Obvious

As explained above, the claimed substance is structurally different from Moriarty's treprostinil free acid because the claimed substance has an improved and different impurity profile. Even if the Board views an improvement in impurity profile of, e.g., [redacted], as a close relationship between the substances of the present claims and of Moriarty, there is no obviousness because there was not a known or obvious process for making the claimed free acid substance. *See In re Hoeksema*, 399 F.2d 269, 274 (C.C.P.A. 1968)("the absence of a known or obvious process for making the claimed compounds overcomes any presumption that the compounds are obvious based on close relationships between their structures and those of prior art compounds"). For the reasons set forth in the previous sections, conducting a salt-formation purification step on the known treprostinil free acid of Moriarty would not have been obvious, so the mere existence of a "close relationship" in the products cannot be used to deny patentability.

5. Additional Claim Limitations Are Not Disclosed by the Cited Prior Art

In addition to the reasons above, certain dependent claims would also not have been obvious in light of the combination of Phares, Moriarty, Eğe, and Kawakami. Claim 6 requires the acid in step (d) to be either HCl or H_2SO_4 and

claim 15 requires the acid to be HCl. Similarly, claim 21 requires step (d) is performed. Phares, Moriarty, and Kawakami all do not disclose the use of either HCl or H_2SO_4 and do not disclose converting a carboxylic acid salt back to its salt form using an acid. Ex. 2020 at ¶115. "Eğe cites HCl as an example in the conversion of benzoic acid, but as described above, a POSA would not have looked to Eğe to further purify a complex carboxylic acid such as treprostinil from its stereoisomers and other impurities and would have no reasonable expectation of success by using HCl based on this disclosure." *Id.* In addition to the reasons above, claims 6, 15, and 21 would not be obvious in light of any combination of the cited prior art.

Like claim 2, claim 10 requires that the product be 99.5% pure and that step (d) be performed. The only purity limitation disclosed in any cited prior art reference is in Moriarty and, as explained above, that purity cannot be directly compared to the purity recited by the claims. Similarly, Moriarty does not perform steps (c) or (d). *Id.* at ¶116. A POSA would have no motivation to look to Phares, Kawakami or Eğe to improve the purity to at least 99.5% and, given that none of these references disclose a purity amount, would have no reasonable expectation of success in achieving that purity. *Id.* Finally, claim 22 requires an extra step of forming a pharmaceutically acceptable salt from the product of step (d). SteadyMed and Dr. Winkler cite no evidence whatsoever for this additional step.

"In fact, none of the references cited even suggest converting a carboxylic acid to a salt form, then regenerating the carboxylic acid, then forming a pharmaceutically acceptable salt from that." *Id.* at ¶117. For this additional reason, claim 22 is not obvious in light of the combination of Phares, Moriarty, Kawakami, or Eğe.

Consequently, SteadyMed has not carried its burden on Ground 3.

VIII. SECONDARY CONSIDERATIONS REBUT ANY POSSIBLE CASE OF OBVIOUSNESS

SteadyMed has not established a *prima facie* case of obviousness. Thus, UTC is not obligated to provide evidence of objective indicia of non-obviousness. Nonetheless, objective indicia of non-obviousness confirm that the claims of the '393 patent would not have been obvious and, in fact, represent a surprising solution to the problem of minimizing impurities and providing a safer and purer treprostinil product.

A. Long-Felt Unmet Need

At the time of the invention, there was a long-felt need to have a more efficient synthesis to produce treprostinil in a more pure form and in a cost-effective manner. *See generally*, Ex. 2022 at ¶¶31, 65. Treprostinil has five chiral centers resulting in 32 possible diastereomers, so the potential for diastereomeric impurities is high; only the treprostinil stereoisomer has the desired pharmaceutical effect. Ex. 2013, at pp. 11, ll. 18-25, pp. 15, ll. 1-pp. 16, ll. 8, pp. 19, ll. 14-25.

Treprostinil is also a very potent drug so any diastereomeric impurities would also potentially be potent. *Id.*; Ex. 2022 at ¶54. Specifically, the FDA as a matter of course seeks to minimize all impurities in drug substances and particularly in highly potent drug substances such as treprostinil. Ex. 2022 at ¶¶ 31, 54. The reduction and removal of several types of impurities met the long-felt need expressed by the FDA to minimize impurities as much as possible. *Id.* at ¶¶ 31, 75. Additionally, because the '393 patent product was so successful, it resulted in a change in the drug specification submitted to FDA. *Id.* at ¶¶66-67. The change indicated that the assay purity of the new drug substance made by the '393 patent process increased in purity from an assay range of [redacted] to [redacted] - a full [redacted] increase in assay purity. *Id.* at ¶ 70. The range of assay values of [redacted] as well as the amount above 100% does not indicate an error associated with the measurement, but just the acceptable value of this measurement approved by the FDA. *Id.* at ¶¶ 69-70. The fact that UTC submitted a [redacted] increase in assay purity to FDA is considered a "major" change by FDA. *Id.* at ¶ 72. *See Knoll Pharm. Co., Inc. v. Teva. Pharm. USA, Inc.*, 367 F.3d 1381, 1385 (Fed.Cir. 2004) (while FDA approval is not determinative of nonobviousness, it can be relevant in evaluating the objective indicia of nonobviousness). In fact, even a change as small as 0.1% of impurities can have an impact on a drug substance. *See, e.g., id.* at ¶¶ 32, 45. Given that FDA consistently wants drug substances to have fewer

impurities and in less amounts, the '393 patent invention met that need by further reducing and removing certain specific impurities and by increasing the overall assay purity of the drug substance.

B. Unexpected Results

The results of the claimed inventions in the '393 were unexpected. The use of a salt form of treprostinil to further purify the treprostinil acid in a cheaper and better way than the previously used methods of purification was an unexpected result. Moreover, it was unexpected that the salt purification step reduced not only diastereomeric impurities, but also certain non-acidic impurities as well. *See, supra*, Section XI.B.1; Ex. 2020 ¶¶94-97, 102, 108-109. Indeed, Eğe itself predicted that a salt formation followed by regeneration using an acid would remove only basic and neutral impurities. *Id.* at ¶107. The unpredictability of this result is supported by the fact that the salt purification step did not reduce all non-acidic impurities; in fact, the '393 product has slightly increased levels of one such impurity, [redacted]. Ex. 2020 ¶96. Thus, a person of skill in the art would not have expected the results of the '393 patent to be so successful at reducing the levels of so many impurities.

IX. Conclusion

For the foregoing reasons, the Board should hold that SteadyMed has failed to carry its burden attacking the patentability of the instituted claims because none

of the prior art cited by SteadyMed anticipates or renders obvious any claim of the '393 patent.

Respectfully submitted,

Date: July 6, 2016

/Stephen B. Maebius/
Stephen B. Maebius
Reg. No. 35,264

CERTIFICATE OF COMPLIANCE

This Paper contains 11,230 words according to the word processing program in which it was created, excluding the portions exempted by 37 C.F.R. ¶42.24(a)(1). Accordingly, this Paper complies with the requirements of 37 C.F.R. § 42.24(b)(1).

Date: July 6, 2016

Signature: /Stephen B. Maebius/
Stephen B. Maebius

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing Patent Owner Response and accompanying exhibits was served on counsel of record for Petitioner on July 6, 2016 by filing through the Board's PRPS system and by delivering a copy via email to Stuart Pollack and Lisa Haile (the counsel of record for the Petitioner) at the following address:

Steadymed-IPR@dlapiper.com

Date: July 6, 2016 Signature: /Stephen B. Maebius/
Stephen B. Maebius

4814-0612-4340.3

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
Patent 8,497,393

DECLARATION OF ROBERT M. WILLIAMS, Ph.D., IN SUPPORT OF PATENT OWNER RESPONSE TO PETITION

TABLE OF CONTENTS

I have been retained by the law firm of Wilson Sonsini Goodrich & Rosati ("WSGR") as an expert consultant to United Therapeutics Corporation ("UTC") in connection with the above-identified matter to provide expert testimony concerning U.S. Patent No. 8,497,393 ("the '393 Patent", Ex. 1001) by Batra *et al.*, entitled "Process to prepare Treprostinil, the active ingredient in Remodulin," issued on July 30, 2013. At the request of Counsel for UTC, I hereby submit this expert declaration.

I. Qualifications and Background

A. Education and Experience

1. I am a tenured University Distinguished Professor of Chemistry at Colorado State University (CSU). I currently serve as the Director for the Colorado Center for Drug Discovery. I also served as co-Director (Experimental Therapeutics) for the Infectious Diseases Supercluster Initiative and also served as co-Director for the Cancer Supercluster Initiative at CSU. My *curriculum vitae* is attached hereto as Exhibit A (Ex. 2021).

2. I received a B.A. in Chemistry from Syracuse University in 1975, and did laboratory research in the field of synthetic organic chemistry under the guidance of the recent Nobel Laureate Professor Ei-ichi Negishi. In 1979, I received both a Master's degree and Ph.D. degree in Organic Chemistry from the Massachusetts Institute of Technology (MIT) under the direction of Professor William H. Rastetter. Upon graduating from MIT, I spent one year (1979-80) as a postdoctoral fellow at Harvard University in the laboratories of the Nobel Laureate, the late Professor Robert B. Woodward, whose laboratory was subsequently managed by Professor Yoshito Kishi.

3. Subsequent to my fellowship at Harvard, I served as an Assistant Professor at Colorado State University from 1980–84. I was tenured and promoted early, to the rank of

Associate Professor in 1985, and in 1988, I was promoted to the rank of Full Professor. In 2002, I was named a University Distinguished Professor, which is my current position. University Distinguished Professor is the highest academic rank at Colorado State University, and there are a maximum of twelve University Distinguished Professors at any given time out of a faculty of 1,200. This is a lifetime appointment until retirement, whereupon Emeritus status is granted. In addition to my positions at Colorado State University, I was a Visiting Professor of Chemistry at Harvard University from 1994–95, at which time I was sponsored by Professor Stuart L. Schreiber and taught a sophomore organic chemistry course for pre-medical students, Chem 17. I was also a Visiting Professor of Chemistry at the University of California at Berkeley in 1990 and worked in the laboratory of Professor Peter G. Schultz.

4. I have extensive experience in the field of synthetic organic chemistry and medicinal chemistry with an emphasis on biologically active compounds including anti-tumor agents, heterocycles, antibiotics, anti-fungal agents, anti-viral agents, immunomodulators, amino acids, peptides and alkaloids, among many other classes of biologically active organic substances. My organic chemistry research interests include the total synthesis of novel natural and synthetic products, heterocyclic chemistry, asymmetric synthesis, synthetic methodology, process chemistry, and reaction mechanisms. I have extensive experience in the synthesis, chemistry, conformational analysis, biochemical activity, and biological activity of a range of organic compounds.

5. My research laboratory at Colorado State University has worked extensively on the chemistry and biology of numerous drugs over my career, including Quinocarcin (Quinocarmycin citrate), Tetrazomine, Bioxalomycin, Ecteinascidin 743 (Yondelis® or trabectidin), Renieramycin, Cribrostatin-4, Jorumycin, the Mitomycins, FR900482, FK973,

FK317, FK228 (Romidepsin), Largazole, Stephacidins A and B, Avrainvillamide, Spirotryprostatins, TMC-95A/B, Rottlerin, and Antimycin, amongst many others.

6. I have been the Principal Investigator on numerous research grants from Federal agencies, such as the National Institutes of Health (NIH) and the National Science Foundation (NSF) as well as from various Foundations, and corporations to synthesize biologically active compounds on both small laboratory scale as well as larger industrial scales.

7. I held a funded research collaboration with the Infectious Diseases Research Institute (IDRI), in Seattle, Washington, to develop several novel adjuvants for the treatment and prevention of autoimmune diseases, infectious diseases and cancer (2010).

8. From 1991-1993, I held a research grant from Symphony Pharmaceuticals, located in Philadelphia, Pennsylvania, to prepare anti-HIV drugs based on inhibition of the HIV protease. I supervised a graduate student who prepared several very potent peptide isosteres that exhibited in vitro activity against HIV.

9. I have taught undergraduate and graduate courses in organic chemistry, organic synthesis, biosynthesis, biological chemistry, drug design, and the synthesis of natural products. I have also lectured at numerous professional conferences, universities, and in corporate R&D laboratories in those areas.

10. I am a Scientific Founder, Acting President, and Chair of the Scientific Advisory Board of Cetya Therapeutics, a company that is developing several drugs, including drugs for the treatment of various cancers, multiple myeloma, autoimmune diseases, and hemoglobinopathies. I also direct all of the process scale synthesis optimization and drug formulation studies being conducted on Cetya's HDAC inhibitors. This includes injectable formulations as well as oral formulations. Specifically, I directed and supervised post-doctoral researchers in my laboratory

(on behalf of Cetya Therapeutics) to formulate the poorly water-soluble drug Largazole, including a myriad of synthetic analogs of Largazole prepared in my laboratory, as a polysorbate-80/ethanol co-solvent excipient system. This formulation has been used in animal studies for obtaining critical dose-escalation and pharmacokinetic data. I have also specifically directed and supervised the formulation of Largazole and related analogs in various PEG-based (polyethylene glycol) excipient systems. This work is currently being conducted in collaboration with oncologist Dr. Douglas Thamm of the Colorado State University Animal Cancer Center, pharmacologist Dr. Dan Gustafson of the Colorado State University Animal Cancer Center, Dr. Kimberly Stegmaier of the Dana-Farber Cancer Institute/Harvard Medical School and Dr. James E. Bradner of the Dana-Farber Cancer Institute/Harvard Medical School. The animal studies commenced in 2010, and the drug formulation studies are being conducted in my laboratory at Colorado State University under my direction.

11. I was a Scientific Founder, Member of the Scientific Advisory Board, and Member of the Corporate Board of Directors for Xcyte Therapies, a company devoted to developing *ex vivo* T-cell therapies for treating cancer, autoimmune, and infectious diseases, including HIV. As a Scientific Founder and Member of the Board of Directors of Xcyte Therapies, I was deeply involved in writing the patents and developing formulation strategies for both topical and injectable drugs based on FK228 (Romidepsin).

12. As a Scientific Founder and Acting Vice-President of Discovery Chemistry of HemaQuest Pharmaceuticals (Seattle, Washington), I have directed the pre-clinical and clinical synthesis, scale-up and formulation studies of several of the companies' drugs. These include both water-soluble drugs and hydrophobic, poorly water-soluble drugs for therapeutic applications in both cancer and hemoglobinopathies. I directed both the medicinal chemistry

efforts as well as the pre-process optimization work for potential industrial-scale syntheses of our lead drug candidates.

13. In addition, I am a Scientific Founder and member of the Scientific Advisory Board of Sapientia Therapeutics, located in Philadelphia, Pennsylvania. I am the acting Director of the Medicinal Chemistry, Process Chemistry and Drug Formulation efforts of this company to develop novel small-molecule inhibitors of protein kinase C-delta for autoimmune diseases, cancer and scleroderma. My laboratory has synthesized the first lead compounds, which are protein kinase C-delta (PKC-Δ) inhibitors and are water-insoluble substances. Under my direction we have engaged in early scale-up and route optimization for our leading drug candidates.

14. As a chemist with expertise in structure-activity studies and synthesis of biologically active agents, I have been retained to consult for a number of pharmaceutical and biopharmaceutical companies for both drug discovery and process research applications over the past thirty years. I consulted for Ajinomoto Co., Japan from 2002-2014 in the general area of process chemistry in the manufacture of amino acids, their derivatives, pharmaceutical intermediates and peptide synthesis. I served as a consultant for Cubist Pharmaceutical Company (2000–03) in the general field of antibacterial agents. I consulted for NewBiotics, Inc. (2001–02) in the general fields of anti-infective agents and anti-cancer agents. I consulted for Hoffman-La Roche, Inc. (1989–92) in the field of cephalosporin-fluoroquinolone dual-action antibacterial agents, as well as on a project concerned with inhibitors of diaminopimelic acid (DAP) biosynthesis as potential antibacterial agents. I consulted for W.R. Grace (1985–90) in the area of specialty chemicals and pharmaceutical intermediates process manufacturing and process development. I was a Scientific Founder, Member of the Scientific Advisory Board,

Consultant and sub-contractor for Microcide Pharmaceutical Co. (Microcide) in their drug discovery and early process research efforts. Microcide was a biopharmaceutical company devoted to developing antibacterial agents against a range of drug-resistant bacterial and fungal infectious diseases. In addition, I have consulted for EPIX Medical, G. D. Searle, Nutrasweet, and Boehringer-Ingelheim, among others. The consulting work I performed for Nutrasweet (1990-1991), was concerned with large-scale manufacturing process chemistry for Aspartame.

15. I was a co-organizer of a special Symposium on process chemistry at The International Chemical Congress of Pacific Basin Societies, PacifiChem 2015 (December 15-18. Honolulu, Hawaii) entitled: *"New Horizon of Process Chemistry by Scalable Reactions and Technology."*

16. I have directed the research activities of more than sixty PhD students and eighty post-doctoral fellows; most of my former co-workers have gone on to successful careers in the pharmaceutical industry in both process research and medicinal chemistry.

17. I have delivered numerous named and plenary lectures at Universities, corporations, and scientific societies on the synthesis, chemistry, biology, and mechanism of action of numerous classes of therapeutic agents, as detailed in my *curriculum vitae* attached hereto as Exhibit A.

18. I have published more than 315 scientific research articles, authored numerous chapters in books, and have written a well-known textbook on the synthesis of optically active amino acids. I have particular expertise in the large-scale industrial synthesis of amino acids and their derivatives. I am also a named inventor on seventeen issued U.S. patents and published patent applications. My publications and patents are listed on my CV, provided in Exhibit 2021.

19. I currently serve on the Editorial board for *Chemistry & Biology*. I have served as Editor for the *Organic Chemistry Series* published by Pergamon Press and Elsevier (1997-2012), and *Mini Reviews in Organic Chemistry* (Bentham Science). I have also served as an editor for several other journals in the past, including *Tetrahedron: Asymmetry*, *Tetrahedron Publications*, *Amino Acids*, and the *Journal of the American Chemical Society*.

20. I am a member of the American Chemical Society, the Japan Antibiotics Research Association, the International Society of Heterocyclic Chemistry, and the American Association for the Advancement of Science. I am a Member of the University of Colorado Cancer Center, located in Aurora, Colorado. I have served as organizer or co-organizer of numerous scientific meetings and symposia, and served as the Vice President of the International Society of Heterocyclic Chemistry, Chairing the 2003 International Congress of Heterocyclic Chemistry.

21. I serve on the Scientific Advisory Board of Arch Therapeutics, located in Boston, Massachusetts, that is developing self-assembling peptides for wound healing and surgical closure.

22. I have also served on the Scientific Advisory Boards for a number of other companies. I currently serve on the External Advisory Committee for the Puerto Rico Alliance for the Advancement of Biomedical Research Excellence. I was a Scientific Founder, Director of Chemistry, and member of the Scientific Advisory Board for HemaQuest Pharmaceuticals. I was a Founding Scientist and Member of the Scientific Advisory Board of Microcide Pharmaceuticals from 1993 to 1998.

23. I have expertise in drug formulation for injectable, topical and oral medications. I have directed research programs, both through my academic laboratory at Colorado State University as well as through my various consulting engagements and as a research director

and/or consultant for companies developing medicines for numerous therapeutic indications. I have consulted on many aspects of pharmaceutical drug discovery, development, formulation, and manufacturing. This includes basic discovery and optimization, early process research, large-scale manufacturing, and drug formulation.

24. I have served as a consultant for a number of companies for both drug discovery and process research applications, including, for example, W.R. Grace Company (1985-1990, fine chemicals synthesis); Symphony Pharmaceuticals (1991-1993, anti-HIV drugs); G.D. Searle Co. (1988-1990, memory and learning enhancement agents based on NMDA receptor antagonists); Nutrasweet Co. (1990-1991, artificial sweeteners); EPIX Medical (1993-1997, MRI imaging and contrast agents); Hoffman-La Roche, Inc. (1989-1992, cephalosporin-fluoroquinolone dual-action antibacterial agents); Boehringer-Ingelheim Pharmaceuticals (1992-1993, antiviral agents); Cubist Pharmaceutical Company (2000-2003, macrocyclic peptide antibacterial agents); NewBiotics, Inc. (2001-2002, anti-infective agents and anti-cancer agents); Microcide Pharmaceutical Co. (1993-1998, analogs of macrocyclic anti-fungal agents related to echinocandin, cephalosporins, and quinolones); Xcyte Therapies (1996-2006, T-cell activation); Ajinomoto Co, Japan (2002-2014, amino acids, peptides, and other specialty chemicals); HemaQuest Pharmaceuticals (2006-2014, short chain fatty acids for treating hemoglobinopathies); Sapientia Therapeutics (2012-present, small-molecule inhibitors of protein kinase C-delta); Arch Therapeutics (2010-present, self-assembling peptides for wound healing); and most recently, Cetya Therapeutics (2012-present, histone deacetylase inhibitors as therapeutic agents for treating cancers, multiple myeloma, autoimmune diseases, and hemoglobinopathies).

25. Under my direction, my laboratory developed the technology for the asymmetric synthesis of amino acids in 1985 that was commercialized by Aldrich Chemical Co. in 1988. My laboratory devised several large-scale (multi-kilogram) process routes for the manufacture of the so-called "Williams Lactone" that has been sold by Sigma-Aldrich Chemical Company since 1988. Early manufacturing was conducted in China by several of my former co-workers at the Chengdu Institute of Organic Chemistry.

26. I have been awarded numerous prizes and awards including the NIH Research Career Development Award (1984-89), the Eli Lilly Young Investigator Award (1986), the Merck, Sharp & Dohme Academic Development Award (1991), an award from the Japanese Society for the Promotion of Science Fellowship (1999), the Arthur C. Cope Scholar Award sponsored by The American Chemical Society (2002), the Multiple Myeloma Research Foundation Senior Award (2010), the ACS Ernest Guenther Award in the Chemistry of Natural Products sponsored by Givoudan and The American Chemical Society (2011), an award from the Japanese Society for the Promotion of Science Long-term Fellowship (2012-2013), and the Organic Synthesis Award from the local Rocky Mountain section of the American Chemical Society (2012).

27. I have testified numerous times as an expert witness in process chemistry patent litigation in the following matters: Great Lakes Chemical *versus* Archimica SPA. Civil Action No. 99–728-JJF; Ranbaxy Laboratories *versus* Abbott Laboratories. Case No. 04 C 8078; Lundbeck *versus* Infosint. 06 Civ. 2869 (LAK); United Therapeutics Corp. *versus* Sandoz, Inc. C.A. Nos.: 12-1617 (PGS)(LHG) and 13-316 (PGS) (LHG); Gilead Sciences, Inc. and Emory University *versus* Cipla, Limited. Civil Action No.: 1:12-cv-06350-RJS; United Therapeutics

Corp. *versus* Teva Pharmaceuticals, USA, Inc. C.A. No.: 3:14-cv-05498 (PGS)(LHG); United Therapeutics Corp. *versus* Sandoz, Inc. C.A. No.: 3:14-cv-05499 (PGS)(LHG).

B. Materials Considered

28. In forming my opinions in this report, I have relied upon my professional experience and personal knowledge. I have also reviewed a number of documents in this case including all documents cited by the SteadyMed and UTC as well as the materials I have cited in this declaration. In this report, I have provided representative citations to exemplary documents that I have relied upon in reaching my opinions. If I am provided additional information or documents in this proceeding, I may offer further opinions regarding the additional information.

II. Legal Standards Provided By Counsel

29. I have been informed by Counsel that, during an *inter partes* review (IPR), a petitioner must prove invalidity by a preponderance of the evidence. Accordingly, I understand that the burden is on a petitioner to prove invalidity, rather than a patent owner to prove validity. I have been informed by Counsel that because each claim defines a separate invention, the validity of each claim in a patent is addressed independently of the validity of the other claims in that patent.

30. I have also been informed by Counsel that the claims of the '393 patent are "product-by-process" claims. I have also been informed by Counsel that when evaluating the validity of a patent claim, the "product" of product-by-process claims must include structural and/or functional differences over the prior art, even if they are not explicitly claimed.

A. The Person of Ordinary Skill in the Art

31. I have been informed by Counsel that a patent is to be interpreted from the perspective of a hypothetical person referred to as the person of ordinary skill in the art ("POSA")

to which the patent pertains. I am further informed that a determination of the level of ordinary skill is based on, among other things, the type of problems encountered in the art, prior art solutions to those problems, rapidity with which innovations are made, sophistication of the art, and the educational level of active workers in the field. I have been informed that in any particular case, every factor may not be present, and one or more factors may predominate. I understand the person of ordinary skill in the art is presumed to know all prior art that is reasonably relevant to the subject matter of the claimed invention.

32. I understand from Counsel that the validity of a patent claim must be assessed from the perspective of a POSA at the time of the invention.

33. Given the complexity of the chemistry involved in the '393 patent, it is my opinion that a POSA with respect to the patent-in-suit would have had, at the time of the claimed invention, a doctorate degree in chemistry, pharmaceutics, pharmaceutical sciences, medicine, or a related discipline. Alternatively, the POSA may have had a lesser degree in one of those fields, with correspondingly more experience. To the extent necessary, a POSA may have collaborated with others of skill in the art, such that the individual and/or team collectively would have had experience in synthesizing and analyzing complex organic compounds. It is my understanding that a patent is to be interpreted from the perspective of a person of ordinary skill in the art at the time of the patent's priority date.

34. I understand that SteadyMed's expert Dr. Winkler has opined that a POSA would have "a master's degree or a Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively, a person of ordinary skill would include an individual with a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." Ex. 1009 at ¶14.

35. All of my opinions regarding validity contained in this report are expressed from the view of a POSA at the time of the priority date of the '393 patent. These opinions apply equally whether my definition of a POSA or Dr. Winkler's is applied.

B. Anticipation

36. I understand from Counsel that anticipation requires that each and every element of a claim is set forth in a single prior art reference, and that these elements are arranged or combined in that reference in the same way as recited by the claim. I further understand from Counsel that if there is any difference between the prior art reference and the claimed invention, there is no anticipation by that reference. Further, I understand that there is no anticipation if the elements disclosed in a prior art reference must be combined with the knowledge of one skilled in the art to achieve the subject matter of the claim. I understand that for a prior art reference to be anticipatory, it must enable a POSA to make or practice the invention without undue experimentation.

37. I also understand from Counsel that if the single prior art reference is missing a claimed feature, the reference may inherently anticipate if that missing feature is necessarily present in the single prior art reference.

38. I also understand from Counsel that if there are structural or functional differences in the product of the product by process claims of the invention from the product of the prior art that arise from the process in which it was made, those differences may be evidence of no anticipation even if those differences are not explicitly claimed.

C. Obviousness

39. I understand from Counsel that obviousness requires that a POSA would have been able to arrive at the claimed invention by modifying a single prior art reference or by

combining two or more prior art references. I also understand from Counsel that obviousness analysis must be conducted from the point of view of a POSA at the time of the invention, and that it is improper to employ hindsight or consider the inventors' own path to the invention as proof of obviousness.

40. Counsel has also informed me that obviousness requires that a POSA would have had a reasonable expectation of success in achieving the claimed invention.

41. I understand from Counsel that four factual issues are relevant to obviousness analysis: the scope and content of the prior art; the level of ordinary skill in the field of the art at the time of the invention; the differences between the claimed invention and the prior art; and various objective indicia of non-obviousness.

42. I understand from Counsel that, in addition to considering the prior art, certain objective indicia may also provide evidence that a claimed invention is not obvious. I am informed by Counsel that these objective indicia, which are also referred to as secondary considerations, may include factors such as commercial success, unexpected results, the resolution of long-felt but previously unmet needs, skepticism by others prior to achieving the invention, failure of others to achieve the invention, praise from others for the invention, and copying by others.

43. I understand from Counsel that, like anticipation, if there are structural or functional differences in the product of the product by process claims of the invention from the product of the prior art that arise from the process in which it was made, those differences may be evidence of non-obviousness even if those differences are not explicitly claimed.

III. Summary of Opinions

44. It is my opinion that the term "product" as it is used in the claims of the '393 patent should be construed using UTC's construction: "a substance resulting from a chemical reaction."

45. It is my opinion that the term "[a] product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" as it is used in the claims of the '393 patent should be construed using UTC's construction: "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof."

46. It is also my opinion that none of the claims of the '393 patent are anticipated by or rendered obvious by the prior art.

47. My opinions and the bases for them are based on information that I know, that I have reviewed, and that I am currently aware exists. I reserve the right to supplement or amend my opinions in light of any additional evidence, testimony, or other information that may be provided to me after the date of this declaration. Additionally, I may use the cited materials to assist me in preparing demonstratives such as graphics and animations if I am asked to testify.

IV. The '393 Patent

48. The '393 patent is directed to an improved treprostinil product and improved process for making the product. I understand from Counsel that the priority date for the '393 patent is December 17, 2007.

49. The synthesis of treprostinil is complex as several improvements resulting in improved products are disclosed in the '393 patent itself. The structure of treprostinil has five chiral centers (stereogenic centers) resulting in 32 possible stereoisomers of treprostinil.

50. The '393 patent has two independent claims: Claims 1 and 9. Claim 1 requires "a product comprising a compound of formula I…or a pharmaceutically acceptable salt thereof," in which formula I can be several structures including treprostinil. Claim 9 requires "[a] product comprising a compound having formula IV…or a pharmaceutically acceptable salt thereof," in which is the structure of treprostinil. Both Claims 1 and 9 then specify that the product is prepared by a process comprising (a) alkylating a compound of Formula II or V [a benzindene triol structure] with an alkylating agent to produce a compound of Formula III or VI [a benzindene nitrile intermediate], (b) hydrolyzing the product of formula III or VI of step (a) with a base, (c) contacting the product of step (b) with a base B to form a salt of Formula Is or IVs [indicating a salt form of treprostinil with an HB+ counterion], and (d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I or IV. Dependent Claim 7 further identifies the specific structure of Formula I of the product of Claim 1 as treprostinil. Because the other possible structures of Claim 1 are not at issue here, I will consider these Claims 1, 7, and 9 together in my analysis. Likewise, I will consider the following dependent claims together that have similar claim limitations.

51. Dependent Claims 2 and 10 provide a further purity limitation. Claim 2 further requires "[t]he product of claim 1 wherein the purity of compound of formula I in said product is at least 99.5%." Similarly, Claim 10 requires "[t]he product of claim 9, wherein the purity of product of step (d) is at least 99.5%." Thus, step (d) must be performed in claim 10, but both of these claims require a purity of at least 99.5%.

52. Dependent Claims 3 and 11 provide a further limitation on what alkylating agent may be used. Claim 3 requires the alkylating agent be $Cl(CH_2)_wCN$, $Br(CH_2)_wCN$, or $I(CH_2)_wCN$. Claim 11 requires the alkylating agent be $Cl(CH_2)_wCN$.

53. Dependent Claims 4 and 12 specify what base may be used in step (b). Claim 4 requires the base in step (b) to be KOH or NaOH and Claim 12 requires the base to be KOH.

54. Dependent Claims 5, 13, 14, 17 and 18 specify what the base B in step (c) may be selected from certain specific bases. Claims 5, 13, and 17 limit base B to the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. Claims 14 and 18 specify that the base B is diethanolamine.

55. Dependent Claims 6 and 15 specify what acid is used in step (d). Claim 6 specifies the acid is HCl or H_2SO_4. Claim 15 specifies the acid is HCl.

56. Dependent Claims 8 and 16 specify that the process does not include purifying the compound of formula III or VI produced in step (a).

57. Dependent Claims 19 and 20 depend on Claims 1 and 9, respectively. Each dependent claim further specifies the base in step (b) is KOH or NaOH and the base in step (c) is selected from the same group specified in Claims 5, 13, and 17.

58. Claim 21 depends on Claim 1 and requires that step (d) is performed. Claim 22 depends on Claim 21 and further requires that the product comprises a pharmaceutically acceptable salt formed from the product of step (d).

V. Claim Construction

59. I understand from Counsel that different claim constructions for certain terms used in the claims of the '393 patent have been proposed by SteadyMed and UTC, and that the U.S. Patent and Trademark Office ("PTO") has entered a preliminary claim construction for certain terms.

60. I agree with UTC's construction of the term "product" as "a substance resulting from a chemical reaction" which is consistent with the plain and ordinary meaning of the term.

61. In the chemical context, "product" generally refers to the real world outcome or result of a reaction:

Generalized Chemical Reaction

Reactants → Products

I agree with UTC that the '393 patent itself distinguishes "product" to identify it as what comes at the end of a chemical process or chemical reaction. Prelim. Resp. at pp.17-18.

62. I also agree with the consistent definitions given by the several textbooks cited by UTC all referring to "product" as the result of a chemical reaction. *Id.* at 19.

63. In fact, I have used the term "product" consistently in my own publications to refer to the real world result of a chemical reaction. *See, e.g.*, Williams, et.al., *Asymmetric, Stereocontrolled Total Synthesis of Paraherquamide A*, J. Am. Chem. Soc. 2003, 125, 12172-178. ("However, the reaction was very slow and gave the desired cyclization product 64 in only 25% yield, accompanied by products from competing pathways.") (Ex. 2026); Williams, et.al., *Stereocontrolled Total Synthesis of (+)-Paraherquamide B*, J. Am. Chem. Soc. 1996, 118, 557-579 ("Compound 66 was refluxed in benzene with 20 equiv of sodium hydride, resulting in a very clean and high yielding cyclization reaction furnishing the desired product 68 in 93% yield.") (Ex. 2027); Williams, et.al., *Synthetic Studies on Et-743. Assembly of the Pentacyclic Core and a Formal Total Synthesis*, J. Org. Chem. 73.24 (2008): 9594-9600. ("The scarcity of the natural product from marine sources renders Et-743 an important target for synthesis.") (Ex. 2028).

64. Dr. Winkler also uses the term "product" as the result of a chemical reaction in his own publications and confirmed that understanding during his deposition. *See, e.g.*, Winkler, J., et.al., *A Pauson-Khand Approach to the Synthesis of Ingenol*, Org. Lett., 2005, 8, 1489-1491 at Abstact ("Pauson-Khand cyclization of dioxanone photoadduct 21 leads to the formation of a single product in good yield.") (Ex. 2029); *see also* Ex. 2051 at 155:12-157:3.

65. Specifically, Dr. Winkler confirmed that "the product of a chemical reaction would be essentially all of the substances that result from the treatment of a particular reactant with a particular set of reagents." Ex. 2051 at 155:2-11. This is consistent with UTC's definition as well as how Dr. Walsh interpreted the product in his Declaration submitted during prosecution of the '393 Patent. Ex. 1002 at 346-347 (showing the products containing certain other substances as impurities).

66. I disagree with the PTO's preliminary construction and SteadyMed's construction of "product" as "a chemical composition." I believe that this proposed definition is too broad and does not accurately describe the term as it is customarily used in the art and in the context of how it is defined in the '393 patent. In the chemical context, there can be no "product" if there is no corresponding reaction, process, or synthesis that it refers to. A "chemical composition" could be used to describe the starting materials, solvents, reagents, catalysts, and even the glassware used during a chemical reaction as there is no limitation on SteadyMed's construction of the term "product" on how it relates to the chemical reaction at issue.

67. In the '393 patent and each of the references I describe above, the word "product" is exclusively used to describe a substance resulting from a chemical reaction, and it is not used to describe any and all "chemical compositions."

68. SteadyMed's construction is therefore inconsistent with the understanding of a POSA and inconsistent with the '393 patent specification regarding the term "product" because "a chemical composition" is not an accurate and specific definition of the term.

69. For the reasons I previously described regarding the term "product", a POSA would understand the plain and ordinary meaning of the claim term "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof," as UTC's construction: "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof." This definition is consistent with how a POSA would understand the term and is consistent with its plain and ordinary meaning.

70. I disagree with the PTO's preliminary construction and SteadyMed's construction of "[a] product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" as "a chemical composition that includes, but is not limited to, a compound of Formula I, or a pharmaceutically acceptable salt thereof, and that may also include other non-mentioned substances (including impurities), additives, or carriers, without limitation as to the types of or relative amounts thereof." I believe that this proposed definition is too broad and does not accurately describe the term. The entirety of the '393 patent is directed to an improved product with lower amounts of impurities and therefore the product includes its own impurity profile which provides a high level of purity and does not indiscriminately include other substances and impurities "without limitation as to the types of or relative amounts thereof."

VI. Phares Does Not Anticipate Claims 1-5, 7-9, 11-14, or 16-20 of the '393 Patent

71. I have reviewed Dr. Winkler's opinions alleging that Phares (Ex. 1005) inherently anticipates Claims, 1-5, 7-9, 11-14, and 16-20. I have also reviewed the Institution Decision in which the Board credited Dr. Winkler's opinion regarding this lack of physical differences

between the treprostinil products of the '393 patent and Phares. Paper 12 at 23-31. I disagree. Additionally, the Board credited Dr. Winkler's opinion that Phares discloses the same process for synthesizing treprostinil as the '393 patent. Paper 12 at 29-30. This is not true. Because no synthesis of treprostinil is disclosed in Phares, the diethanolamine salt described would have an unknown impurity profile and therefore cannot anticipate any claim of the '393 patent.

A. The Product Disclosed in Phares is Physically Different Than the Products Disclosed in the '393 Patent Claims

72. In order for Phares to anticipate any claim of the '393 patent, Phares must disclose every claim limitation of the product. Phares does not disclose the same product as claimed in the '393 patent.

73. Contrary to Dr. Winkler's opinion, the polymorph form and purity of the treprostinil diethanolamine salt is not the same as that claimed in the '393 patent. Specifically, Phares discloses samples made for a polymorph screen, not large scale batches. *See, e.g.*, Ex. 1005 at 85-86. In fact Phares notes several different conditions to form polymorph A including preparation using fast evaporation, slow evaporation, freeze drying, heating, and slow cooling in a variety of solvent systems including water and ethanol; water, toluene, and tetrahydrofuran. *Id.* Once polymorph A is prepared, Phares then further states that polymorph form B must be made from polymorph A, listing several conditions under which polymorph B is prepared. *Id.* Phares further notes that the polymorph B sample that was used for characterization was made from heated slurries of form A in 1,4-dioxane and toluene. *Id.* at 87. In fact, it is not clear which sample of polymorph form A was further used to create the characterized sample of polymorph B that Dr. Winkler discusses. Ex. 1009 at ¶¶58-61.

74. The '393 patent does not discuss that polymorph A must be formed first. *See, e.g.*, Ex. 1001 at col. 12-13 and 15. The '393 patent also does not describe the use of 1,4 dioxane or toluene and only describes forming the diethanolamine salt followed by cooling and filtering the salt with ethyl acetate and ethanol, and then drying. *Id.* Thus, the treprostinil diethanolamine salt formed in Phares required an extra step to first form polymorph A, under different reaction conditions with different solvents.

75. It is well-known that the use of different solvent systems in forming different crystal forms can have a significant effect on the melting point of a substance as well as other characteristics including purity. *See, e.g.*, R. Adhiyaman, et.al., *Crystal modification of dipyridamole using different solvents and crystallization conditions*, Int'l J. Pharm.321, 2006, 27-34 at 33 ("Adhiyaman") ("In conclusion, it can be said that the crystallization conditions and medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves.") (Ex. 2030). Given that the samples of polymorph B described in Phares are prepared in a completely different way under different conditions than those described in the '393 patent, their melting points and other analytical data cannot be directly compared.

76. Furthermore, the only data that Dr. Winkler relies upon to conclude that the polymorph B sample of treprostinil diethanolamine salt in Phares has a "higher purity than the '393 product" is that the recorded melting point was higher in one sample than the melting point of the diethanolamine salt sample of the '393 patent. Ex. 1009 at ¶¶ 59-60. This is incorrect for several reasons. First, as mentioned above, the different solvents and conditions used to form the salt can greatly affect the melting point – which is the only purported evidence

that Dr. Winkler cites for purity. Second, there is absolutely no actual purity data disclosed in Phares for the diethanolamine salt or treprostinil free acid and a POSA would not have concluded based on a single melting point example of polymorph B prepared under unknown conditions (e.g., recrystallization solvent and recrystallization conditions are not identified) would be of a higher purity than the known purity of the '393 patent. Third, even if the diethanolamine salt samples were prepared under the same work-up and purification conditions, a higher melting point does not mean that the substance must be of a higher purity. *See*, Ex. 2030 at Fig. 5 showing modified crystals in several different solvents had a higher melting point than the pure dipyridamole). Fourth, the DSC curve cited by Dr. Winkler in Fig. 21 of Phares (Ex. 1009 at ¶59) shows a broad melting peak with a range of close to 10 degrees which is indicative of a lower purity substance. *See*, Marti, E., *Purity determination by differential scanning calorimetry*, Thermochimica Acta, 5(1972) 173-220 at 214 ("The melting of diphenyl is extremely sharp because of the purity level; on the other hand, the melting region of phenacetin-benzamide is rather broad.") (Ex. 2031). Additionally, the DSC data provided does not describe the sample size, the rate of temperature increase as a function of time and does not compare this with an authentic standard of known purity melted under identical conditions. It is known in the art that sample size, rate of heating, the recrystallization solvent(s) used, and the conditions under which the crystalline sample was obtained can significantly affect the DSC data. Dr. Winkler's conclusion based on this single vague and incompletely described DSC data is not scientifically sound.

77. Dr. Winkler also points to the brief description of the formation of the treprostinil diethanolamine salt (Ex. 1009 at ¶¶50-54), but that description does not indicate what treprostinil free acid was used to make it. While the Board agreed with Dr. Winkler regarding the similarity

of the products of Phares and the '393 patent, the source of the treprostinil used to make treprostinil diethanolamine is very important and would greatly affect the impurity profile and other analytical characteristics, including DSC, of the sample.

78. In fact, Phares itself describes several references that could be used to make treprostinil, but does not identify which one, if any, was used to make the sample for the treprostinil diethanolamine salt. *See, e.g.*, Ex. 1005 at 9 ("Compounds of the present invention can also be provided by modifying the compounds found in U.S. Patent Nos. 4,306,075 ("the '075 patent", Ex. 2032) and 5,153,222 ("the '222 patent", Ex. 2033) in like manner."). The '075 patent, for example, discloses a very different and less pure treprostinil product than that of Moriarty (Ex. 1004). *See, e.g.*, Ex. 1004 at 1892-93. Thus, without knowing the source of the treprostinil used in Phares to make the treprostinil diethanolamine salt, the resulting product could have a very different purity and impurity profile and would necessarily have a distinct impurity profile if it were made by a different process than that disclosed in the '393 patent.

B. Phares Does Not Disclose Several Other Claim Limitations

79. Dr. Winkler alleges that Phares discloses the same synthesis to make treprostinil diethanolamine as the synthesis described in the '393 patent and the Board credited his opinion on this point. *See*, Ex. 1009 at ¶¶51-57; Paper 12 at 29-30. I disagree. First, there is no description whatsoever in Phares of how to make treprostinil free acid. Instead, Dr. Winkler points to the synthesis of the enantiomer of treprostinil ((-) treprostinil) which is a completely different synthesis for a different stereoisomer. Ex. 1009 at ¶57. Winkler alleges that because certain steps are used in forming the enantiomer, those steps are inherently disclosed for use with treprostinil. Ex. 1009 at ¶¶56-57.

80. I understand the Board decision did not address the additional limitations of independent Claims 1 and 9 nor the dependent claim limitations in its anticipation analysis because "the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product." Paper 12 at 31. I disagree with this assertion. Even if Phares used the synthesis of Moriarty to make treprostinil, there are significant differences between the product of Moriarty and the product of the '393 patent. *See*, Section VII(A) below. Because the products are different, the process differences are relevant to the anticipation analysis.

81. The synthesis for the enantiomer of treprostinil disclosed in Phares, however, is different than the synthesis of treprostinil disclosed in the '393 patent. First, contrary to Dr. Winkler's claims, the earlier part of the synthesis used in Phares to make the enantiomer is not the same synthesis disclosed in Moriarty. Specifically, the Moriarty reference obviously does not describe the synthesis of the enantiomer of treprostinil, but also does not include the Mitsunobu inversion step described by Phares wherein the stereochemistry of the secondary alcohol moiety has to be chemically reversed. Ex. 1005 at 40. In fact, because (S)-2-methyl-CBS-oxazaborolidine is used on structure 5, the resulting structures 6-11 are diastereoisomers of the intermediates used in the synthesis of the '393 patent. As a result, intermediate products of formulas (II) and (III) of Claim 1 and intermediate products of formulas (V) and (VI) of Claim 9 of the '393 patent are not disclosed in Phares. Thus, because steps (a) – (c) of *every claim* of the patent requires these products, Phares cannot anticipate any claim of the '393 patent.

82. Second, Claim 2 requires a specific purity of 99.5%. As I discussed above, there are no specific purity measurements disclosed in Phares and a single broad melting point determination with a large melting point range does not provide evidence that the purity of the

treprostinil diethanolamine sample is at least 99.5%. *See*, Section VI(A) above. For this additional reason, Phares does not anticipate Claim 2. The purity of that sample was not calculated from the DSC data as no control to an authentic standard of known purity was performed or reported.

83. SteadyMed claims that because the synthesis of the enantiomer of treprostinil in Phares does not describe a purification step, that the claim limitation of Claims 8 and 16 that the process does not include purifying the compound of Formula III (or VI) produced in step (a) is satisfied. That is not correct. In fact, Phares does not disclose any specific details of those steps whatsoever. Indeed, if the same synthesis from Moriarty was used as Dr. Winkler suggests, purification at step (a) is specifically described in that reference. Ex. 1004 at 1901-1902. Regardless of what synthesis was used, however, the fact remains that compounds of Formula III and VI do not appear in Phares as described above.

84. Under my interpretation of the highly pure product described in each of the claims of the '393 patent, Phares does not anticipate Claims 1-5, 7-9, 11-14, or 16-20 because it does not disclose the highly-pure product of the '393 patent, the synthesis of treprostinil, nor compounds of structures (II) and (III) from independent Claim 1 or structures (V) and (VI) from independent Claim 9, which are required by all of the claims.

VII. None of the Claims of the '393 patent Are Rendered Obvious by the Prior Art

85. I understand that the Board cited additional grounds for unpatentability including obviousness based on the combination of Moriarty and Phares and obviousness based on the combination of Moriarty, Phares, Kawakami (Ex. 1007), and Eğe (Ex. 1008). I disagree that any claim of the '393 patent is rendered obvious by any combination of these references.

A. The Product of the '393 Patent Is Structurally Different Than the Product of the Prior Art

86. In his declaration, Dr. Winkler expresses his opinion that "the '393 patent processes do not result in a physically different or unique product than that disclosed in the prior art." Ex. 1009 at ¶71. I am aware that, in the Institution Decision, the Board credited Dr. Winkler's opinion regarding this lack of physical differences between the treprostinil products of the '393 patent and the prior art. Paper 12 at 16-17. I disagree with Dr. Winkler's opinion for at least the following reasons.

87. Dr. Winkler appears to base his opinion on a comparison between the '393 patent process batches identified in the declaration submitted by Dr. David Walsh, one of the inventors of the '393 patent, during prosecution (Walsh Declaration), and a single prior art process batch identified in a particular prior art publication by Moriarty . Ex. 1009 at ¶¶63-71. However, Dr. Winkler's comparison suffers from several critical flaws.

88. First, and most fundamentally, there is no basis for comparing the "purity" reported in Moriarty with the purity discussed in the Walsh Declaration. When purity is determined by comparison of a sample to a reference standard such as assay purity (*see, e.g.*, ICH Guidance For Industry: Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients (2001) ("Q7A") at 28-29 (Ex. 2034); see also Reviewer Guidance: Validation of Chromatographic Methods (1994) ("Reviewer Guidance") at 5-8) (Ex. 2035), one cannot directly compare the purity values of two samples in any meaningful way unless each value was achieved by comparison to the same reference standard. Neither the Walsh Declaration nor Moriarty identifies a specific reference standard. While Moriarty notes that the

treprostinil product obtained was compared to an authentic sample of UT-15, there is no mention of any such comparison in the Walsh Declaration.

89. Instead, with respect to the Walsh Declaration, purity must be understood not with respect to any reference standard, but with respect to the amount of total impurities reported as detected in each of the sample batches. The term "purity" must also be understood with respect to the amount of total impurities detected in the context of the '393 patent itself; wherever assay purity is referred to, the '393 patent specifies that the number indicated refers to "HPLC (Assay)." For each of the representative batches discussed in the Walsh Declaration, impurity data is presented in the same way, and thus the purity of these samples can properly be compared to each other; the same cannot necessarily be said of the sample data reported in Moriarty.

90. Second, Dr. Winkler concludes from Example 4 of the '393 patent that the instrumentation used to measure purity "can have variations of at least 0.4%," and thus any detected difference less than that can be attributed to experimental error. Ex. 1009 at ¶¶69-70. Dr. Winkler bases his estimate of experimental error on the statement "that Example 4's Batch 1 had an HPLC Assay of 100.4%, which is obviously greater than the 100% value theoretically achievable." Ex. 1009 at ¶70. This is unsupported and appears to arise from Dr. Winkler's fundamental misunderstanding of how assay purity values are calculated. HPLC assay values are calculated with respect to a reference standard; thus, any time that the sample you are measuring has a greater purity than the reference standard, the assay value will exceed 100%. As such, it is incorrect to conclude that an assay value of 100.4% must indicate an error of at least 0.4%. Dr. Winkler's conclusion on this point is therefore fundamentally flawed.

91. This explains why the assay value for drug specification submitted to the FDA changed from a range of [redacted] to [redacted]. *See*, Ex. 2003 at 6. This change was not due to

an increase in impurities, but because the purity of the product using the '393 patent process improved (as compared to the already-established reference standard) thus moving the acceptability range to a higher purity specification. *Id.* The letter notes that the scope of the range remained unchanged which simply indicates the acceptability criteria was increased, and does not index an error rate or limit of detection. Indeed, the change to the specification is further evidence that the product of the '393 patent is physically different than the product of Moriarty.

92. Indeed, Dr. Winkler's conclusion is contradicted by the impurity data actually measured for the treprostinil product made by both the '393 patent process and the prior art process according to Moriarty. For both processes, impurities are reported with specific numbers unless the amount detected fell below 0.05%; in cases where some amount of an impurity less than 0.05% was detected, it was reported as simply "less than 0.05%" or "< 0.05%." This means that the level of detection for measuring impurities in these treprostinil samples was somewhere between 0 and 0.05%, not something in excess of 0.4% as Dr. Winkler erroneously concludes.

93. Third, as Dr. Winkler himself points out, there is the possibility for "significant batch-to-batch variations in the impurity profile of each batch of treprostinil." Dr. Walsh stated that the data presented in his declaration came from representative samples of each synthetic process. Ex. 1002 at 346-347. However, there is no such indication that the purity data reported in Moriarty comes from a representative sample of the prior art process. Due to the possibility of batch-to-batch variations, if a small number of batches are to be used as the basis for comparison, it is critical that those batches be representative of their respective products and processes. Thus while one could reasonably rely on a comparison between the representative batches presented in

the Walsh Declaration, one could not reasonably add the batch discussed in Moriarty to that comparison. It is exactly this scientifically unsound comparison to Moriarty upon which Dr. Winkler bases his opinion.

94. Ideally, to avoid the risk of batch-to-batch variations unintentionally biasing the data, a comparison should be made between the average impurities detected in treprostinil products made by the '393 patent process and treprostinil products made by the prior art process. To this end, I have prepared a chart containing impurity data for 56 samples of treprostinil product as produced by the prior art process according to Moriarty through 2004 (the date of the publication), attached as Appendix A to this declaration[1], and another chart containing impurity data for 122 samples of treprostinil product as produced by the '393 patent processes, attached as Appendix B to this declaration. I have prepared these charts using impurity data from release testing of samples of the respective treprostinil products that were produced by or for UTC for the purposes of obtaining regulatory approval and/or commercial sale. *See* Appendix A, Appendix B; Ex. 2005; Ex. 2036; Ex. 2037; Ex. 2052; Ex. 2053. As the purpose of these charts is to calculate the average impurities – both specific and total – found in the treprostinil products of each process, I have necessarily assigned a value of zero where the level of impurities was

[1] I am aware that UTC's Process Optimization Report for treprostinil prepared according to the '393 process included Table 2, which provided average impurity data for 96 batches of treprostinil made according to the prior art process. UT Ex. 2005, at 7. However, Table 2 does not provide exact values for four of the eight impurities under consideration, [redacted] and does not identify the underlying batch data. *Id.* As such, I have prepared my own chart using data on 56 treprostinil samples made by the prior art method and have based my analysis, including my calculations of average for total and individual impurities, upon this chart. While I believe my chart allows for a more precise comparison between Moriarty treprostinil products and '393 treprostinil products, the averages presented in the Process Optimization Report still show significant differences between '393 treprostinil products and the Moriarty treprostinil products. Specifically, Table 2 of the Process Optimization Report shows that on average [redacted] was detectable in these 96 batches, and that these 96 batches contained higher average levels of [redacted], and total impurities as compared to the averages for the '393 treprostinil product. Ex. 2005 at 7; Appendix B.

reported as "ND" (Not Detected), and a value of 0.05 where the level of impurities was reported as being less than 0.05%. From these data, I have found the following average impurity levels:

Moriarty Process Impurities (Average Percent Detected)								
1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substance**
0.0473	0.0407	0.2545	0.1646	0.1025	0.0405	0.0889	0.1028	**0.9545**
'393 patent Process Impurities (Average Percent Detected)								
[redacted]								

95. These averages make clear that the '393 patent process does result in a treprostinil product that is physically different from the prior art treprostinil product. In terms of total volume of impurities, the Moriarty process resulted in [redacted] times the amount of impurities that is achieved with the '393 patent process.

96. The products from the two processes also differ significantly with respect to the individual impurities in each product's impurity profile. Notably, the '393 patent process produces a treprostinil product that does not contain any detectable amounts of [redacted]. Additionally, the '393 patent process produces a treprostinil product that, on average, contains only [redacted] each of [redacted] and [redacted] and only [redacted] of [redacted]; as compared to the Moriarty process, this represents greater than a [redacted] reduction in each of the [redacted] and [redacted] impurities and a [redacted] reduction in the [redacted] impurity. The '393 patent process also produces a treprostinil product that, on average, has significantly reduced amounts of several other identified impurities; as compared to the average of the Moriarty process, the '393 patent process produces a treprostinil product with less than [redacted] the amount of [redacted], approximately [redacted] the amount of [redacted], and approximately [redacted] the amount of

[redacted]. Conversely, the '393 patent process produces a treprostinil product which actually contains slightly more [redacted] impurity than was detected in the treprostinil product of the Moriarty process.

97. Looking past the average data, it is also worth noting that, out of all the batches of treprostinil product made by the '393 patent process which I reviewed, [redacted] was only detected in a single batch [redacted]) and [redacted] was also only detected in a single batch ([redacted]), and both impurities were only detected at a level of 0.05% or less. Furthermore, batches [redacted] and [redacted] were both identified as "optimization batches" (as distinguished from commercial batches) and thus are not properly representative of treprostinil products made by the '393 patent process.

98. From these data, it is clear that the treprostinil product produced by the '393 patent process has a markedly different impurity profile than the treprostinil product of the Moriarty prior art process, and as such is physically distinct from the prior art product. Moreover, it could not have been obvious that employing the process of the '393 patent would result in a reduction of impurities as compared to the Moriarty process. Indeed, the '393 patent process actually results in an [redacted] in one detected impurity, [redacted]. Furthermore, it is also clear that the treprostinil product produced by the '393 patent process has a higher average purity than the Moriarty product. The treprostinil product of the '393 patent has an average purity of [redacted] while the Moriarty product has an average purity of 99.05%. Thus, the treprostinil product of the '393 patent has an average purity that is [redacted] higher than that of Moriarty's.

99. Therefore, it is my opinion that the treprostinil product produced by the process used in the '393 patent Claims 1 and 9 is physically different than the treprostinil product produced by Moriarty.

B. Claims 1-5, 7-9, 11-14, and 16-20 Are Not Rendered Obvious by the Combination of Moriarty and Phares

100. As described above, the product of Moriarty is physically different than the product of the '393 patent process. Even if the Moriarty synthesis was used to make treprostinil, a POSA would not have been motivated to make the diethanolamine salt identified in Phares.

101. Specifically, the '393 patent notes that the salt formation step results in an improved and more pure treprostinil product. Given that Moriarty discloses the use of column chromatography for purification, a POSA would not have been motivated to create the salt form in Phares as Phares does not disclose any benefit or increased purity as a result of using the diethanolamine salt. In fact, Phares does not allege that the diethanolamine salt is superior in any way to the treprostinil product of Moriarty and instead identifies other earlier treprostinil disclosures as a means to create the treprostinil used to form the diethanolamine salt. *See*, Section VI(A) above.

102. Additionally, a POSA would not have had a reasonable expectation of success in making the higher purity treprostinil product claimed in the '393 patent by the use of a salt formation step. As identified above, the impurities of treprostinil include [redacted] ([redacted]), [redacted] ([redacted]), the [redacted] starting material ([redacted]), and the [redacted]. As described above, the '393 patent process essentially eliminated the [redacted] impurities [redacted], and [redacted] impurity [redacted], but did not eliminate another [redacted] which likely has the same [redacted] as the other

stereoisomers. Similarly, the [redacted] impurity increased while the [redacted] impurity decreased. A POSA would have expected that all of the stereoisomers would remain as salt impurities, but that is not the case. Instead, the impurity profile of the '393 patent process yields an unexpected result by removing [redacted] while [redacted] impurity and [redacted] another. A POSA could not have predicted this outcome based on the salt formation described in Phares.

103. Regarding Claim 2, neither Moriarty nor Phares discloses treprostinil or treprostinil diethanolamine at a purity of 99.5%. As described above, Phares does not disclose any purity measurement (see Section VI above) and the purity measurement identified in Moriarty does not identify how the measurement was taken (see Section VII(A) above). Regardless of the purity identified in Moriarty, a further analysis of all batches made by the Moriarty process up to the time of the reference itself reveals an average purity of 99.05% while the average purity of the '393 patent batches is [redacted]. Given that the error rate must be below 0.05% for these measurements (see Section VII(A) above), the '393 patent process batches are significantly better in terms of overall purity. For this additional reason, Claim 2 is not rendered obvious by the combination of Moriarty and Phares.

104. Regarding Claims 8 and 16, Phares does not disclose any synthesis for treprostinil and therefore cannot disclose whether purification was needed for step (a). (*See*, Section VI(B) above). As previously described, Moriarty specifically discloses that purification is performed at step (a). See Section VII(B) above). In fact and most significantly, the '393 patent itself identifies that as a distinguishing feature over the prior art. *See, e.g.*, Ex. 1001 at Example 6. For this additional reason, Claims 8 and 16 are not rendered obvious by the combination of Moriarty and Phares.

C. Claims 6, 10, 15, 21, and 22 Are Not Rendered Obvious by the Combination of Moriarty, Phares, Kawakami, and Ege

105. Each of Claims 6, 10, 14, 21, and 22 require the additional step (d) of independent Claims 1 and 9 which is to react the salt formed in step (c) with an acid to form the compound of formula I or IV (treprostinil). Claim 22 further requires a pharmaceutically acceptable salt formed from the product of step (d). Step (d) is not disclosed in any way in Moriarty, Phares, Kawakami, or Eğe. Additionally, it is my opinion that it would not have been obvious to combine these references to arrive at the claimed inventions of Claims 6, 10, 15, 21, or 22.

106. First, there is no teaching or suggestion to perform step (d) in either Moriarty or Phares and similarly no reference to reverting back to treprostinil free acid from any treprostinil salt. Given that the purification techniques disclosed in Moriarty include chromatography and recrystallization after many years of research to optimize the process of making treprostinil, a POSA would not have been motivated to use a salt purification technique disclosed in an undergraduate chemistry textbook. More importantly, a POSA would not have had a reasonable expectation of success in further purifying the treprostinil product of Moriarty by using such a technique. To the extent a POSA was motivated to further purify treprostinil, a POSA would have focused on the known impurities and investigated methods of removing those. At the time of the invention, it was known that the formation of diastereomers occurred in the formation of treprostinil. *See*, Ex. 1004 at 1897-99. Thus, a POSA would have focused on how to remove those types of impurities.

107. Eğe simply discloses that "carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties

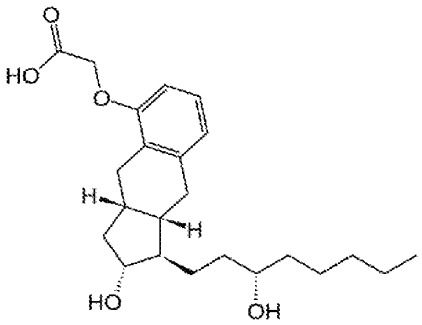
of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds." Ex. 1008 at 8. This disclosure, however, would not have provided a POSA with a motivation to make the treprostinil free acid disclosed in Moriarty, convert that to the salt form of Phares, then convert the salt form back to the free acid.

108. First, Eğe does not provide any detail regarding how this reaction could be applied to more complex carboxylic acids or if it even could be applied. Specifically, the only carboxylic acid referenced in Eğe as an example is benzoic acid, a very simple aromatic acid, which is structurally very different from treprostinil acid. Indeed, benzoic acid has no chiral centers and therefore no stereoisomers and there is no suggestion in Eğe that this step could be used in purifying more complex carboxylic acids such as treprostinil which have stereoisomeric impurities. Second, Eğe specifically notes that "these properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds," therefore Eğe would not apply to purifying carboxylic acids with stereoisomeric impurities because each stereoisomer would necessarily be an acidic impurity. As described above, the impurities that are removed from the '393 patent product include some, but not all acidic impurities and some but not all neutral impurities. *See*, Section VII(B) above. For these reasons a POSA would not have been motivated to combine Eğe with either Moriarty or Phares and would not have had a reasonable expectation of success in further purifying treprostinil using the acid reformation step described in Eğe.

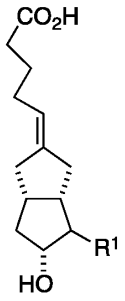
109. Indeed, given that Eğe predicts that only neutral and basic impurities would be removed, the actual average impurity profile for the '393 patent product is an unexpected result given that some but not all neutral impurities are removed as well as some but not all acidic impurities. *See*, Section VII(B) above.

110. Kawakami similarly does not provide any motivation for combining with either Phares or Moriarty and a POSA would not have had a reasonable expectation of success in preparing the products of Claims 6, 10, 15, 21, or 22 by combining these references.

111. Kawakami discloses the purification of a methanoprostacyclin derivative by forming the dicyclohexyl amine salt then regenerating the free acid to achieve a "fairly high" purity. Ex. 1007 at 6. Treprostinil and methanoprostacyclin, however, are very different structures:



Treprostinil



methanoprostacyclin compound in Kawakami

112. As shown here, the methanoprostacylin compound in Kawakami is a two-fused ring structure which is different than the three-fused ring structure of treprostinil that also includes an aromatic ring absent in the Kawakami methanoprostacyclin. These differences matter because a POSA would not have looked to Kawakami (or Eğe) if they were looking for additional purification techniques for treprostinil because neither reference discloses how to remove stereoisomeric impurities.

113. Instead, Kawakami provides a purification method for separating E and Z isomers of a starting material that is otherwise free of impurities, and not diastereomers that result from the various chiral centers that treprostinil was known to have as impurities. In fact, treprostinil

contains no mixture of E and Z isomers because it does not contain a carbon-carbon double bond that is capable of forming E and Z isomers. Indeed, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of a much more complex compound with multiple chiral centers such as treprostinil could be isolated from entirely different impurities and then converted back to the free acid form. In fact, nothing in Kawakami suggests that this method could be used for a substance that was already fairly pure such as the treprostinil disclosed in Moriarty.

114. Similarly, Kawakami uses a dicyclohexylamine salt and does not use a diethanolamine salt, nor any salt counterion disclosed in the '393 patent. A POSA would have had no reason to combine the synthesis of Moriarty, use the salt only disclosed by Phares, and convert back to the free acid based on the teaching of Kawakami because Kawakami uses a different salt to separate a different structure from different types of impurities. Even if a POSA did combine these references in this way, a POSA would not have had a reasonable expectation of success in forming a more pure treprostinil product because Kawakami does not provide any information regarding the high level of purity required by the '393 patent and does not describe the separation of the types of stereoisomeric impurities known to be present in the treprostinil product. Dr. Winkler's obviousness analysis using these combinations is flawed and suffers from hindsight analysis.

115. Claim 6 requires the acid in step (d) be either HCl or H_2SO_4 and Claim 15 requires the acid to be HCl. Claim 21 requires that step (d) is performed. Phares, Moriarty, and Kawakami all do not disclose the use of either HCl or H_2SO_4 in converting a salt back to a carboxylic acid of any kind. Eğe cites HCl as an example in the conversion of benzoic acid, but as described above, a POSA would not have looked to Eğe to further purify a complex

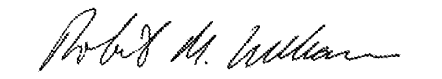
carboxylic acid such as treprostinil from its stereoisomers and other impurities and would have no reasonable expectation of success by using HCl based on this disclosure. For this additional reason, Claims 6 and 15 would not have been rendered obvious by any combination of Phares, Moriarty, Kawakami or Eğe. Similarly, given the deficiencies described above regarding Eğe and Kawakami, Claim 21 would not have been rendered obvious by any combination of Phares, Moriarty, Eğe, or Kawakami.

116. Claim 10 requires that step (d) is performed and further requires the product to be at least 99.5% pure. The only purity limitation disclosed in any of the cited prior art references is to Moriarty in which neither step (c) or (d) is performed. There is absolutely no other disclosure of a purity of at least 99.5% in any other cited prior art reference. A POSA looking to improve the purity of treprostinil above that level would have had no reason to look to Phares, Kawakami, or Eğe and based on their disclosures, would have had no reasonable expectation of success in making a treprostinil product with that level of purity as it simply is not present in the prior art allegedly disclosing step (d).

117. Claim 22 depends on Claim 21 and further requires a pharmaceutically acceptable salt be formed from the product of step (d). Dr. Winkler cites no evidence for this additional step in the prior art. In fact, none of the references cited even suggest converting a carboxylic acid to a salt form, then regenerating the carboxylic acid, then forming a pharmaceutically acceptable salt from that. It is my opinion that there is no evidence in the prior art supporting the additional claim limitation of Claim 22 and therefore no combination of Moriarty, Phares, Kawakami, or Eğe would render this claim obvious.

I declare under penalty of perjury that the foregoing is true and correct.

Date: July 6, 2016



Robert M. Williams, Ph.D.

APPENDIX A

Sample of product of Moriarty process	Impurities (Percent Detected)									Data Source
	1AU90	2AU90	3AU90	750W93	751W93	97W86	ethyl ester	methyl ester	Total Related Substances	
LRX-97J01	0.3	0.3	0.4	1.2	0.7	0.1	0	0.7	5.4	Ex. 2052, pp. 25-27
LRX-98A01	0.4	0.07	0.5	0.1	0.09	0.2	0	0.3	4.4	Ex. 2052, pp. 25-27
LRX-98B01	0.4	0.1	1	0.1	0.06	0.2	0	0.3	4.8	Ex. 2052, pp. 25-27
UT15-98H01	0.2	0.07	0.4	0.6	0.3	0	0	1.2	3.6	Ex. 2052, pp. 25-27
UT15-98I01	0.2	0.07	0.4	0.6	0.4	0.05	0	0.8	3.8	Ex. 2052, pp. 25-27
UT15-98I001	0.3	0.06	0.4	0.8	0.4	0	0	0.8	3.5	Ex. 2052, pp. 25-27
UT15RP-98K001	0.1	0.06	0.3	0.4	0.2	0	0	0.1	1.6	Ex. 2052, pp. 25-27
UT15-RP99D002	0.05	0.05	0	0.2	0.1	0.05	0.1	0.05	0.4	Ex. 2052, pp. 28-30
UT15-99E001	0.05	0.05	0.2	0.1	0.1	0	0	0.05	0.7	Ex. 2052, pp. 28-30
UT15MIX-99G001	0.05	0.05	1.1	0.3	0.2	0.6	0.6	0.05	2.8	Ex. 2052, pp. 28-30
UT15-99H001	0.05	0.05	0	0.5	0.3	0	0.1	0.06	1.0	Ex. 2052, pp. 28-30; Ex. 2036, pp. 2-3
UT15-000701	0	0.05	0.1	0.06	0.05	0	0	0.05	0.2	Ex. 2053, p. 19; Ex. 2036, pp. 88-89
UT15-000801	0	0.05	0.2	0.07	0.05	0	0	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 91-92
UT15-000802	0	0.05	0.1	0.1	0.07	0	0	0.05	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 94-95
UT15-000803	0	0.05	0.2	0.2	0.09	0	0	0.05	0.6	Ex. 2053, p. 19; Ex. 2036, pp. 100-101
UT15-000901	0	0.05	0.3	0.05	0.05	0	0.05	0.05	0.05	Ex. 2053, p. 19; Ex. 2036, pp. 33-34
UT15-000902	0	0.05	0.2	0.1	0.06	0	0.05	0.05	0.5	Ex. 2053, p. 19; Ex. 2036, pp. 97-98

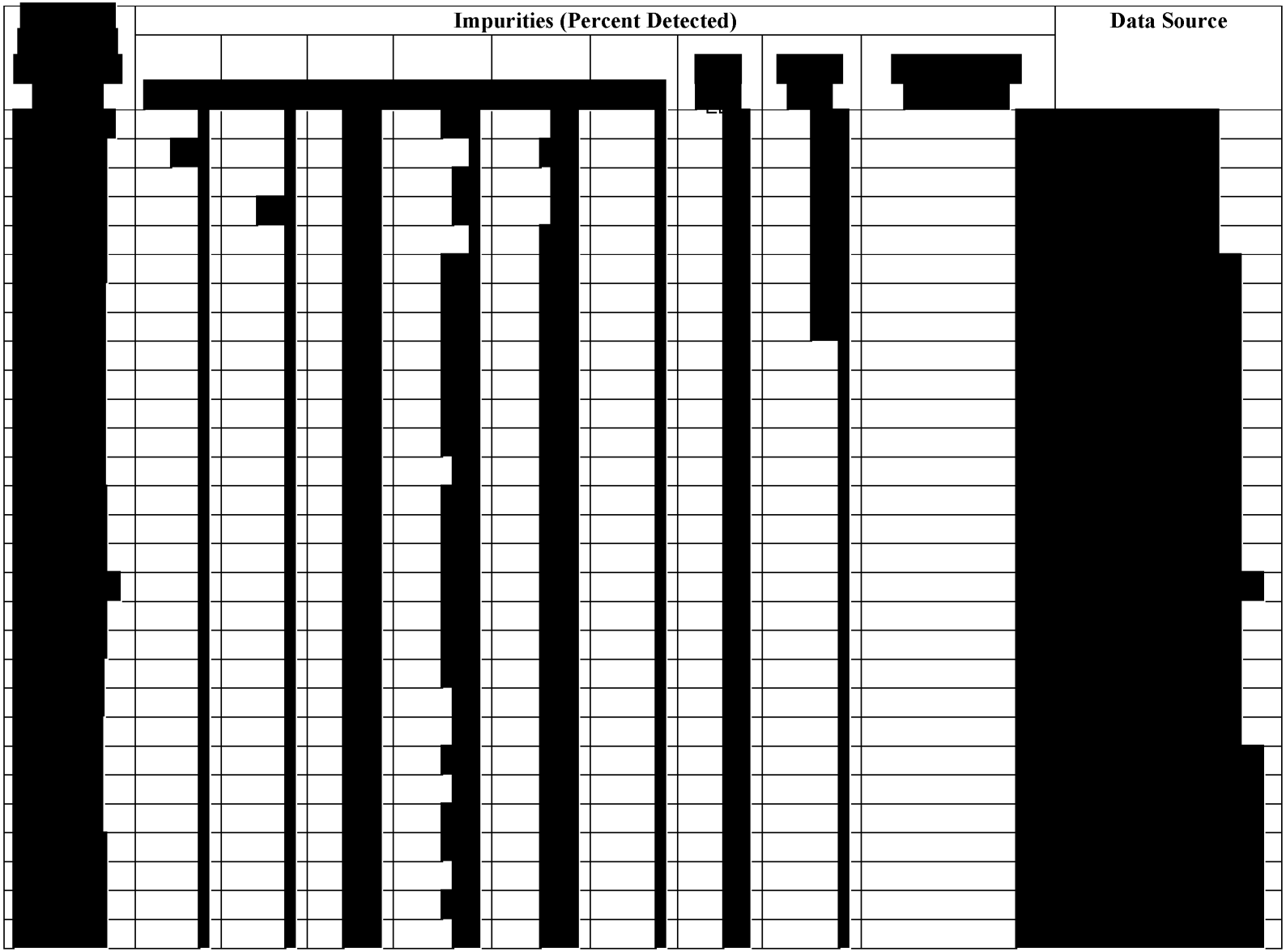
UT15-001001	0.05	0.05	0.2	0.09	0.06	0	0.05	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 35-36
UT15-010201	0	0.05	0.2	0.09	0.05	0.05	0	0	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 37-38
UT15-010202	0	0.05	0.2	0.09	0.05	0.05	0	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 39-40
UT15-010203	0.2	0.05	0.3	0.4	0.2	0.08	0.05	0.05	1.5	Ex. 2053, p. 19; Ex. 2036, pp. 41-42
UT15-010301	0	0.05	0.3	0.09	0.05	0.05	0.05	0	0.5	Ex. 2053, p. 19; Ex. 2036, pp. 43-44
UT15-010302	0.05	0	0.2	0.05	0.05	0.05	0.08	0	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 45-46
UT15-010303	0	0	0.2	0.1	0.05	0.05	0	0	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 47-48
UT15-010801-RP	0	0.05	0.1	0.2	0.1	0.05	0.2	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 60-61
UT15-010802	0.05	0.05	0.2	0.05	0.05	0	0.05	0.05	0.2	Ex. 2053, p. 20; Ex. 2036, pp. 50-52
UT15-010803	0.05	0.05	0.2	0.1	0.06	0	0.07	0.05	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 52-53
UT15-010901	0	0.05	0.2	0.1	0.08	0.07	0.09	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 54-55
UT15-010902	0	0.05	0.2	0.05	0.05	0	0.1	0	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 56-57
UT15-011001	0	0.05	0.3	0.08	0.05	0.05	0.1	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 58-59
UT15-020101	0	0.05	0.2	0.05	0.05	0	0.05	0	0.4	Ex. 2053, p. 20
UT15-020201	0	0.05	0.2	0.1	0.1	0	0.1	0	0.4	Ex. 2053, p. 20
UT15-020202	0	0.05	0.1	0.1	0.1	0.05	0.2	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 62-63
UT15-020203	0	0	0.05	0.05	0.05	0	0.1	0.05	0.2	Ex. 2053, p. 20; Ex. 2036, pp. 64-65

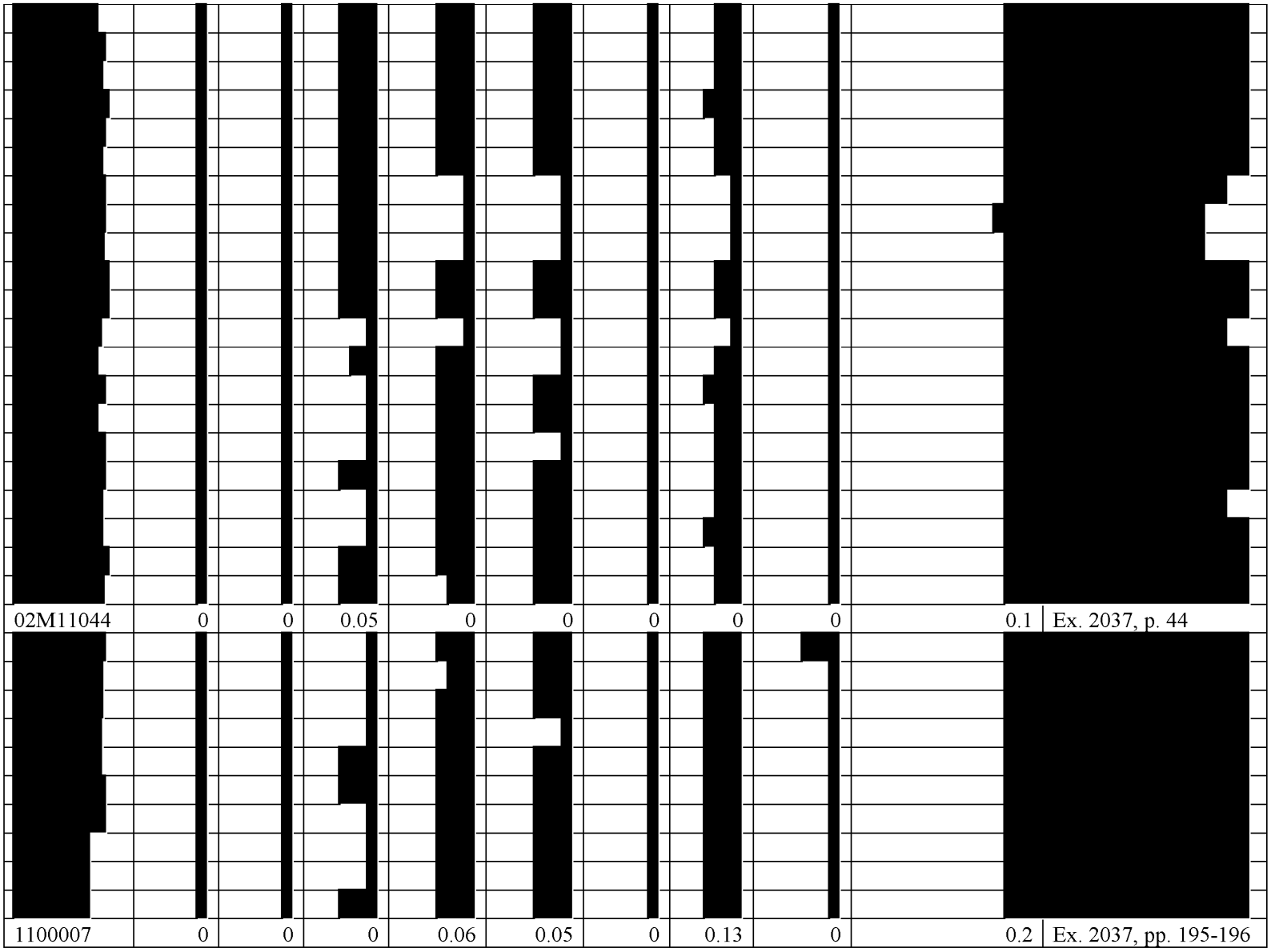
UT15-020301	0	0.05	0.2	0.05	0.05	0	0.1	0	0.3	Ex. 2053, p. 20; Ex. 2036, pp. 66-67
UT15-020302	0	0.05	0.2	0.06	0.05	0	0.1	0	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 68-69
UT15-020303	0	0.05	0.2	0.05	0.05	0	0.1	0	0.3	Ex. 2053, p. 20; Ex. 2036, pp. 70-71
UT15-021001	0	0	0.4	0.1	0.08	0.05	0.1	0.05	0.8	Ex. 2053, p. 21; Ex. 2036, pp. 72-73
UT15-021002	0	0.05	0.3	0.06	0.05	0.05	0.2	0.05	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 74-76
UT15-021003	0	0	0.4	0.05	0.05	0	0.1	0.05	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 78-79
UT15-021101	0	0	0.2	0.09	0.06	0	0.1	0	0.5	Ex. 2053, p. 21; Ex. 2036, pp. 80-82
UT15-021102	0	0	0.1	0.2	0.1	0.07	0.1	0	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 83-85
UT15-030401	0	0	0.3	0.06	0.05	0	0.2	0.05	0.5	Ex. 2053, p. 21; Ex. 2036, pp. 31-32
UT15-030501	0	0	0.3	0.1	0.07	0	0.1	0.05	0.6	Ex. 2036, pp. 29-30
UT15-030502	0	0	0.3	0.1	0.06	0	0.1	0.05	0.6	Ex. 2036, pp. 27-28
UT15-030503	0	0	0.3	0.2	0.1	0.05	0.2	0.05	0.9	Ex. 2036, pp. 25-26
UT15-030504	0.05	0.05	0.2	0.06	0.05	0.05	0.1	0.05	0.4	Ex. 2036, pp. 23-24
UT15-030601	0.05	0.05	0.2	0.05	0.05	0.05	0.09	0.05	0.3	Ex. 2036, pp. 21-22
UT15-030602	0.05	0.05	0.2	0.06	0.05	0.05	0.1	0.05	0.4	Ex. 2036, pp. 19-20
UT15-031001	0	0	0.2	0.2	0.08	0.05	0.1	0.05	0.6	Ex. 2036, pp. 17-18
UT15-031002	0	0	0.2	0.05	0.05	0	0.1	0	0.4	Ex. 2036, pp. 15-16
UT15-031003	0	0	0.2	0.1	0.06	0.05	0.2	0.05	0.6	Ex. 2036, pp. 13-14
UT15-031101	0	0	0.2	0.05	0.05	0	0.2	0	0.5	Ex. 2036, pp. 11-12
UT15-031102	0	0	0.1	0.1	0.06	0.05	0.1	0.05	0.4	Ex. 2036, pp. 8-10
UT15-031201	0	0	0.2	0.09	0.05	0	0.1	0.05	0.4	Ex. 2036, pp. 6-7

UT15-031202	0	0	0.2	0.07	0.05	0	0.2	0.05	0.5	Ex. 2036, pp. 4-5
Average	**0.0473**	**0.0407**	**0.2545**	**0.1646**	**0.1025**	**0.0405**	**0.0889**	**0.1028**	**0.9545**	
	1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substances**	

Note: For impurities reported as not detected ("ND") a value of 0 has been assigned; for impurities reported as <0.05, a value of 0.05 has been assigned.

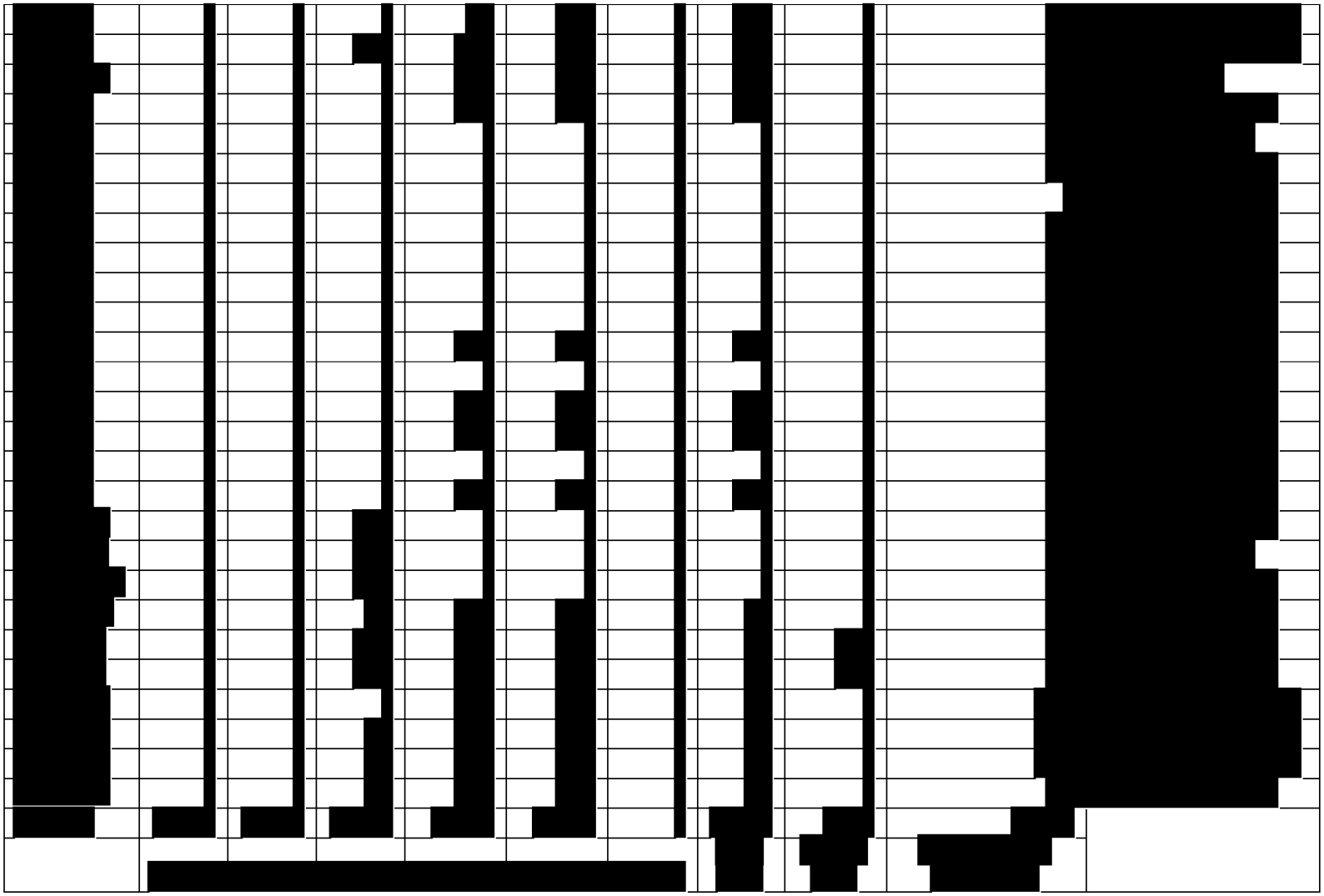
APPENDIX B





02M11044	0	0	0.05	0	0	0	0	0	0.1	Ex. 2037, p. 44
1100007	0	0	0	0.06	0.05	0	0.13	0	0.2	Ex. 2037, pp. 195-196

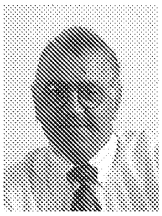
4851-2371-9220.1



Note: For impurities reported as not detected ("ND") a value of 0 has been assigned; for impurities reported as <0.05, a value of 0.05 has been assigned.

CURRICULUM VITAE

Robert Michael Williams, Ph.D.
University Distinguished Professor of Chemistry
Department of Chemistry, Colorado State University, Fort Collins, CO 80523
Phone (970) 491-6747; FAX (970)-491-3944
e-mail: robert.williams@colostate.edu
Webpage: http://rwindigo1.chm.colostate.edu/



Personal Information:
Date of birth: February 8, 1953
Married: Jill Janssen Williams
Children: Ridge Janssen Williams (born February 23, 2001)
Rainier Valentine Williams (born August 20, 2005)

Education:
B.A., Chemistry (with highest distinction), May, 1975. Syracuse University, Syracuse, NY. Thesis under Professor Ei-ichi Negishi on *"A Stereoselective Synthesis of Partially Substituted 1,2,3-Butatriene Derivatives via Hydroboration".*

Ph.D., Organic Chemistry, June, 1979. Massachusetts Institute of Technology, Cambridge, MA. Thesis advisor, Dr. W. H. Rastetter. Thesis title: *"Epidithiapiperazinedione Syntheses".*

Postdoctoral Fellow, September 1979-September 1980, Harvard University, Cambridge, MA. The late Professor R. B. Woodward group (Y. Kishi, principal investigator). *Total synthesis of erythromycin A.*

Honors and Awards:
Organic Synthesis Award, Local Rocky Mountain ACS Section, Reaching New Heights (2012)
JSPS Invitation Fellowship Program for Research in Japan (Long-Term) 2012-2013
Ernest Guenther Award in the Chemistry of Natural Products, American Chemical Society (2011)
Multiple Myeloma Research Foundation Senior Award (2009-2011)
University Distinguished Professor, Colorado State University (2002)
Arthur C. Cope Scholar Award, American Chemical Society (2002)
Japanese Society for the Promotion of Science (JSPS) Fellowship (1999)
Merck & Co. Academic Development Award (1991-93)
Fellow of the Alfred P. Sloan Foundation (1986-88)
Eli Lilly Young Investigator Grantee (1986-88)
NIH Research Career Development Awardee (1984-89)
Phi Beta Kappa, Syracuse University (1975)
Kanner Prize for Chemistry and Physics, Syracuse University (1974)

Research and Professional Experience:
Director, Colorado Center for Drug Discovery (C2D2), April 2012 -present
Co-Director, Infectious Diseases Supercluster in Developmental Therapeutics at CSU
Co-Director, Cancer Supercluster in Developmental Therapeutics at CSU
Member, University of Colorado Cancer Center 2004-present
University Distinguished Professor, Colorado State University 2002-present
Professor, Colorado State University; Fort Collins, Colorado, January 1988-2002
Associate Professor, Colorado State University; Fort Collins, Colorado, July 1985-December 1987
Assistant Professor, Colorado State University; Fort Collins, Colorado, September 1980-June 1985
Visiting Professor, University of California, Berkeley, CA September 1990-December 1990
Visiting Professor of Chemistry, Harvard University, Cambridge, Mass. Sept. 15, 1994-Feb. 15, 1995

Professional Society Memberships:
American Association for the Advancement of Science
American Chemical Society
Japan Antibiotics Research Association
International Society of Heterocyclic Chemistry
Phi Beta Kappa

Editorial:

(1) Associate Editor, *Tetrahedron: Asymmetry* (2006-2008)

(2) Member, Editorial Board *"Chemistry and Biology"* Current Biology, Ltd. (1994-present).

(3) Member, Editorial Advisory Board for *Mini-Reviews in Organic Chemistry*, Bentham Science Pubs. (2004-2012).

(4) Co-Editor for the *"Organic Chemistry Series",* Elsevier (Sir J.E. Baldwin co-Ed.) 1997-2012.

(5) Editor-in-Chief for *"Amino Acids"*, Springer-Verlag (1991-1998).

(6) Acting Associate Editor for the *Journal of the American Chemical Society*; Sept. 1983-Jan. 1984.

Consulting Activities:

Cetya Therapeutics, Fort Collins, Colorado (2012-present)

Sapientia Therapeutics, Philadelphia, Pennsylvania (2012-present)

Arch Therapeutics, Cambridge, Massachusetts (2010-present)

HemaQuest Pharmaceuticals, Boston, Massachusetts (2006-2014)

Ajinomoto Co., Yokohama Japan (2002-present)

Xcyte Therapies, Inc., Seattle, Washington (1996-2006)

NewBiotics, Inc., SanDiego, California (2001-2002)

Cubist Pharmaceutical Co., Cambridge, Massachusetts (2000-2003)

Microcide Pharmaceuticals, Mountainview, California (1993-1998)

Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut (1992-1993)

Hoffman-LaRoche, Inc., Nutley, New Jersey (1989-1992)

EPIX Medical, Cambridge, Massachusetts (1993-1997)

W.R. Grace Company, Columbia, Maryland (1985-1990)

G.D. Searle Co., St. Louis, Missouri (1988-1990)

Nutrasweet Co., Skokie, Illinois (1990-1991)

Symphony Pharmaceutical Co., Philadelphia, Pennsylvania (1991-1993)

Venture Capital Corporate Boards and Scientific Advisory Boards:

(1) Founding Scientist, Acting President, Member of the Board of Directors and Member of the Scientific Advisory Board of Cetya Therapeutics, Fort Collins, Colorado 2012-present.

(2) Founding Scientist and Member of the Scientific Advisory Board for Sapientia Therapeutics (2012-present).

(3) Consultant and Member of the Scientific Advisory Board for Arch Therapeutics, Cambridge, Mass. 2010-present.

(4) Founding Scientist, Acting Vice-President of Discovery Chemistry, consultant and Member of the Scientific Advisory Board of HemaQuest, Seattle, Washington. 2006-present.

(5) Founding Scientist and Member of the Corporate Board of Directors for Xcyte Therapies, Seattle, Washington. 1996-2006.

(6) Scientific Advisory Board Member of Xcyte Therapies, Seattle, Washington. 1996-2006.

(7) Scientific Advisory Board Member of NewBiotics, Inc., San Diego, CA. 2001-2004.

(8) Founding Scientist and Scientific Advisory Board Member for Microcide Pharmaceuticals, Inc., Mountainview, California 1993-1998.

Advisory Boards:

Member of the Advisory Board of the Los Alamos Stable Isotope Resource (1999-2007)

Member of the Advisory Board for the *Puerto Rico Alliance for the Advancement of Biomedical Research Excellence* (PRAABRE) (2006-present)

Teaching

Graduate

C651 - "Molecular Basis for Drug Action and Design: Mechanism-Based Enzyme Inhibitors"
C651 - "Total Synthesis of Natural Products"
C651 - "Biosynthesis of Primary and Secondary Metabolites"
C541 - "Spectroscopic Methods"
C545 - "Advanced Organic Synthesis I"
C549 – "Advanced Organic Synthesis II"
C-547 – "Chemical Biology"

Undergraduate

C340H - "Honors Organic Chemistry I"
C341 - "Organic Chemistry I"
C343 - "Organic Chemistry II"
C245 - "Organic and Biological Chemistry"
C345 - "Organic Chemistry I"
C346 – "Organic Chemistry II"
Chem 17 (Harvard University, 1994 - 1995)

Ph.D. Theses Directed:

Robert W. Armstrong	(1984)	Chester Yuan	(1997)	Xiangna Jia	(2009)
Andrew O. Stewart	(1987)	Bradley Herberich	(1999)	Daniel A. Gubler	(2009)
Peter J. Sinclair	(1987)	Jeffrey Cao	(2000)	Tenaya Newkirk	(2009)
Lynn K. Maruyama	(1987)	Kathleen M. Halligan	(2000)	Cameron Burnett	(2009)
Paul P. Ehrlich	(1989)	Jack D. Scott	(2001)	Brandon J. English	(2010)
Myeong-Nyeo Im	(1991)	Emily M. Stocking	(2001)	Jennifer M. Finefield	(2011)
James A. Hendrix	(1992)	Paul R. Sebahar	(2003)	Timmy McAfoos	(2011)
Sean C. Esslinger	(1993)	Ted C. Judd	(2003)	Ryan J. Rafferty	(2011)
Glenn J. Fegley	(1993)	Steven R. Lenger	(2003)	Guojun Pan	(2011)
Gregory F. Miknis	(1993)	Duane E. DeMong	(2003)	Paul T. Schuber	(2011)
Timothy D. Cushing	(1993)	Brian K. Albrecht	(2003)	Guojun Pan	(2011)
Mark E. Flanagan	(1995)	Ryan E. Looper	(2004)	Timothy R. Welch	(2011)
Steve Rubenstein	(1996)	Yuyin Chen	(2006)	Jennifer M. Bubb	(2012)
Jennifer Travers	(1995)	Alan W. Grubbs	(2006)	Aaron Pearson	(2013)
Samuel B. Rollins	(1997)	Siyuan Chen	(2007)	Alberto Jimenez	(2013)
Scott R. Rajski	(1997)	Chandele Gray	(2008)	Michelle Sanchez	(2014)

Masters Theses Directed:

Tracy N. Tippie	(1995)	Christie Kosogof	(2001)	Annie Youngman	(2009)
Paul B. Gansle	(1997)	Meriah W.N. Valente	(2006)	Andrea Geiser	(2009)
David M. Bender	(1998)	Nick Gearhart	(2008)	Tatyana Sabodash	(2011)
				Marie Trujillo	(2013)

Reviewer for Journals:

Journal of the American Chemical Society
Chemical Society Reviews
Journal of Organic Chemistry
Canadian Journal of Chemistry]
Archives of Biochemistry and Biophysics
Bioorganic and Medicinal Chemistry Letters
Bioorganic and Medicinal Chemistry
Amino Acids
Science
Tetrahedron
Tetrahedron Letters
Synthesis
Organic Letters
Chemistry & Biology
Accounts of Chemical Research
Chemical Communications
Angewandte Chemie
Chemical Reviews
Syn. Lett.
Nature Protocols
Nature Chemistry

Reviewer for Proposals:

National Institutes of Health Synthetic and Biological Chemistry B (SBCB) Study Section 2005
National Institutes of Health Medicinal Chemistry Study Section (MCHA) (2002-2005)
National Science Foundation
National Institutes of Health Medicinal Chemistry Study Section (Feb. 1987)
National Institutes of Health BNP Study Section (Feb. 1989)
Research Corporation
Petroleum Research Fund (ACS)
State Board of Education, Idaho
Ad-hoc reviewer for the Medicinal Chemistry Study Section (MCHA) February, 1987
Ad-hoc reviewer for the Bioorganic and Natural Products Chemistry Study Section (BNP) Feb., 1990
Member, NIH T-32 Chemistry / Biology Interface Training Grant Study Section, 1993
Ad-hoc reviewer for the Medicinal Chemistry Study Section (MCHA) October, 1996

Committee Memberships (Colorado State University)

Mis-conduct in Science Investigation Committee
Council of Research Associate Deans
University Distinguished Professors Selection Committee
Colorado State University Patent Committee
Graduate Operations Committee, Department of Chemistry
Graduate Admissions Committee, Department of Chemistry
Colorado State University Biosafety Committee
Promotion and Tenure Committee, Department of Chemistry
Executive Committee, Department of Chemistry
Industrial Liaison Committee, Department of Chemistry
Awards Committee, Department of Chemistry

Current Research Funding:

***Active Grants* (Robert M. Williams, PI: all projects)**

(1)	2RO1 CA70375-13 (NIH/NCI) Title: *"Total Synthesis and Biosynthesis of Bioactive Substances"*	08/01/14 – 07/31/19 $268,515 Annual Amount $1,292,938 Total Award
(2)	1 RO1 CA152314-01 (NIH/NCI) Title: *"Multiple Myeloma and Cancer Therapies via Largazole Analogs"*	09/01/10 – 12/30/15 $338,173 Annual Amount $1,599,830 Total Award

Chair, Organizer or Co-organizer of Scientific Meetings
Robert M. Williams, Professor of Chemistry

(1) The 1987 NSF Workshop on Environmental Chemistry, Stanford Sierra Camp, Lake Tahoe, California, Sept. 25-27, 1987.

(2) The 1989 Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, December 17-22, 1989. Co-organizer of a Symposium entitled: *"Recent Developments in the Chemistry of Amino Acids".*

(3) Special Bilateral U.S.-Britain Workshop entitled: *"Asymmetric Synthesis"* July 3-8, 1990. Pingree Park, Colorado. Co-organizer of this workshop.

(4) NSF-JSPS Bilateral Seminar entitled: *"Selectivity in Synthetic and Bio-Organic Chemistry",* Tokyo, Japan, June 3-7, 1991. Co-organizer of this bilateral seminar.

(5) The 3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic Amino Acids via Chiral Glycinates".* Co-organizer of this meeting.

(6) The John K. & Dolores Stille Symposium on *"Biological Chemistry",* September 12, 1997. Organizer of this symposium.

(7) The 60th Birthday Celebration for Professor Yoshito Kishi, Harvard University. April 13, 1997, Harvard University. Co-organizer of this symposium and reception.

(8) The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Organizer of a Symposium entitled: *"Frontiers in Antibiotics: Synthesis, Design and Mode of Action".*

(9) *The 19th International Congress of Heterocyclic Chemistry*, Fort Collins, Colorado. August, 2003. Vice-President, Chairman and Organizer of this meeting.

(10) The PacifiChem 2005 Congress, Honolulu, Hawaii, December 15-20, 2005. Co-Chair, Symposium entitled: *"Total Synthesis of Natural Products"*

(11) The 70th Birthday Celebration for Professor Yoshito Kishi, Harvard University. April 13, 2007, Harvard University. Co-organizer of this symposium and reception.

(12) *The Albert & Joan Meyers Symposium*. October 24, 2008, Colorado State University. Co-organizer of this symposium and reception.

(13) *The PacifiChem 2010 Conference*, Honolulu, Hawaii. Organizer of a Symposium entitled: *"Natural Products Synthesis"*

(14) *Robert Burns Woodward Memorial Symposium*. National ACS Meeting in Boston, Massachusetts, August 22-26, 2010. Principal Co-organizer.

Robert M. Williams
Brief Biographical Sketch

Robert M. Williams was born in New York in 1953 and was raised in Huntington, Long Island by Edith and Valentine Williams. He attended Syracuse University from 1971-1975 and received the B.A. degree in Chemistry in 1975. While at Syracuse, he did undergraduate research with the recent Nobel Laureate, Prof. Ei-ichi Negishi in the area of hydroboration methodology. He then moved to Cambridge, Massachusetts and entered the Ph.D. program at MIT and obtained his Ph.D. degree in 1979 under the supervision of Prof. William H. Rastetter. His doctoral studies were concerned with the total synthesis of two fungal metabolites, gliovictin and hyalodendrin. Following completion of his doctoral studies, he joined the laboratories of the late Prof. R.B. Woodward in 1979 whose postdoctoral group was subsequently managed by Professor Yoshito Kishi. His postdoctoral work was concerned with the completion of the total synthesis of erythromycin A. Upon completion of his postdoctoral tenure at Harvard, he joined the faculty at Colorado State University in 1980. He was promoted to Associate Professor with tenure in 1985, Full Professor in 1988, and University Distinguished Professor in 2002, his current position. Dr. Williams has received several Honors and Awards including the NIH Research Career Development Award (1984-1989); The Eli Lilly Young Investigator Award (1986); Fellow of the Alfred P. Sloan Foundation (1986); the Merck, Academic Development Award (1991), the Japanese Society for the Promotion of Science Fellowship (1999), the ACS Arthur C. Cope Scholars Award (2002), the ACS Ernest Guenther Award in the Chemistry of Natural Products (2011) and the Japanese Society for the Promotion of Science Long-term Fellowship (2012-2013). In 2002, he was named one of the twelve University Distinguished Professors, the highest honor and rank that Colorado State University bestows upon faculty. He spent a sabbatical at the University of California, Berkeley in 1990 in the laboratories of Professor Peter G. Schultz and was a visiting Professor at Harvard University in 1994 where he spent a sabbatical with Professor Stuart L. Schreiber and also taught Chem 17. He serves on the Editorial Board of the journal *Chemistry & Biology* and was an Editor for the journal *Amino Acids* (1991-1998). He has served as a Series co-Editor for *The Organic Chemistry Series*, published by Pergamon Press/Elsevier with Professor Sir Jack E. Baldwin of Oxford. Dr. Williams was a Scientific Founder and member of the Scientific Advisory Board of Microcide Pharmaceutical Co. from 1993-1998 located in Mountainview, California and was a Founding Scientist, Member of the Scientific Advisory Board and Member of the Board of Directors of Xcyte Therapies, located in Seattle, Washington from 1995-2006. Dr. Williams is currently a Scientific Founder, consultant, and member of the Scientific Advisory Board for HemaQuest Pharmaceuticals, located in Seattle, Washington from 2006-present and also serves as a consultant and Member of the Scientific Advisory Board for Arch Therapeutics, Cambridge, Mass. 2010-present. Dr. Williams is a Scientific Founder, Member of the Board of Directors, Chairman of the Scientific Advisory Board and acting President of Cetya Therapeutics, located in Fort Collins, Colorado. Dr. Williams is a Scientific Founder and Member of the Scientific Advisory Board of Sapientia Therapeutics, located in Philadelphia, Pennsylvania. Dr. Williams was named as Director for the Colorado Center for Drug Discovery (C2D2) in April, 2012.

Dr. Williams' research results from the interplay of synthetic organic chemistry, microbiology, biochemistry and molecular biology. Dr. Williams research interests have included the total synthesis of natural products, the asymmetric synthesis of amino acids and peptide isosteres, studies on anti-tumor drug-DNA interactions, design and synthesis of antibiotics and DNA-cleaving molecules, combinatorial phage libraries and biosynthetic pathways. He has utilized natural products synthesis to probe and explore biomechanistic and biosynthetic problems with a particular emphasis on antitumor and antimicrobial antibiotics. He has developed technology for the asymmetric synthesis of α-amino acids and peptide isosteres that has been commercialized by Aldrich Chemical Co. and has written a monograph on this subject.

Personal Interests: Downhill, cross-country, randonee and telemark skiing; rock electric & acoustic guitars; backpacking; mountain biking; running; bicycling; technical rock climbing; scuba diving; water skiing; surfing; sailing; photography; oil painting; oriental carpets; fine woodworking, furniture design and construction; golf; squash racquets; gardening; orchids; salt water aquarist.

PUBLICATIONS

Robert M. Williams, Colorado State University

1. [ʃ] *A Stereoselective Synthesis of Partially Substituted 1,2,3-Butatriene Derivatives via Hydroboration*, Yoshida, T.; Williams, R.M.; Negishi, E-i., *J. Am. Chem. Soc.*, **1974**, *96*, 3688-3690.

2. [ʃ] *A Stereoselective Synthesis of cis-Alkenylboranes*, Negishi, E-i.; Williams, R.M.; Lew, G.; Yoshida, T., *J. Organomet. Chem.*, **1975**, *92*, C4-C6.

3. [¶] *An Efficient Synthesis of d,l-Gliovictin: Construction of the Hydroxymethyl Moiety via a 3-Formyl-2,5-Piperazinedione*, Williams, R.M.; Rastetter, W.H., *Tetrahedron Lett.,* **1979**, 1187-1190.

4. [¶] *Synthesis of the Fungal Metabolites (±)-Gliovictin and (±)-Hyalodendrin,* Williams, R.M.; Rastetter, W.H., *J. Org. Chem.,* **1980**, *45*, 2625-2631.

5. [§] *Asymmetric Total Synthesis of Erythromycin I,* Woodward, R.B.; Logusch, E.; Nambiar, K.P.; Sakan, K.; Ward, D.E.; Au-Yeung, B.W.; Balaram, P.; Browne, L.J.; Card, P.J.; Chen, C.H.; Chenevert, R.B.; Fliri, A.; Frobel, K.; Gais, H.J.; Garratt, D.G.; Hayakawa, K.; Heggie, W.; Hesson, D.P.; Hoppe, D.; Hoppe, I.; Hyatt, J.A.; Ikeda, D.; Jacobi, P.A.; Kim, K.S.; Kobuke, Y.; Kojima, K.; Krowicki, K.; Lee, V.J.; Leubert, T.; Malchenko, S.; Martens, J.; Mathews, R.S.; Ong, B.S.; Press, J.B.; RajanBabu, T.V.; Rousseau, G.; Sauter, H.M.; Suzuki, M.; Tatsuta, K.; Tolbert, L.M.; Truesdale, E.A.; Uchida, I.; Ueda, Y.; Uyehara, T.; Vasella, A.T.; Vladuchick, W.C.; Wade, P.A.; Williams, R.M.; Wong, H.N.-C. *J. Am. Chem. Soc.*, **1981**, *103*, 3210-3213.

6. [§] *Asymmetric Total Synthesis of Erythromycin II,* Woodward, R.B.; Logusch, E.; Nambiar, K.P.; Sakan, K.; Ward, D.E.; Au-Yeung, B.W.; Balaram, P.; Browne, L.J.; Card, P.J.; Chen, C.H.; Chenevert, A.; Fliri, A.; Frobel, K.; Gais, H.J.; Garratt, D.G.; Hayakawa, K.; Heggie, W.; Hesson, D.P.; Hoppe, D.; Hoppe, I.; Hyatt, J.A.; Ikeda, D.; Jacobi, P.A.; Kim, K.S.; Kobuke, Y.; Kojima, K.; Krowicki, K.; Lee, V.J.; Leubert, T.; Malchenko, S.; Martens, J.; Mathews, R.S.; Ong, B.S.; Press, J.B.; RajanBabu, T.V.; Rousseau, G.; Sauter, H.M.; Suzuki, M.; Tatsuta, K.; Tolbert, L.M.; Truesdale, E.A.; Uchida, I.; Ueda, Y.; Uyehara, T.; Vasella, A.T.; Vladuchick, W.C.; Wade, P.A.; Williams, R.M.; Wong, H.N.-C., *J. Am. Chem. Soc.*, **1981**, *103*, 3213-3215.

7. [§] *Asymmetric Total Synthesis of Erythromycin III,* Woodward, R.B.; Logusch, E.; Nambiar, K.P.; Sakan, K.; Ward, D.E.; Au-Yeung, B.W.; Balaram, P.; Browne, L.J.; Card, P.J.; Chen, C.H.; Chenevert, R.B.; Fliri, A.; Frobel, K.; Gais, H.J.; Garratt, D.G.; Hayakawa, K.; Heggie, W.; Hesson, D.P.; Hoppe, D.; Hoppe, I.; Hyatt, J.A.; Ikeda, D.; Jacobi, P.A.; Kim, K.S.; Kobuke, Y.; Kojima, K.; Krowicki, K.; Lee, V.J.; Leubert, T.; Malchenko, S.; Martens, J.; Mathews, R.S.; Ong, B.S.; Press, J.B.; RajanBabu, T.V.; Rousseau, G.; Sauter, H.M.; Suzuki, M.; Tatsuta, K.; Tolbert, L.M.; Truesdale, E.A.; Uchida, I.; Ueda, Y.; Uyehara, T.; Vasella, A.T.; Vladuchick, W.C.; Wade, P.A.; Williams, R.M.; Wong, H.N.-C., *J. Am. Chem. Soc.*, **1981**, *103*, 3215-3217.

[ʃ] Syracuse University

[¶] MIT

[§] Harvard University

**Colorado State University*

1981

8. **Bicyclomycin Synthetic Studies: Utilization of Bridgehead Carbanions*, Williams, R. M., *Tetrahedron Lett.,* **1981**, *22*, 2341-2344.

1982

9. **A New and Efficient Cyclization Reaction to Construct the Bicyclomycin Ring System: Synthesis of N,N'-Dimethyl-4-des-methylene Bicyclomycin*, Williams, R.M.; Anderson, O.P.; Armstrong, R.; Josey, J.; Meyers, H.; Eriksson, C., *J. Am. Chem. Soc.*, **1982**, *104*, 6092-6099.

1983

10. **C-Glycosidation of Pyridyl Thioglycosides*. Williams, R.M.; Stewart, A.O., *Tetrahedron Lett.*, **1983**, *24*, 2715-2718.

11. **Regioselective Functionalization of Bicyclic Piperazinedione Bridgehead Carbanions*. Williams, R.M.; Dung, J-S.; Josey, J.; Armstrong, R.W.; Meyers, H., *J. Am. Chem. Soc.*, **1983**, *105*, 3214-3220.

12. **Improved Synthesis and Absolute Configuration of (+)- and (-)-2,2,4-Trimethyl-1,3-dioxolane-4-carboxaldehyde*. Dung, J-S.; Armstrong, R.W.; Anderson, O.P.; Williams, R.M., *J. Org. Chem.,* **1983**, *48*, 3592-3594.

1984

13. **Metal-Mediated Concomitant Silyl Ether Cleavage/Cyclization Reactions to Construct Bicyclic Piperazinediones and a New Polymer-Supported Hg(II) Perchlorate*. Dung, J-S.; Armstrong, R.W.; Williams, R.M., *J. Org. Chem.,* **1984**, *49*, 3416-3419.

14. **Alternate Preparation of Methyl-3-amino-2,3,6-trideoxy-α-D-arabino-hexopyranoside and Chiral Intermediates for the Synthesis of Thienamycin*. Stewart, A.O.; Williams, R.M., *Carbohydrate Res.*, **1984**, *135*, 167-173.

15. **Stereocontrolled Total Synthesis of (±)- and (+)-Bicyclomycin: New Carbon-Carbon Bond-Forming Reactions on Electrophilic Glycine Anhydride Derivatives*. Williams, R.M.; Armstrong, R.W.; Dung, J-S., *J. Am. Chem. Soc.*, **1984**, *106*, 5748-5750.

1985

16. **Synthesis and Antimicrobial Evaluation of Bicyclomycin Analogs*. Williams, R.M.; Armstrong, R.W.; Dung, J-S., *J. Med. Chem.*, **1985**, *28*, 733-740.

17. **Unusual Bridgehead Hydroxylations via Selenoxides: Evidence for Bridgehead Carbocations*. Williams, R.M.; Dung, J-S., *Tetrahedron Lett.*, **1985**, *26*, 37-38.

18. **A Divergent Generalized Synthesis of Unsymmetrically Substituted 2,5-Piperazinediones*. Williams, R.M.; Armstrong, R.W.; Maruyama, L.K.; Dung, J-S.; Anderson, O.P., *J. Am. Chem. Soc.*, **1985**, *107*, 3246-3253.

19. **Stereocontrolled Total Synthesis of (±)- and (+)-Bicyclomycin.* Williams, R.M.; Armstrong, R.W.; Dung, J-S., *J. Am. Chem. Soc.*, **1985**, *107*, 3253-3266.

20. **C-Glycosidation of Pyridyl Thioglycosides.* Stewart, A.O.; Williams, R.M., *J. Am. Chem. Soc.,* **1985**, *107*, 4289-4296.

21. **Thiolate Additions to Bicyclomycin and Analogues: A Structurally Novel Latent Michael-Acceptor System*. Williams, R.M.; Tomizawa, K.; Armstrong, R.W.; Dung, J-S., *J. Am. Chem. Soc.,* **1985**, *107*, 6419-6421.

1986

22. **Electrophilic Glycinates: New and Versatile Templates for Asymmetric Amino Acid Synthesis*. Sinclair, P.J.; Zhai, D.; Reibenspies, J.; Williams, R.M., *J. Am. Chem. Soc.,* **1986**, *108*, 1103-1104.

23. **Promising Cyclization Reactions to Construct the Ring Systems of Brevianamides A,B*. Williams, R.M.; Glinka, T., *Tetrahedron Lett.*, **1986**, *27*, 3581-3584.

24. **Asymmetric Synthesis of (R)- and (S)-[2-$^2H_1$]-Glycine.* Williams, R.M.; Zhai, D.; Sinclair, P.J., *J. Org. Chem.*, **1986**, *51*, 5021-5022.

25. **Synthesis of a Bicyclo[4.1.1] β-Lactam: A Novel, Anti-Bredt β-Lactam.* Williams, R.M.; Lee, B., *J. Am. Chem. Soc.,* **1986**, *108*, 6431-6433.

1987

26. **A New Synthetic Approach to 1-Hydroxymethyl-8-methoxy-1,2,3,4-tetrahydro-isoquinolin-4-one*. Williams, R.M.; Zhai, W.; Ehrlich, P.P.; Hendrix, J., *J. Org. Chem.*, **1987**, *52*, 2615-2617.

27. **Mechanism, Biological Relevance and Structural Requirements for Thiolate Additions to Bicyclomycin and Analogues: A Unique Latent Michael Acceptor System*. Williams, R.M.; Tomizawa, K.; Armstrong, R.W.; Dung, J-S., *J. Am. Chem. Soc.,* **1987**, *109*, 4028-4035.

28. **Synthesis of Functionalized Bicyclic Dioxopiperazines via Intramolecular Epoxide Opening*. Williams, R.M.; Maruyama, L.K., *J. Org. Chem.,* **1987**, *52*, 4044-4047.

1988

29. **Alkynylation of Mixed Haloacetals with Organotin Acetylides*. Zhai, D.; Zhai, W.; Williams, R.M., *J. Am. Chem. Soc.,* **1988**, *110*, 2501-2505.

30. **Practical Asymmetric Synthesis of α-Amino Acids through Carbon-Carbon Bond Constructions on Electrophilic Glycine Templates*. Williams, R.M.; Sinclair, P.J.; Zhai, D.; Chen, D., *J. Am. Chem. Soc.,* **1988**, *110*, 1547-1557.

31. **Bicyclomycin: Synthetic, Mechanistic and Biological Studies*. Williams, R.M.; Durham, C.A., *Chemical Reviews*, **1988**, *88*, 511-540.

32. **Asymmetric Synthesis of β-Carboxy Aspartic Acid (Asa).* Williams, R.M.; Sinclair, P.J.; Zhai, W., *J. Am. Chem. Soc.,* **1988**, *110*, 482-483.

33. **Synthesis of Verruculotoxin*. Williams, R.M.; Brunner, E.J.; Sabol, M.R., *Synthesis,* **1988**, 963-966.

34. **Versatile New Approach to the Synthesis of Monosubstituted and Bicyclic Piperazine-2,5-diones: Unusual* in situ *Generation and Enolate Addition to a Cumulene*. Williams, R.M.; Kwast, A., *J. Org. Chem.,* **1988**, *53*, 5785-5787.

35. **Asymmetric Synthesis of α-Amino Acids: Comparison of Enolate vs Cation Functionalization of N-BOC-5,6-Diphenyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-ones*. Williams, R.M.; Im, M-N., *Tetrahedron Lett.,* **1988**, *29*, 6075-6078.

36. **Versatile, stereocontrolled, Asymmetric Synthesis of E-Vinylglycine Derivatives*. Williams, R.M.; Zhai, W., *Tetrahedron,* **1988**, *44*, 5425-5430.

37. **Facial Selectivity of the Intramolecular S_N2' Cyclization: Stereocontrolled Total Synthesis of Brevianamide B*. Williams, R.M.; Glinka, T.; Kwast, E., *J. Am. Chem. Soc.,* **1988**, *110*, 5927-5929.

38. **Synthesis and X-Ray Crystal Structure Determination of 1,3-Bridged β-Lactams: Novel, Anti-Bredt β-Lactams*. Williams, R.M.; Lee, B-H.; Miller, M.M.; Anderson, O.P., in *Recent Advances in the Chemistry of β-Lactam Antibiotics,* Royal Society of Chemistry, London **1988** pp. 106-118.

1989

39. **Synthesis and X-Ray Crystal Structure Determination of 1,3-Bridged β-Lactams: Novel, Anti-Bredt β-Lactams*. Williams, R.M.; Lee, B.H.; Miller, S.; Anderson, O.P., *J. Am. Chem. Soc.,* **1989**, *111*, 1073-1081.

40. **Carbanion-Mediated Oxidative Deprotection of Non-Enolizable Benzylated Amides*. Williams, R.M.; Kwast, E., *Tetrahedron Lett.,* **1989**, *30*, 451-454.

41. **Remarkable, Enantio-Divergent Biogenesis of Brevianamide A and B.* Williams, R.M.; Kwast, E.; Coffman, H.; Glinka, T., *J. Am. Chem. Soc.,* **1989**, *111*, 3064-3065.

42. **Synthetic studies on FR900482: Promising Method to Construct the Bicyclic Hydroxylamine Hemi-Ketal Ring System*. Yasuda, N.; Williams, R.M., *Tetrahedron Lett.,* **1989**, *30*, 3397-3400.

43. **Synthetic Studies on Paraherquamide: Regioselectivity of Indole Oxidation.* Williams, R.M.; Glinka, T.; Kwast, E., *Tetrahedron Lett*., **1989**, *30*, 5575-5578.

44. **Synthesis of Optically Active α-Amino Acids*, Williams, R.M., Pergamon Press, **1989**, Oxford, (Organic Chemistry Series, Baldwin, J.E., Series Editor). 410 pp.

1990

45. **Synthesis of Ethynylglycine (FR900130).* Williams, R.M.;Aldous, D.J.; Aldous, S.C., *J. Chem. Soc. Perkin Trans I Comm.,* **1990**, 171-172.

46. **Asymmetric Synthesis of Arylglycines*. Williams, R.M.; Hendrix, J.A., *J. Org. Chem.,* **1990**, *55*, 3723-3728.

47. **Asymmetric, Stereocontrolled Total Synthesis of (-)-Brevianamide B*. Williams, R.M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J.K., *J. Am. Chem. Soc.,* **1990**, *112*, 808-821.

48. **General Synthesis of β,γ-Alkynylglycine Derivatives.* Williams, R.M.; Aldous, D.J.; Aldous, S.C., *J. Org. Chem.,* **1990**, *55*, 4657-4663.

49. **Synthetic Studies on Aspirochlorine (A30641).* Williams, R.M.; Miknis, G.F., *Tetrahedron Lett.,* **1990**, *31*, 4297-4300.

50. **Synthetic Studies on Paraherquamide: Synthesis of the 2H-1,5-Benzodioxepin Ring System*. Williams, R.M.; Cushing, T.D., *Tetrahedron Lett.,* **1990**, *31*, 6325-6328.

51. **Identification of a Novel Structural Class of Positive Modulators of the N-Methyl-D-Aspartate Receptor, Whose Actions are Mediated Through the Glycine Recognition Site*. Monahan, J.B.; Hood, W.F.; Compton, R.P.; Cordi, A.A.; Williams, R.M., *Eur. J. Pharmacol.,* **1990**, *189*, 373-379.

1991

52. ∫*Highly Stereoselective Synthesis of Conjugated E,E- and E,Z-Dienes, E-Enynes and E-1,2,3-Butatrienes via Alkenylborane Derivatives*. Negishi, E.; Yoshida, T.; Abramovitch, A.; Lew, G.; Williams, R.M., *Tetrahedron,* **1991**, *47*, 343-356.

53. **Synthesis, Conformation, Crystal Structures and DNA Cleavage Abilities of Tetracyclic Analogs of Quinocarcin*. Williams, R.M.; Glinka, T.; Gallegos, R.; Ehrlich, P.; Flanagan, M.E.; Coffman, H.; Park, G., *Tetrahedron (Symposium-in-Print),* **1991**, *47*, 2629-2642.

54. **Synthesis of Tri-Substituted Furans: Mild Ti(IV)-Mediated Couplings to Acetals. Construction of the Epoxy Hemi-Amido Ketal of Fusarin C*. Williams, R.M.; Esslinger, C.S., *Tetrahedron Lett.,* **1991**, *32*, 3635-3638.

55. **Novel Ring Contractions via 2,3-Wittig Rearrangements: Synthesis of (2-Desoxy)-2-Methylene Bicyclomycin*. Williams, R.M.; Sabol, M.R.; Kim, H.; Kwast, A., *J. Am. Chem. Soc.,* **1991**, *113*, 6621-6633.

56. **Asymmetric Synthesis of 2,6-Diamino-6-hydroxymethylpimelic Acid: Assignment of Stereochemistry*. Williams, R.M.; Im, M-N.; Cao, J., *J. Am. Chem. Soc.,* **1991**, *113*, 6976-6981.

57. **Asymmetric Synthesis of 1-Aminocyclopropane Carboxylic Acid Derivatives*. Williams, R.M.; Fegley, G.J., *J. Am. Chem. Soc.,* **1991**, *113*, 8796-8806.

58. *Asymmetric Synthesis of Mono-Substituted and α,α-Disubstituted α-Amino Acids via Diastereoselective Glycine Enolate Alkylations*. Williams, R.M.; Im, M-N., *J. Am. Chem. Soc.,* **1991**, *113*, 9276-9286.

59. *Cannizzaro-Based O_2-Dependent Cleavage of DNA by Quinocarcin.* Williams, R.M.; Glinka, T.; Flanagan, M.E.; Gallegos, R.; Coffman, H.; Pei, D., *Advances in New Drug Development*, The Pharmaceutical Society of Korea, Seoul **1991**, 38-50.

1992

60. *Cannizzaro-Based O_2-Dependent Cleavage of DNA by Quinocarcin.* Williams, R.M.; Glinka, T.; Flanagan, M.E.; Gallegos, R.; Coffman, H.; Pei, D., *J. Am. Chem. Soc.,* **1992**, *114*, 733-740.

61. *Asymmetric Synthesis of α-Amino Acids*. Williams, R.M., *Aldrichimica Acta,* **1992**, *25*, 11-25.

62. *Preparation of 4-Methoxy-4'-nitrobiphenyl.* Stille, J.K.; Echavarren, A.M.; Williams, R.M.; Hendrix, J.A., *Org. Synth.,* **1992**, *71*, 97-106.

63. *Asymmetric Synthesis of Arylglycines.* Williams, R.M.; Hendrix, J.A., *Chem. Rev.,* **1992**, *92*, 889-917.

64. *DNA Cross-Linking Studies on FR-900482: Observations on the Mode of Activation*. Williams, R.M.; Rajski, S.R., *Tetrahedron Lett.,* **1992**, *33*, 2929-2932.

65. *Asymmetric Synthesis of 2,6-Diaminopimelic Acids.* Williams, R.M.; Yuan, C., *J. Org. Chem.,* **1992**, *57*, 6519-6527.

66. *Asymmetric Synthesis of S-(-)-Cucurbitine*. Williams, R.M.; Fegley, G.J., *Tetrahedron Lett.,* **1992**, *33*, 6755-6758.

67. *Asymmetric [1,3]-Dipolar Cycloaddition Reactions: Synthesis of Highly Substituted Proline Derivatives*. Williams, R.M.; Zhai, W.; Aldous, D.J.; Aldous, S.C., *J. Org. Chem.*, **1992**, *57*, 6527-6532.

68. *Design and Synthesis of Biologically Active Peptide Mimics*. Williams, R.M., *The Development and Utilization of Biologically Active Peptides*, Technomic Publishing Co., Lancaster, PA **1992**, p. 187-215.

69. *Design of Bioactive Peptides Based on Immunoglobulin Structure*. Greene, M.I.; Kieber-Emmons, T.; Weiner, D.; Williams, R.M.; Williams, W.V., CA 2091258 A1, April 2, **1992**.

70. *Design of Bioactive Peptides Based on Immunoglobulin Structure.* Greene, M.I.; Kieber-Emmons, T.; Weiner, D.; Williams, R.M.; Williams, W.V., WO 1992004914 A1, April 2, **1992**.

1993

71. *Total Synthesis of Aspirochlorine*. Williams, R.M.; Miknis, G.F., *J. Am. Chem. Soc.,* **1993**, *115*, 536-547.

72. *Biosynthesis of the Brevianamides: Quest for a Biosynthetic Diels-Alder Cyclization*. Sanz-Cervera, J.-F.; Glinka, T.; Williams, R.M., *J. Am. Chem. Soc.,* **1993**, *115*, 347-348.

73. *Biosynthesis of Brevianamides A and B: In Search of the Biosynthetic Diels-Alder Construction*. Sanz-Cervera, J.F.; Glinka, T.; Williams, R.M., *Tetrahedron,* **1993**, *49*, 8471-8482.

74. *Determination of DNA Cross-Linking Sequence Specificity of FR66979: Observations on the Mode of Action of the FR900482 Class of Anti-tumor Compounds*. Williams, R.M.; Rajski, S.R., *Tetrahedron Lett.,* **1993**, *44*, 7023-7026.

75. *Stereocontrolled Total Synthesis of (+)-Paraherquamide B*. Cushing, T.D.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.,* **1993**, *115*, 9323-9324.

76. *Asymmetric Synthesis of 1S,2R-(+)-2-Phenyl-1-Aminocyclopropane-1-Carboxylic Acid*. Williams, R.M.; Fegley, G.J., *J. Org. Chem.,* **1993**, *58*, 6933-6935.

77. **Stereoselective Synthetic and Mechanistic Chemistry of Bicyclomycin*. Williams, R.M., *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Editor, Elsevier Pub., **1993**, Amsterdam, Vol. 12.

78. **Vinyl Glycine Derivatives for Memory and Learning Enhancement or Treatment of a Cognitive Disorder*. Cordi, A.A.; Monahan, J.B.; Williams, R.M., U.S. Patent No. 5,208,260, May 4, **1993**.

1994

79. **O_2-Dependent Cleavage of DNA by Tetrazomine.* Williams, R.M.; Flanagan, M.E.; Tippie, T., *Biochemistry,* **1994**, *33*, 4086-4092.

80. **Asymmetric Synthesis of γ-D(L)-Glutamyl-L-meso-Diaminopimelic Acid Dipeptide.* Williams, R.M.; Yuan, C., *J. Org. Chem.,* **1994**, *59*, 6190-6193.

81. **A New Method for Hydroxymethylene Peptide Isostere Synthesis: Asymmetric Synthesis of Statine*. Williams, R.M.; Colson, P-J.; Zhai, W., *Tetrahedron Lett.,* **1994**, *35*, 9371-9374.

82. **FR66979 Requires Reductive Activation to Cross-Link DNA Efficiently.* Huang, H.; Rajski, S.R.; Williams, R.M.; Hopkins, P.B., *Tetrahedron Lett.,* **1994**, *35*, 9669-9672.

1995

83. **Netropsin and Spermine Conjugates of a Water-Soluble Quinocarcin Analog: Analysis of Sequence-Specific DNA Interactions*. Flanagan, M.E.; Rollins, S.B.; Williams, R.M., *Chem. Biol.,* **1995**, *2*, 147-156.

84. **Asymmetric Synthesis of α-Amino Acids*. Williams, R.M., *Advances in Asymmetric Synthesis*, JAI Press **1995**, Volume 1 (pp 45-94) A. Hassner, Ed.

85. **Studies on the Biosynthesis of Taxol: Total Synthesis of Taxa-4(20),11(12)-diene and Taxa-4(5),11(12)-diene. The First Committed Biosynthetic Intermediate*. Rubenstein, S.M.; Williams, R.M., *J. Org. Chem.,* **1995**, *60*, 7215-7223.

86. **Synthetic Studies on Quinocarcin: Total Synthesis of (+)-Quinocarcinamide via Dipole Cycloaddition of an Azomethine Ylide Generated by Novel NBS Oxidation*. Flanagan, M.E.; Williams, R.M., *J. Org. Chem.,* **1995**, *60*, 6791-6797.

87. **Amino Acid Synthesis,* Williams, R.M., *Molecular Biology and Biotechnology. A Comprehensive Desk Reference.* VCH, Weinheim, Meyers, R.A., Ed., **1995**, Volume 1, pp 28-33.

88. *Development of GM-CSF Antagonist Peptides.* VonFeldt, J.M.; Monfardini, C.; Fish, S.; Rosenblum, H.; Kieber-Emmons, T.; Williams, R.M.; Kahn, S.A.; Weiner, D.B.; Williams, W.V., *Pept. Res.,* **1995**, *8*, 20.

1996

89. **Amino Acid Synthesis*. Williams, R.M., *The Encyclopedia of Molecular Biology and Molecular Medicine,* VCH, Weinheim, R.A. Meyers, Ed., **1996**, Volume 1, pp 52-58.

90. **Stereocontrolled Total Synthesis of (+)-Paraherquamide B*. Cushing, T.D.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.,* **1996**, *118*, 557-579.

91. **Asymmetric Synthesis of (2S,3S,6S)-, (2S,3S,6R)-, and (2R,3R,6S)-2,3-Methano-2,6-diaminopimelic Acids. Studies Directed to the Design of Novel Substrate-based Inhibitors of L,L-Diaminopimelate Epimerase*. Williams, R.M.; Fegley, G.J.; Pruess, D.L.; Schaeffer, F., *Tetrahedron,* **1996**, *52*, 1149-1164.

92. **Photochemical Cycloaromatization Reactions of ortho-Dialkynylarenes. A New Class of DNA Photocleaving Agents*. Funk, R.L.; Young, E.R.R.; Williams, R.M.; Flanagan, M.A.; Cecil, T.R., *J. Am. Chem. Soc.,* **1996**, *118*, 3291-3292.

93. **An Efficient Method for the Preparation of Amidinoureas*. Williams, R.M.; Yuan, C., *Tetrahedron Lett.,* **1996**, *37*, 1945-1948.

94. **Studies on the Biosynthesis of Paraherquamide: Origin of the β-Methylproline Ring*. Stocking, E.; Sanz-Cervera, J.F.; Williams, R.M.; Unkefer, C.J., *J. Am. Chem. Soc.*, **1996**, *118*, 7008-7009.
Errata: *J. Am. Chem. Soc.,* ***1997****, 119, 9588.*

95. ** Studies on the Total Synthesis of Paraherquamide A. Stereocontrolled, Asymmetric Synthesis of α-Alkyl-β-Hydroxyproline Derivatives*. Williams, R.M.; Cao, J., *Tetrahedron Lett.,* **1996**, *37*, 5441-5444.

96. **Cytochrome P-450-Catalyzed Hydroxylation of Taxa-4(5),11(12)-diene to Taxa-4(20),11(12)-diene-5-α-ol: The First Oxygenation Step in Taxol Biosynthesis*. Hefner, J.; Rubenstein, S.M.; Ketchum, R.E.B.; Gibson, D.M.; Williams, R.M., Croteau, R. *Chem. Biol.,* **1996**, *3*, 479-489.

1997

97. **FR900482, a Close Cousin of Mitomycin C that Exploits Mitosene-Based Cross Linking*. Williams, R.M.; Rajski, S.R.; Rollins, S.B., *Chem. Biol.,* **1997**, *4*, 127-137.

98. **Biosynthesis of the Brevianamides: A Theoretical Study of the Biosynthetic Diels-Alder Cyclization*. Domingo, L.R.; Sanz-Cervera, J.F.; Williams, R.M.; Picher, M.T.; Marco, J.A., *J. Org. Chem.,* **1997**, *62*, 1662-1667.

99. **Synthetic Studies on FR900482. Synthesis of a Photo-triggered Pro-Mitosene.* Rollins, S.B.; Williams, R.M., *Tetrahedron Lett.*, **1997**, *38*, 4033-4036.

100. **An Efficient Synthesis of (S)-meta-Tyrosine.* Bender, D.M.; Williams, R.M., *J. Org. Chem.,* **1997**, *62*, 6690-6691.

101. **Total Synthesis of the Anti-MRSA Peptide Antibiotics TAN-1057A-D.* Yuan, C.C.; Williams, R.M., *J. Am. Chem. Soc.*, **1997**, *119*, 11,777-11,784.

102. *Biologically Active Compounds and Methods of Constructing and Using the Same*. Greene, M.I.; Williams, W.V.; Weiner, D.B.; Cohen, J.A.; Kieber-Emmons, T.; Williams, R.M.; U.S. Patent No. 5,637,677, June 11, **1997**.

1998

103. **Biomimetic Diels-Alder Cyclizations for the Construction of the Brevianamide, Paraherquamide and VM55599 Ring Systems.* Williams, R.M.; Sanz-Cervera, J.F.; Sancenon, F., Marco, J.A.; Halligan, K.; *J. Am. Chem. Soc.*, **1998**, *120,* 1090-1091.

104. **Synthesis and Antimicrobial Evaluation of TAN-1057A/B Analogs.* Williams, R.M.; Yuan, C.; Lee, V.J.; Chamberland, S., *J. Antibiot.*, **1998**, *51*, 189-201.

105. **FR-66979 Covalently Cross-Links the Binding Domain (BD) of the High Mobility Group I/Y(HMG I/Y) Proteins to DNA.* Rajski, S.R., Rollins, S.B.; Williams, R.M., *J. Am. Chem. Soc.*, **1998**, *120*, 2192-2193.

106. **Asymmetric Synthesis of Differentially Protected 2,7-Diaminosuberic Acid, A Ring-Closure Metathesis Approach.* Williams, R.M.; Liu, J., *J. Org. Chem.*, **1998**, *63*, 2130-2132.

107. **General Asymmetric Synthesis of Hydroxymethylene and Hydroxyethylene Peptide Isosteres.* Williams, R.M.; Aoyagi, Y., *Tetrahedron (Symposium-in-Print),* **1998**, *54*, 10419-10433.

108. **Synthetic Studies on Tetrazomine: Assignment of Stereochemistry of the β-Hydroxypipecolic Acid.* Scott, J.D.; Tippie, T.N.; Williams, R.M., *Tetrahedron Lett.* **1998**, *39*, 3659-3662.

109. **Efficient Asymmetric Synthesis of (S)-2-Methylasparagine*. Aoyagi, Y.; Williams, R.M., *Syn. Lett.* **1998**, 1099-1101.

110. **Biomimetic Diels-Alder Cyclizations for the Construction of the Brevianamide, Paraherquamide, Sclerotamide, Asperparaline and VM55599 Ring Systems*. Williams, R.M.; Sanz-Cervera, J.F.; Sancenón, F.; Marco, J.A.; Halligan, K., *Bioorg. & Med. Chem.* **1998**, *6*, 1233-1241.

111. **DNA Interstrand Cross-Link Formation Induced by Bioxalomycin α_2.* Williams, R.M.; Herberich, B., *J. Am. Chem. Soc.*, **1998**, *120*, 10272-10273.

112. **Asymmetric Synthesis of 1-BOC-3- and 4-Hydroxypyrrolidines.* Aoyagi, Y.; Williams, R.M., *Tetrahedron*, **1998**, *54*, 13045-13058.

113. **DNA Interstrand Cross-Linking Agents as Antitumor Drugs*. Rajski, S.R.; Williams, R.M., *Chem. Rev.* **1998**, *98*, 2723-2796.

1999

114. **Asymmetric Syntheses of Unnatural Amino Acids and Hydroxyethylene Peptide Isosteres.* Williams, R.M., Humana Press, *Peptidomimetics Protocols,* Kazmierski, W., Ed., Totowa, **1999,** Vol. 23**,** Chapter 19, pp. 339-356.

115. **Reverse versus Normal Prenyl Transferases in Paraherquamide Biosynthesis Exhibit Distinct Facial Selectivities.* Stocking, E.M.; Sanz-Cervera, J.F.; Williams, R.M., *Angew. Chem. Int. Ed.* **1999**, *38*, 786-789.

116. **DNA Cross-Linking by a Phototriggered Dehydromonocrotaline Progenitor.* Williams, R.M.; Tepe, J., *J. Am. Chem. Soc.,* **1999**, *121*, 2951-2955.

117. **Synthetic Studies on Asperparaline A. Synthesis of the Spriosuccinimide Ring System.* Gonzalez, F.; Williams, R.M., *Tetrahedron Lett.* **1999**, *40*, 4519-4522.

118. **Reductive Activation of a Hydroxylamine Hemi-acetal Derivative of Dehydromonocrotaline. The First Reductively Activated Pyrrolizidine Alkaloid Capable of Cross-linking DNA.* Tepe, J.J.; Williams, R.M., *Angew. Chemie Int. Ed Engl.* **1999**, *38*, 3501-3503.

2000

119. **Synthesis of the First Photo-triggered Pro-Mitosene Based on FR-900482.* Williams, R.M.; Rollins, S.B.; Judd, T.C., *Tetrahedron*, **2000**, *56*, 521-532.

120. **Total Synthesis of VM55599. Utilization of an Intramolecular Diels-Alder Cycloaddition of Potential Biogenetic Relevance.* Stocking, E.M.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.* **2000**, *122*, 1675-1683.

121. **Synthesis of Stable and Radioisotopomers of Taxa-4(5),11(12)-diene, Taxa-4(20),11(12)-diene and Taxa-4(20),11(12)-dien-5-α-ol. Early Intermediates in Taxol Biosynthesis.* Rubenstein, S. M.; Vazquez, A.; Williams, R.M., *J. Labeled Cmpd & Radiopharm.* **2000**, *43*, 481-491.

122. **Synthesis of a Netropsin Conjugate of a Water-Soluble Epi-quinocarcin Analog: The Importance of Stereochemistry at Nitrogen.* Herberich, B.; Scott, J.D.; Williams, R.M., *Bioorg. Med. Chem.*, **2000**, *8,* 523-532.

123. **Biosynthesis of Prenylated Alkaloids Derived from Tryptophan.* Williams, R.M.; Sanz-Cervera, J.F.; Stocking, E., in *Topics in Current Chemistry*, Volume on *Biosynthesis-Terpenes and Alkaloids*, Leeper, F.; Vederas, J.C., Eds., Springer-Verlag, Berlin, **2000**, *209*, 97-173.

124. **The Asymmetric Total Synthesis of (+)- and (-)-Spirotryprostatin B.* Sebahar, P.; Williams, R.M., *J. Am. Chem. Soc.* **2000**, *122*, 5666-5667.

125. **Observations on the Covalent Cross-Linking of the Binding Domain (BD) of the High Mobility Group I/Y (HMG I/Y) Proteins to DNA by FR66979.* Rajski, S.R.; Williams, R.M., *Bioorg. Med. Chem.* **2000**, *8*, 1331-1342.

126. **Asymmetric, Stereocontrolled Total Synthesis of Paraherquamide A*. Williams, R.M.; Cao, J.; Tsujishima. H., *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2540-2544.

127. **A Synthesis of 3´,5´-Dideoxy-5´-Aminouridine.* Bender, D.M.; Hennings, D.D.; Williams, R.M., *Synthesis* **2000**, 399-402.

128. **Synthesis and Evaluation of Microtubule Assembly Inhibition and Cytotoxicity of Prenylated Derivatives of cyclo-L-Trp-L-Pro.* Sanz-Cervera, J.F.; Stocking, E.M.; Usui, T.; Osada, H.; Williams, R.M., *Bioorg. Med. Chem.* **2000**, *8*, 2407-2415.

129. **Reverse Prenyl Transferases Exhibit Poor Facial Discrimination in the Biosyntheses of Paraherquamide, Brevianamide and Austamide.* Stocking E.M.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.* **2000**, *122*, 9089-9098.

130. **Synthesis of (S,S)- and (R,R)- 2-Amino-3-methylaminobutanoic Acid (AMBA).* Hennings, D.D.; Williams, R.M., *Synthesis* **2000**, 1310-1314.

131. **A Synthetic Model for the [4+2] Cycloaddition in the Biosynthesis of the Brevianamides, Paraherquamides, and Related Compounds.* Sanz-Cervera, J.F.; Williams, R.M.; Marco, J.A.; López-Sánchez, J.M.; González, F.; Martínez, M.E.; Sancenón, F., *Tetrahedron* **2000**, *56*, 6345-6358.

132. **FR900482 Class of Anti-tumor Drugs Cross-links Oncoprotein HMG I/Y to DNA in Vivo.* Beckerbauer, L.; Tepe, J.; Cullison J.; Reeves, R.; Williams, R.M., *Chem. Biol.* **2000**, *7*, 805-812.

133. **Synthetic Studies on Tetrazomine: Lipase PS Resolution of Racemic cis-β-Hydroxypipecolic Acid.* Scott, J.D.; Williams, R.M., *Tetrahedron Lett.* **2000**, *41*, 8413-8416.

134. **Intramolecular Proton Transfer in the Cyclization of Geranylgeranyl Diphosphate to the Taxadiene Precursor of Taxol Catalyzed by Recombinant Taxadiene Synthase.* Williams, D.C.; Caroll, B.J.; Jin, Q.; Rithner, C.D.; Lenger, S.R.; Floss, H.G.; Coates, R.A.; Williams, R.M.; Croteau, R., *Chem. Biol.* **2000**, *7*, 969-977.

135. **Studies on the Biosynthesis of Taxol. Synthesis of Taxa-4(20), 11(12)-dien-2α, 5α-diol.* Vazquez, A.; Williams, R.M., *J. Org. Chem.* **2000**, *65*, 7865-7869.

136. **Lipase TL®-Mediated Kinetic Resolution of Benzoin: Facile Synthesis of (1R, 2S)-erythro-2-Amino-1,2-diphenylethanol.* Aoyagi, Y.; Agata, N.; Shibata, N.; Horiguchi, M.; Williams, R.M., *Tetrahedron Lett.* **2000**, *41*, 10159-10162.

137. **Antibiotic for Methicillin Resistant Bacteria*. Williams, R.M.; Yuan, C., U.S. Patent No. 6,110,925, August 29, **2000**.

138. **Antibiotic for Methicillin Resistant Bacteria*. Williams, R.M.; Yuan, C., U.S. Patent No. 6,162,925, December 19, **2000**.

2001

139. **An Efficient Asymmetric Synthesis of (2S, 3S)-β-Hydroxyornithine.* DeMong, D.E.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42,* 183-185.

140. **Construction of the A-ring of Cylindrospermopsin via an Intramolecular Oxazinone-N-oxide Dipolar Cycloaddition.* Looper, R.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42,* 769-771.

141. **Sequential Staudinger/Pictet-Spengler Cyclization Reactions for the Construction of Densely Functionalized Tetrahydroisoquinolines.* Herberich, B.; Vazquez, A.; Kinugawa, M.; Jin, W.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42,* 543-546.

142. **Studies on the Biosynthesis of Paraherquamide. Synthesis and Incorporation of a Hexacyclic Indole Derivative as an Advanced Metabolite.* Stocking, E.M.; Sanz-Cervera, J.F.; Williams, R.M., *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 1296-1298.

143. **Studies on the Biosynthesis of Paraherquamide: Concerning the Mechanism of Oxidative Cyclization of L-Isoleucine to β-Methylproline.* Stocking, E.M.; Martinez, R.A.; Silks, L.A.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.* **2001**, *123*, 3391-3392.

144. **Molecular Cloning of a Cytochrome P450 Taxane 10-beta-Hydroxylase cDNA from* Taxus *and Functional Expression in Yeast.* Schoendorf, A.; C. Rithner, C.; Williams R.M.; Croteau, R., *Proc. Nat. Acad. Sci. USA* **2001**, 98, 1501-1506.

145. **Total Synthesis of (-)-Tetrazomine and Determination of Its Stereochemistry.* Scott, J.D.; Williams, R.M. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 1463-1465.

146. **Entry into the Bi-aryl Moiety of the TMC-95 Proteosome Inhibitors via the Stille Protocol.* Albrecht, B.K.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42*, 2755-2757.

147. **Stereocontrolled Asymmetric Synthesis of α-Hydroxy-β-Amino Acids. A Stereodivergent Approach.* Aoyagi, Y.; Jain, R.P.; Williams, R.M., *J. Am. Chem. Soc.* **2001**, *123*, 3472-3477.

148. **Asymmetric Synthesis of (S)-(+)-Carnitine and Analogs.* Jain, R.P.; Williams, R.M., *Tetrahedron (Symposium-in-Print),* **2001**, *57*, 6505-6509.

149. **The Asymmetric Synthesis of (2S,3R)-Capreomycidine.* DeMong, D.E.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42*, 3529-3532.

150. **Asymmetric Synthesis of (R)-(-)-Carnitine.* Jain, R.P.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42*, 4437-4440.

151. **Studies on the Biosynthesis of Paraherquamide: Construction of the Amino Acid Framework.* Stocking, E.M.; Sanz-Cervera, J.F.; Unkefer, C.J.; Williams, R.M., *Tetrahedron.* **2001**, *57*, 5303-5320.

152. **DNA Cross-linking by a Phototriggered Pyrrolic Progenitor Developed from Monocrotaline.* Kosogof, C.; Tepe, J.J.; Williams, R.M., *Tetrahedron Lett.* **2001**, *42*, 6641-6643.

153. **Taxol Biosynthesis: Differential Transformations of Taxadien-5α-ol and Its Acetate Ester by Cytochrome P 450 Hydroxylases from* Taxus *Suspension Cells.* Wheeler, A.L.; Long, R.M.; Ketchum, R.E.B.; Rithner, C.; Williams R.M.; Croteau, R., *Arch. Biochem. Biophys.* **2001**, *390*, 265-278.

154. **Asymmetric Synthesis of [2,3-$^{13}C_2$, ^{15}N]-4-Benzyloxy-5,6-diphenyl-2,3,5,6-tetrahydro-4H-oxazine-2-one via Lipase TL®-Mediated Kinetic Resolution of Benzoin: General Procedure for the Synthesis of [2,3-$^{13}C_2$, ^{15}N]-L-Alanine.* Aoyagi, Y.; Iijima, A.; Williams, R.M., *J. Org. Chem.* **2001**, *66*, 8010-8014.

155. **Asymmetric Synthesis of (+)-Hypusine.* Jain, R.P.; Albrecht, B.K.; DeMong, D.E.; Williams, R.M., *Org. Lett.* **2001**, *3*, 4287-4289.

156. **Taxol Biosynthesis: Taxane 13α-Hydroxylase is a Cytochrome P450-Dependent Monooxygenase.* Jennewein, S.; Rithner, C.D.; Williams, R.M.; Croteau, R., *Proc. Nat. Acad. Sci. USA* **2001**, *98*, 13595-13600.

2002

157. **Differential Effects of FR900482 and FK317 on Apoptosis, IL-2 Gene Expression and Induction of Vascular Leak Syndrome.* Beckerbauer, L.; Tepe, J.J.; Eastman, R.A.; Mixter, P.; Williams, R.M.; Reeves, R., *Chem. Biol.* **2002**, *9,* 427-441.

158. **Asymmetric Total Synthesis of (-)-VM55599: Establishment of the Absolute Stereochemistry and Biogenetic Implications.* Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.* **2002**, *124*, 2556-2559.

159. **Total Synthesis of (-)-Tetrazomine. Determination of the Stereochemistry of Tetrazomine and the Synthesis and Biological Activity of Tetrazomine Analogs.* Williams, R.M.; Scott, J.D., *J. Am. Chem. Soc.* **2002**, *124*, 2951-2956.

160. **Synthetic Studies Towards Paraherquamide F: Synthesis of the 1,7-Dihydropyrano[2,3-g]indole Ring System*. Cox, R.J.; Williams, R.M., *Tetrahedron Lett.* **2002**, *43*, 2149-2152.

161. **The First Asymmetric Synthesis of (2S)- and (2R)-Amino-3,3-Dimethoxypropanoic Acid.* DeMong, D.E.; Williams, R.M., *Tetrahedron Lett.* **2002**, *43*, 2355-2357.

162. **DNA Interstrand Cross-link Formation by Reductive Activation of Dehydropyrrolizidine Progenitors.* Tepe, J.J.; Kosogof, C.; Williams, R.M., *Tetrahedron* **2002**, *58*, 3553-3559.

163. **Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics.* Scott, J.D.; Williams, R.M., *Chem. Rev.* **2002**, *102*, 1669-1730.

164. **The Synthesis and Biosynthesis of the Paraherquamides: An Intriguing Story of the Biological Diels-Alder Construction.* Williams, R.M., *Chem. Pharm. Bull.* **2002**, *50*, 711-740 (invited review).

165. **Asymmetric, Stereocontrolled Total Synthesis of (+)- and (-)-Spirotryprostatin B via a Diastereoselective Azomethine Ylid [1,3]-Dipolar Cycloaddition Reaction.* Sebahar, P.R.; Osada, H.; Usui, T.; Williams, R.M., *Tetrahedron (Symposium-in-Print)* **2002**, *58*, 6311-6322.

166. **Studies on Taxol Biosynthesis. Preparation of Taxa-4(20), 11(12)-dien-5α-acetoxy-10β-ol by Deoxygenation of a Taxadiene Tetra-acetate Obtained from Japanese Yew*. Horiguchi, T.; Rithner, C.D.; Croteau, R.; Williams, R.M., *J. Org. Chem.* **2002**, *67*, 4901-4903.

167. **Asymmetric Synthesis of Spirooxindole and 3,4 Dehydro-Proline Derivatives via Asymmetric Azomethine Ylide [1,3]-Dipolar Cycloaddition Reactions.* Sebahar, P.; Williams, R.M., *Heterocycles* **2002**, *58*, 563-575.

168. **Asymmetric Synthesis of (+)-Negamycin.* Jain, R.P.; Williams, R.M., *J. Org. Chem.* **2002**, *67*, 6361-6365.

169. **Synthesis and DNA Cross-linking of a Phototriggered FR900482 Mitosene Progenitor.* Judd, T.; Williams, R.M., *Org. Lett.* **2002**, *4*, 3711-3714.

170. **Concise Enantioselective Synthesis of (+)-FR66979 and (+)-FR900482: DMDO-Mediated Construction of the Hydroxylamine Hemi-ketal.* Judd, T.; Williams, R.M., *Angew. Chem. Int. Ed.,* **2002**, *41*, 4683-4685.

171. *Biologically Active Compounds and Methods of Constructing and Using the Same*; Greene, Mark I.; Williams, William V.; Weiner, David B.; Cohen, Jeffrey A.; Kieber-Emmons, Thomas; Williams, Robert M.; U.S. Patent No. 6,372,884 B1, April 16, **2002**.

2003

172. **Studies on Taxol Biosynthesis. Preparation of 2α, 10β-dihydroxy Taxadien-5α-yl Acetate Derivatives by Deoxygenation of a Taxadiene Tetra-acetate Obtained from Japanese Yew*. Horiguchi, T.; Rithner, C.D.; Croteau, R.; Williams, R.M., *Tetrahedron* **2003**, *59*, 267-273.

173. **A Concise Formal Total Synthesis of TMC-95A/B Proteasome Inhibitors.* Albrecht, B.; Williams, R.M., *Org. Lett.* **2003**, *5*, 197-200.

174. **Paraherquamides, Brevianamides and Asperparaline: Laboratory Synthesis and Biosynthesis. An Interim Report.* Cox, R.J.; Williams, R.M., *Acc. Chem. Res.* **2003**, *36*, 127-139.

175. **Asymmetric Synthesis of N-tert-Butoxycarbonyl α-Amino Acids. Synthesis of (5S, 6R)- and (5R, 6S)-4-tert-Butoxycarbonyl-2,3-Diphenylmorpholin-6-one.* Williams, R.M.; Sinclair, P.J.; DeMong, D.; Chen, D.; Zhai, D., *Org. Synth.* **2003**, *80*, 18-30.

176. **Efficient Asymmetric Synthesis of N-tert-Butoxycarbonyl α-Amino Acids Using (tert-Butoxy)Carbonyl-2,3-Diphenylmorpholin-6-one. Preparation of (R)-Allylglycine.* Williams, R.M.; Sinclair, P.J.; DeMong, D.E., *Org. Synth.* **2003**, *80*, 31-37.

177. **Synthetic Studies on Ecteinascidin-743: Constructing a Versatile Pentacyclic Intermediate for the Synthesis of Ecteinascidins & Saframycins.* Jin, W.; Metobo, S.; Williams, R.M., *Org. Lett.* **2003**, *5*, 2095-2098.

178. **Synthetic Studies on Ecteinascidin 743: Asymmetric Synthesis of the Versatile Amino Acid Component.* Jin, W.; Williams, R.M., *Tetrahedron Lett.* **2003**, *44*, 4635-4639.

179. **Studies on the Biosynthesis of Paraherquamide A and VM99955. A Theoretical Study for the Intramolecular Diels-Alder Cycloaddition.* Domingo, L.R.; Zaragozá, R.J.; Williams, R.M., *J. Org. Chem.* **2003**, *68*, 2895-2902.

180. *Taxoid Metabolism: Taxoid 14β-Hydroxylase is a Cytochrome P450-Dependent Monooxygenase.* Jennewein, S.; Rithner, C.D.; Williams, R.M.; Croteau, R., *Arch. Biochem. Biophys.* **2003**, *413*, 262-270.

181. **Chemistry and Biology of Biosynthetic Diels-Alder Reactions.* Stocking, E., Williams, R.M., *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 3078-3115.

182. **Asymmetric Synthesis of (2S, 3R)-Capreomycidine and the Total Synthesis of Capreomycin IB*. 125, 8561, D.E.; Williams, R.M., *J. Am. Chem. Soc.* **2003**, *125*, 8561-8565.

183. **Taxus Metabolomics: Methyl Jasmonate Preferentially Induces Production of Taxoids Oxygenated at C-13 in Taxus x media Cell Cultures.* Ketchum, R.E.B.; Rithner, C.D.; Qiu, D.; You Sun Kim, Y.S.; Williams, R.M.; Croteau R.B., *Phytochem.* **2003**, *62*, 901-909.

184. **Lipase TL®-Mediated Kinetic Resolution of 5-Benzyloxy-1-tert-butyldimethylsilyloxy-2-pentanol in Low Temperature: Concise Asymmetric Synthesis of Both Enantiomers of Piperazic Acid Derivative.* Aoyagi, Y.; Saitoh, Y.; Ueno, T.; Horiguchi, M.; Takeya, K.; Williams, R.M., *J. Org. Chem.* **2003**, *68*, 6899-6904.

185. **Concise Asymmetric Total Synthesis of Spirotryprostatin A.* Onishi, T.; Sebahar, P.R.; Williams, R.M., *Org. Lett.* **2003**, *5*, 3135-3137.

186. **Asymmetric, Stereocontrolled Total Synthesis of Paraherquamide A.* Williams, R.M.; Cao, J.; Tsujishima, H., *J. Am. Chem. Soc.* **2003**, *125*, 12172-12178.

187. **Interstrand Cross-linking of DNA by FK-317 and Its Deacetylated Metabolites FR70496 and FR157471.* Ducept, P.; Williams, R.M., *Biochemistry* **2003**, *42*, 14696-14701.

188. **Studies on the Biosynthesis of Asperparaline A. Origin of the Spiro-succinimide Moiety.* Gray, C.; Sanz-Cervera, J.F.; Williams, R.M., *J. Am. Chem. Soc.* **2003**, *125*, 14692-14693.

189. *Design of Bioactive Peptides Based on Immunoglobulin Structure*. Weiner, David B.; Williams, Robert M.; Kieber-Emmons, Thomas; Williams, William V.; Greene, Mark I.; EP 1310557A1, May 14, **2003**.

2004

190. **Cytochrome P450 Taxadiene 5α-Hydroxylase, a Mechanistically Unusual Monooxygenase Catalyzing the First Oxygenation Step of Taxol Biosynthesis.* Jennewein, S.; Long, R.M.; Williams, R.M.; Croteau, R., *Chem. Biol.* **2004**, *11*, 379-387.

191. **A Concise Total Synthesis of (+)-FR900482 and (+)-FR66979.* Judd, T.C.; Williams, R.M., *J. Org. Chem.* **2004**, *69*, 2825-2830.

192. **Total Synthesis of the TMC-95A/B Proteasome Inhibitors.* Albrecht, B.; Williams, R.M. *Proc. Nat. Acad. Sci. USA* **2004**, *101*, 11949-11954.

193. **Concise Synthesis of the Core Bicyclo[2.2.2]diazaoctane Ring Common to Asperparaline, Paraherquamide and Stephacidin Alkaloids.* Adams, L.A.; Gray, C.R.; Williams, R.M. *Tetrahedron Lett.* **2004**, 45, 4489-4493.

194. **Concise, Asymmetric Total Synthesis of Spirotryprostatin A.* Onishi, T.; Sebahar, P.R.; Williams, R.M. *Tetrahedron* **2004**, *60*, 9503-9515.

195. **A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epi-cylindrospermopsin.* Looper, R.E.; Williams, R.M. *Angew. Chem. Int. Ed.* **2004**, *43*, 2930-2933.

196. **A Concise Asymmetric Synthesis of the ADE Fragment of Nakadomarin A.* Ahrendt, K.A.; Williams, R.M. *Org. Lett.* **2004**, *6*, 4539-4541.

2005

197. **An Improved Synthesis of Optically Pure 4-Boc-5,6-Diphenylmorpholin-2-one and 4-Cbz-5,6-Diphenylmorpholin-2-one.* Dastlik, K.A.; Sundermeier, U.; Johns, D.M.; Chen, Y.; Williams, R.M. *Syn. Lett.* **2005**, 693-696.

198. **Synthesis of the Putative Structure of 7-Deoxycylindrospermopsin: C7-Oxygenation is not Required for the Inhibition of Protein Synthesis.* Looper, R.E.; Runnegar, M.T.C.; Williams, R.M., *Angew. Chem. Int. Ed.* **2005**, *44*, 3879-3881.

199. **Asymmetric Total Synthesis of Jorumycin, Renieramycin G, 3-epi-Jorumycin and 3-epi-Renieramycin G*. Lane, J.W.; Chen, Y.; Williams, R.M., *J. Am. Chem. Soc.* **2005**, *127*, 12684-12690.

200. *Remarkable Diastereomeric Rearrangement of an α-Acyloxy β-ketosulifide to an α-Acyloxy Thioester: A Novel Approach to the Synthesis of Optically Active (2S,3S) β-Amino α-Hydroxy Acids.* Suzuki, T.; Honda, Y.; Izawa, K.; Williams, R.M. *J. Org. Chem.* **2005**, *70*, 7317-7323.

201. **Concise Syntheses of the 1,7-dihydropyrano[2,3-g]indole Ring System of the Stephacidins, Paraherquamides and Norgeamides.* Artman, G. D.; Grubbs, A.W.; Williams, R.M. *Tetrahedron Lett.* **2005**, *46*, 9013-9016.

2006

202. **Improved Synthesis by Mitsunobu Cyclization of the Core Benzazocine Ring Precursor to FR900482 and the Mitomycins.* Williams, R.M.; Ducept, P; Gubler, D.A., *Heterocycles*, **2006**, *67*, 597-619.

203. **A Concise Synthesis of Brevianamide B via a Biomimetically-inspired IMDA Construction.* Adams, L.A.; Valente, M.W.N.; Williams, R.M. *Tetrahedron Symposium-in-Print.* **2006**, *62*, 5195-5200.

204. **Syntheses of the Cylindrospermopsin Alkaloids.* Looper, R.E.; Runnegar, M.T.C.; Williams, R.M., *Tetrahedron* **2006**, *62*, 4549-4562.

205. **Antitumor Activity of Tetrahydroisoquinoline Analogues 3-epi-Jorumycin and 3-epi-Renieramycin G.* Lane, J.W.; Estevez, A.; Mortara, K.; Callan, O.; Spencer, J.R.; Williams, R.M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3180-3183.

206. **Synthetic Studies on ET743. Concise, Asymmetric, Stereocontrolled Construction of the Tetrahydroisoquinoline Core via Radical Cyclization on a Glyoxalimine.* Fishlock, D.; Williams, R.M. *Org. Lett.* **2006**, *8*, 3299-3301.

207. **Synthetic Studies on Quinine: Quinuclidine Construction via a Ketone Enolate-directed Regio- and Diastereoselective Allylic Alkylation.* Johns, D.M.; Mori, M.; Williams, R.M. *Org. Lett*. **2006**, *8*, 4051-4054.

208. **Synthesis of Highly Functionalized γ,δ-Unsaturated Amino Acids.* Chen, S.; Williams, R.M. *Tetrahedron* **2006**, *62*, 11572-11579.

209. **The Concise and Versatile Synthesis of epi-Malbrancheamide and Structurally Related Analogs.* Valente, M.W.N.; Williams, R.M. *Heterocycles* **2006**, *70*, 249-259.

2007

210. **Administering Cultured Taxus Cells with Early Precursors Reveals Bifurcations in the Taxoid Biosynthetic Pathway.* Ketchum, R.E.B.; Horiguchi, T.; Qiu, D.; Williams, R.M.; Croteau, R.B. *Phytochem.* **2007**, 68, 335-341.

211. **Asymmetric Total Synthesis of (-)-Cribrostatin 4 (Renieramycin H)*. Vincent, G.; Williams, R.M. *Angew. Chem. Int. Ed.* **2007**, *46*, 1517-1520.

212. **Coupling of Activated Esters to Gramines in the Presence of Ethyl Propiolate Under Mild Conditions.* Jones, D.T.; Artman III, G.D.; Williams, R.M. *Tetrahedron Lett.* **2007**, *48*, 1291-1294.

213. *Notoamides A-D: Prenylated Indole Alkaloids Isolated from a Marine-derived Fungus, Aspergillus* sp. Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R.M.; Tsukamoto, S. *Angew. Chem. Int. Ed.* **2007**, *46*, 2254-2256.

214. **A Concise Total Synthesis of the Notoamides C and D.* Grubbs, A.W.; Artman III, G.D.; Tsukamoto, S.; Williams, R.M. *Angew. Chem. Int. Ed.* **2007**, *46*, 2257-2261.

215. **A Concise, Biomimetic Total Synthesis of Stephacidin A and Notoamide B.* Greshock, T.J.; Grubbs, A.W.; Tsukamoto, S.; Williams, R.M. *Angew. Chem. Int. Ed.* **2007**, *46*, 2262-2265.

216. **Chiral Lactones*. Onishi, T.; Williams, R.M. U.S. Patent No. 7,173,141 B2, February 6, **2007**.

217. **Formation of the C3-C4 Unsaturated Framework of Cribrostatin 4 via DEAD-Mediated Oxidation of an Allylic Tertiary Amine.* Vincent, G.; Chen, Y.; Lane, J.W.; Williams, R.M. *Heterocycles* **2007**, *72*, 385-398.

218. **Concise, Asymmetric, Stereocontrolled Total Synthesis of Stephacidins A,B and Notoamide B.* Artman, G.D.; Grubbs, A.W.; Williams, R.M. *J. Am. Chem. Soc.* **2007**, *129*, 6336-6342.

219. **Regioselectivity of Pictet-Spengler Cyclization Reactions to Construct the Pentacyclic Frameworks of the Ecteinascidin-Saframycin Class of Tetrahydroisoquinoline Antitumor Antibiotics.* Vincent, G.; Lane, J.W.; Williams, R.M. *Tetrahedron Lett.* **2007**, *48*, 3719-3722.

220. **Effects of Photo-chemically Activated Alkylating Agents of the FR900482 Family on Chromatin*. Subramanian, V.; Ducept, P.; Williams, R.M.; Luger, K. *Chem. Biol.* **2007**, *14*, 553-563.

221. **Concise, Biomimetic Total Synthesis of Marcfortine C*. Greshock, T.J.; Grubbs, A.W.; Williams, R.M. *Tetrahedron* **2007**, *63*, 6124-6130.

222. **Improved Biomimetic Total Synthesis of d,l-Stephacidin A.* Greshock, T.J.; Williams, R.M. *Org. Lett.* **2007**, *9*, 4255-4258.

223. **Synthetic and Biosynthetic Studies on FR900482 and Mitomycin C. An Efficient and Stereoselective Hydroxymethylation of an Advanced Benzazocane Intermediate.* Namiki, H.; Chamberland, S.; Gubler, D.A.; Williams, R.M. *Org. Lett.* **2007**, *9*, 5341-5344.

224. **Preface to Heterocycles Issue Dedicated to Prof. Yoshito Kishi's 70th Birthday*. Williams, Robert M. *Heterocycles* **2007**, *72* 1-3.

2008

225. **Diphenyloxazinones: Versatile Templates for the Asymmetric Synthesis of Amino Acids, Peptide Isosteres and Natural Products.* Burnett, C.; Williams, R.M., *Strategies and Tactics in Organic Synthesis* **2008** Vol.7, Chapter 8, pp 268-327.

226. **Improved Total Synthesis of the Potent HDAC Inhibitor FK228 (FR-901228).* Johns, D.M.; Greshock, T.J.; Noguchi, Y.; Williams, R.M., *Org. Lett.* **2008**, *10*, 613-616.

227. **Rabe Rest in Peace: Confirmation of the Rabe-Kindler Conversion of d-Quinotoxine to Quinine. Experimental Affirmation of the Woodward-Doering Formal Total Synthesis of Quinine.* Smith, A.; Williams, R.M. *Angew. Chem. Int. Ed.* **2008**, *47*, 1736-1740.

228. **Isolation, Structure Elucidation, and Biomimetic Total Synthesis of Versicolamide B and the Isolation of Antipodal (-)-Stephacidin A and (+)-Notoamide B from Aspergillus versicolor.* Greshock, T.J.; Grubbs, A.W.; Jiao, P.; Wicklow, D.T.; Gloer, J.B.; Williams, R.M. *Angew. Chem. Int. Ed.* **2008**, *47*, 3573-3577.

229. **Biomimetic Total Synthesis of Malbrancheamide and Malbrancheamide B.* Miller, K.A.; Welch, T.R.; Greshock, T.J.; Ding, Y.; Sherman, D.H.; Williams, R.M. *J. Org. Chem.* **2008**, *73*, 3116-3119.

230. **Studies on Taxol Biosynthesis. Preparation of Taxadiene-diol- and triol- Derivatives by Deoxygenation of Taxusin.* Horiguchi, T.; Li, H.; Croteau, R.; Williams, R.M. *Tetrahedron* **2008**, *64*, 6561-6567.

231. *Molecular Analysis of A 4-Dimethylallyltryptophan Synthase from Malbranchea aurantiaca.* Ding, Y.; Williams, R.M.; Sherman, D.H., *J. Biol. Chem.* **2008**, *283*, 16068-16076.

232. **Synthetic Studies Towards Paraherquamides E & F and Related ^{13}C-labeled Putative Biosynthetic Intermediates: Stereocontrolled Synthesis of the α-Alkyl-β-Methylproline Ring System.* Sommer, K.; Williams, R.M., *Tetrahedron* **2008**, *64*, 7106-7111.

233. **Synthetic Studies on Et-743. Assembly of the Pentacycle and a Formal Total Synthesis of Et-743.* Fishlock, D.; Williams, R.M. *J. Org. Chem.* **2008**, *73*, 9594-9600.

234. **Total Synthesis and Biological Mode of Action of Largazole: A Potent Class I Histone Deacetylase Inhibitor.* Bowers, A.; West, N.; Taunton, J.; Schreiber, S.L.; Bradner, J.E.; Williams, R.M. *J. Am. Chem. Soc.* **2008**, *130*, 11219-11222.

235. **Studies on Taxol Biosynthesis. Preparation and Tritium Labeling of Biosynthetic Intermediates by Deoxygenation of a Taxadiene Tetra-acetate Obtained from Japanese Yew*. Tohru Horiguchi, T.; Rithner, C.D.; Croteau, R.; Williams, R.M., *J. Labeled Cmpd. & Radiopharm.* **2008**, *51*, 325-328.

236. **Detection of VM55599 and Pre-paraherquamide from Aspergillus Japonicus and Penicillium fellutanum: Biosynthetic Implications.* Ding, Y.; Gruschow, S.; Greshock, T.J.; Finefield, J.; Sherman, D.H.; Williams, R.M., *J. Nat. Prod.* **2008**, *71*, 1574-1578.

237. **Studies on Paraherquamide Biosynthesis: Synthesis of Deuterium-Labeled 7-Hydroxy-Pre-Paraherquamide, a Putative Precursor of Paraherquamides A, E & F.* Sommer, K.; Williams, R.M. *Tetrahedron* **2009**, *65*, 3246-3260. (published on-line September 11, 2008)

238. **Pre-malbrancheamide: Synthesis, Isotopic Labeling, Biosynthetic Incorporation, and Detection in Cultures of Malbranchea aurantiaca.* Ding, Y.; Greshock, T.J.; Miller, K. Sherman, D.H.; Williams, R.M. *Org. Lett.* **2008**, *10*, 4863-4866.

239. **Calmodulin Inhibitory Activity of the Malbrancheamides and Various Analogs.* Miller, K.A.; Figueroa, M.; Valente, M.W.N.; Greshock, T.J.; Mata. R.; Williams, R.M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6479-6481.

240. **A Diastereoselective Intramolecular Pauson-Khand Approach for Construction of the BC Ring System in Tuberostemoninol.* Jia, X.; Williams, R.M. *Tet: Asymm.* **2008**, *19*, 2901-2906.

241. **Foreword to Special Issue on Amino Acids.* Williams, R.M., *Tet: Asymm.* **2008**, *19*, 2753.

2009

242. **Synthesis of Potential Early-Stage Intermediates in the Biosynthesis of the Antitumor Antibiotics FR-900482 and Mitomycin C.* Chamberland, S.; Sherman, D.H.; Williams, R.M. *Org. Lett.* **2009**, *11*, 791-794.

243. **Synthesis and Conformation-Activity Relationships of the Peptide Isosteres of FK228 and Largazole.* Bowers, A.A.; Greshock, T.J.; West, N.; Estiu, G.; Schreiber, S.L.; Wiest, O.; Williams, R.M. Bradner, J.E. *J. Am. Chem. Soc.* **2009**, *131*, 2900-2905.

244. **Synthesis and Histone Deacetylase Inhibitory Activity of Largazole Analogs: Alteration of the Zinc-Binding Domain and Macrocyclic Scaffold* Bowers, A.A.; West, N.; Newkirk, T.; Troutman-Youngman, A.E.; Schreiber, S.L.; Wiest, O.; Bradner, J.E.; Williams, R.M. *Org. Lett.* **2009**, *11*, 1301-1304.

245. **Isolation of Antipodal (-)-Versicolamide B and Notoamides L-N from a Marine-Derived Aspergillus sp.* Tsukamoto, S.; Kawabata, T.; Kato, H.; Greshock, T.J.; Hirota, H.; Ohta, T.; Williams, R.M. *Org. Lett.* **2009**, *11*, 1297-1300.

246. **Isolation of Notoamide E, a Key Precursor in the Biosynthesis of Prenylated Indole Alkaloids in a Marine-Derived Fungus, Aspergillus sp.* Tsukamoto, S.; Kato, H.; Greshock, T.J.; Hirota, H.; Ohta, T.; Williams, R.M. *J. Am. Chem. Soc.* **2009**, *131*, 3834-3835.

247. **Asymmetric, Total Syntheses of (+)- and (-)-Versicolamide B and Biosynthetic Implications.* Miller, K.A.; Tsukamoto, S.; Williams, R.M. *Nature Chemistry* **2009**, *1*, 63-68.

248. **Synthesis of (+/-)-Oleocanthal via a Tandem Intramolecular Michael Cyclization-HWE Olefination*. English, B.J.; Williams, R.M. *Tetrahedron Lett.* **2009**, *50*, 2713-2715.

249. **(3R,7aS)-3-(Trichloromethyl)tetrahydropyrrolo[1,2-c]oxazol-1(3H)-one: An Air and Moisture Stable Reagent for the Synthesis of Optically Active α-Branched Prolines.* Artman, G.D.; Rafferty, R.J.; Williams, R.M., *Org. Synth.* **2009**, *86*, 262-273.

250. **Synthetic Studies Towards the Mitomycins: Construction of the Tetracyclic Core via a Reductive Aminocyclization Reaction*. Gubler, D.A.; Williams, R.M. *Tetrahedron Lett.* **2009**, *50*, 4265-4267.

251. **Toward Palau'amine; $Hg(OTf)_2$-Catalyzed Synthesis of the Cyclopentane Core.* Namba, K.; Kaihara, Y.; Yamamoto, H.; Imagawa, H.; Tanino, K.; Williams, R.M.; Nishizawa, M. *Chem. Eur. J.* **2009**, 6560-6563.

252. **New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products.* ACS Symposium Book Series. Soloshonok, V.A.; Izawa, K., Eds., American Chemical Society, Washington, D.C., Burnett, C.M.; Williams, R.M. **2009**, Chapter 26, 420-442.

253. **Asymmetric Synthesis of the Core of AMPTD, the Key Amino Acid of Microsclerodermins F-I*. Burnett, C.M.; Williams, R.M. *Tetrahedron Lett.* **2009**, 50, 5449-5451.

254. **Discovery, Biological Activity, Synthesis and Potential Therapeutic Utility of Naturally Occurring Histone Deacetylase Inhibitors*. Bowers, A.A.; Newkirk, T.; Williams, R.M., *Natural Product Rep.* **2009**, *26*, 1293-1320.

255. **Synthetic Approaches to the Bicyclo[2.2.2]diazaoctane Ring System Common to the Paraherquamides, Stephacidins and Related Prenylated Indole Alkaloids.* Miller, K.A.; Williams, R.M., *Chem. Soc. Rev.* **2009**, *38*, 3160-3174.

2010

256. *Methods to Characterize the Effect of DNA-Modifying Compounds on Nucleosomal DNA.* Subramanian, V.; Williams, R.M.; Boger, D.L.; Luger, K. *Methods in Molecular Biology*, **2010**, *613*, 173-192.

257. **Recent Updates and Examples of the Stille Bi-Aryl Cross-Coupling Reaction.* Williams, R.M., *Org. Synth.* **2011**, *88*, 197-201.

258. **Synthesis of Notoamide J. A Potentially Pivotal Intermediate in the Biosynthesis of Several Prenylated Indole Alkaloids.*. Finefield, J.; Williams, R.M., *J. Org. Chem.* **2010**, *75*, 2785-2789.

259. **Studies on the Biosynthesis of the Stephacidins and Notoamides. Total Synthesis of Notoamide S.* McAfoos, T.; Williams, R.M. *Heterocycles* **2010**, 82, 461-472.

260. **Genome-based Characterization of Two Prenylation Steps in the Assembly of the Stephacidin and Notoamide Anticancer Agents in a Marine Aspergillus sp.* Ding, Y.; de Wet, J.R.; Cavalcoli, J.; Li, S.; Greshock, T.J.; Miller, K.A.; Finefield, J.M.; Sunderhaus, J.D.; McAfoos, T.; Tsukamoto, S.; Williams, R.M.; Sherman, D.H. *J. Am. Chem. Soc.* **2010**, *132*, 12733-12740.

261. **Synthetic Studies on the Ambiguine Family of Alkaloids: Construction of the ABCD Ring System.* Rafferty, R.J.; Williams, R.M., *Tetrahedron Lett.* **2011**, *52*, 3733-3736.

262. **Synthetic Studies on Palau'amine. Approach to the Cyclopentane Core via an Asymmetric, Intramolecular [1,3]-Dipolar Cycloaddition Reaction.* Namba, K.; Greshock, T.J. Sundermeier, U.; Li, H.; Williams, R.M. *Tetrahedron Lett.* **2010**, *51*, 6557-6559.

263. **A Divergent Strategy for the Synthesis of Secologanin Derived Natural Products.* English, B.J.; Williams, R.M. *J. Org. Chem.* **2010**, *75*, 7869-7876.

264. *Method for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., WO 2010009334 A1, January 21, **2010**.

2011

265. **Biomimetic Synthesis of Alkaloids Derived from Tryptophan: Dioxopiperazine Alkaloids.* Welch. T.; Williams, R.M., chapter in *Biomimetic Organic Synthesis*, Wiley-VCH, Poupon, E.; Nay, B., Editors. **2011**, *1*, 117-148.

266. **Notoamide E: Biosynthetic incorporation into notoamides C and D in cultures of Aspergillus versicolor NRRL 35600.* Finefield, J.; Greshock, T.J.; Sherman. D.H.; Tsukamoto, S.; Williams, R.M. *Tetrahedron Lett.* **2011**, *52*, 1987-1989.

267. **Studies on the Biosynthesis of the Notoamides. Synthesis of an Isotopomer of 6-Hydroxydeoxybrevianamide E and Biosynthetic Incorporation into Notoamide J.* Finefield, J.; Sherman. D.H.; Tsukamoto, S.; Williams, R.M. *J. Org. Chem.* **2011**, *76*, 5954-5958.

268. **Natural Products Synthesis: Enabling Tools to Penetrate Nature's Secrets of Biogenesis and Biomechanism.* Williams, R.M., *J. Org. Chem.* **2011**, *76*, 4221-4259.

269. **Studies on the Biosynthesis of the Stephacidin and Notoamide Natural Products: A Stereochemical and Genetic Conundrum.* Sunderhaus, J.D.; Williams, R.M., *Israel J. Chem.* **2011**, *3-4*, 442-452.

270. **Robert Burns Woodward's Unfinished Symphony: Organic Conducting Materials.* Woodward, R.B.; Cava, M.P.; Lakshmikantham, M.V.; Hoffmann, R.; Williams, R.M., *Tetrahedron* **2011**, *67*, 6771-6797.

271. **Biosynthetic Studies of the Notoamides: Isotopic Synthesis of Stephacidin A and Incorporation into Notoamide B and Sclerotiamide.* Finefield, J.M.; Kato, H.; Greshock, T.J.; Sherman. D.H.; Tsukamoto, S.; Williams, R.M. *Org. Lett.* **2011**, *15*, 3802-3805.

272. *Protein Kinase C-δ Inactivation Inhibits Cellular Proliferation and Decreases Survival in Human Neuroendocrine Tumors.* Chen, Z.; Forman, L.W.; Miller, K.A.; English, B.; Takashima, A.; Bohacek, R.M.; Williams, R.M.; Faller, D.V. *Endocrine Related Cancers* **2011**, 18, 759-771.

273. **Meta-omic Characterization of the Marine Invertebrate Microbial Consortium that Produces the Chemotherapeutic Natural Product ET-743.* Rath, C.M.; Janto, B.; Earl, J.; Ahmed, A.; Hu, F.Z.; Hiller, L.; Dahlgren, M.; Kreft, R.; Yu, F.; Wolff, J.J.; Kweon, H.K.; Christiansen, M.A.; Håkansson, K.; Williams, R.M.; Ehrlich, G.D.; Sherman, D.H., *ACS Chem. Biol.* **2011**, *6*, 1244-1256.

274. *Histone Deacetylase Inhibitors are Potent Inducers of Tumor Latent EBV Infection.* Ghosh, S.K.; Perrine, S.P.; Williams, R.M.; Faller, D.V., *Blood*, **2012**, *119*, 1008-1017 (published on-line Dec. 7, 2011).

275. **Study on the Biosynthesis of the Notoamides: Pinacol-type rearrangement of the Isoprenyl Unit in Deoxybrevianamide E and 6-Hydroxydeoxybrevianamide E.* Tsukamoto, S.; Kato, H.; Nakamura, Y.; Umaoka, H.; Nakahara, T.; Finefield, J.M.; Williams, R.M., *Tetrahedron Lett.* **2011**, *52*, 6923-6926.

276. **Synthetic Studies Towards MPC1001: Preparation of a β-Hydroxy-Tyrosine Derivative.* Schuber, P.T.; Williams, R.M., *Tetrahedron Lett.* **2012**, *53*, 380-382. (published on-line November 22, 2011)

277. **Synthetic Studies on MPC1001: A Dipolar Cycloaddition Approach to the Pyrrolidine Ring System.* Schuber, P.T.; Williams, R.M., *Heterocycles* **2012**, *84*, 1193-1207. (published on-line October 28, 2011; invited manuscript Padwa 75th)

278. **Total Synthesis of Hapalindoles J and U.* Rafferty, R.J.; Williams, R.M. *J. Org. Chem.* **2012**, *77*, 519-524. (published on line November 29, 2011)

279. **Biochemical Characterization of NotB as an FAD-Dependent Oxidase in the Biosynthesis of Notoamide Indole Alkaloids.* Li, S.; Finefield, J., Sunderhaus, J.D.; McAfoos, T., Williams, R.M.; Sherman, D.H. *J. Am. Chem. Soc.* **2012**, *134*, 788-791. (published on-line December 20, 2011)

2012

280. **Enantiomeric Natural Products: Occurrence and Biogenesis.* Finefield, J.; Sherman, D.H.; Kreitman, M.; Williams, R.M., *Angew. Chem. Int. Ed.* **2012**, *51*, 4802-4836. (published on-line May 3, 2012)

281. **Fungal Origins of the Bicyclo[2.2.2]diazaoctane Ring System of Prenylated Indole Alkaloids.* Finefield, J.; Sherman, D.H.; Frisvad, J.; Williams, R.M. *J. Nat. Prod.* **2012**, *75*, 812-833. (published on-line April 15, 2012)

282. **The Early Stages of the Biosynthesis of Taxol: An Interim Report on the Synthesis and Identification of Early Pathway Metabolites.* Guerra, J.; Croteau, R.; Williams, R.M. *Nat. Prod. Rep.* **2012**, *29*, 683-696. (published on-line May 1, 2012)

283. **Comparative Analysis of the Biosynthetic Systems for Fungal Bicyclo[2.2.2]diazaoctane Indole Alkaloids: The (+)/(-)-Notoamide, Paraherquamide and Malbrancheamide Pathways.* Li, S.; Srinivasan, K.; Tran, H.; Yu, F.; Finefield, J.M.; Sunderhaus, J.D.; McAfoos, T.J.; Tsukamoto, S.; Williams, R.M.; Sherman, D.H., *Med. Chem. Comm.* **2012**, *3*, 987-996. (published on-line April 16, 2012)

284. **Discussion Addendum for: Efficient Asymmetric Synthesis of N-tert-Butoxycarbonyl α-Amino Acids using 4-tert-Butoxycarbonyl-5,6-Diphenylmorpholine-2-one: (R)-(N-tert-Butoxycarbonyl)allylglycine.* Looper, R.E.; Williams, R.M., *Org. Synth.* **2012**, *89*, 394-403.

285. **Unified Total Synthesis of Fawcettimine Class Alkaloids: Fawcettimine, Fawcettidine, Lycoflexine and Lycoposerramine B.*. Pan, G.; Williams, R.M. *J. Org. Chem.* **2012**, *77*, 4801-4811. (published on-line April 21, 2012)

286. **Formal Synthesis of Hapalindole O and Synthetic Studies Towards Hapalindoles K and Ambiguine A.* Rafferty, R.J.; Williams, R.M., *Heterocycles* **2012**, *86*, 219-231. (published on-line April 23, 2012; invited article in honor of Prof. Ei-ichi Negishi)

287. **Methods for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., U.S. Patent No. 8,217,076 B2, July 10, **2012**.

288. *An Improved Synthesis of 5-Acylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic Acid from Glycine with Readily Removable Protecting Groups.* Takahashi, D.; Kashiwagi, T.; Onoye, H.; Williams, R.M.; Izawa, K.; *Heterocycles,* **2012**, *85,* 2213-2229.

289. **Studies on the Biosynthesis of Chetomin: Enantiospecific Synthesis of a Putative Late-Stage Biosynthetic Intermediate.* Welch, T.R.; Williams, R.M. *Tetrahedron* **2012**, *68*, 770-773. (published on-line November 3, 2012)

290. **Honoring the 77th Birthday of Professor Ei-ichi Negishi. (Preface to Heterocycles Issue Dedicated to Prof. Ei-ichi Negishi's 77th Birthday)*. Williams, Robert M. *Heterocycles* **2012**, *86* 1-3. (invited article in honor of Prof. Ei-ichi Negishi)

291. **Synthesis and Bioconversions of Notoamide T: A Biosynthetic Precursor to Stephacidin A and Notoamide B.* Sunderhaus, J.D.; McAfoos, T.J.; Finefield, J.M.; Kato, H.; Li, S.; Tsukamoto, S.; Sherman, D.H.; Williams, R.M. *Org. Lett.* **2013**, *15*, 22-25. (published on-line Dec. 12, 2012)

2013

292. **Mitomycinoid Alkaloids: Mechanism of Action, Biosynthesis, Total Syntheses, and Synthetic Approaches.* Bass, P.; Gubler, D.A.; Judd, T.C.; Williams, R.M. *Chem. Rev.* **2013**, *113*, 6816-6863.

293. **Synthetic Studies on Lemonomycin: Construction of the Tetracyclic Core.* Jiménez-Somarribas, A.; Williams, R.M. *Tetrahedron* **2013**, 69, 7505-7512.

294. **Synthesis and HDAC Inhibitory Activity of Isosteric Thiazoline-Oxazole Containing Largazole Analogs.* Guerra-Bubb, J.M.; Bowers, A.A.; Smith, W.B.; Paranal, R.; Estiu, G.; Wiest, O.; Bradner, J.E.; Williams, R.M. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6025-6028.

295. **Method for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., Wiest, O.G., U.S. Patent No. 8,513,290 B2, August 20, **2013**.

296. **A Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs*. Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M., PCT 13/640,815, April 18, **2013**.

297. **Biosynthetic Systems Producing Fungal Indole Alkaloids.* Li, S.; Anand Srinivasan, K.; Williams, R.M.; Sherman, D.H.; WO 2013/152110 A1, International Patent Application October 10, **2013**.

298. **Efficient Synthesis of the Cyclopentanone Fragrances (Z)-3-(2-oxopropyl)-2-(pent-2-en-1-yl) cyclopentanone and Magnolione.* Pan, G.; Williams, R.M., *Tetrahedron* **2014**, 70, 276-279.

2014

299. **Epidithiodioxopiperazines: Occurrence, Synthesis and Biogenesis.* Welch, T.R.; Williams, R.M. *Nat. Prod. Rep.* **2014**, *31*, 1376-1404.

300. *Protein Kinase Cδ is a Therapeutic Target in Malignant Melanoma with NRAS Mutation or BREF Inhibitor-Resistance*. Takashima, A.; English, B.J.; Chen., Z.; Cao, J.; Cui, R.; Williams, R.M.; Faller, D.V. *ACS Chem. Biol.* **2014**, *9*, 1003-1014.

301. **Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs.* Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M. U.S. Patent No. 8,815,562 B2, August 26, **2014**.

302. **Synthetic Studies Towards Zetekitoxin AB: Preparation of 4,5-epi-11-Hydroxysaxitoxinol.* Pearson, A.D.; Williams, R.M. *Tetrahedron* **2014**, *70*, 7942-7949.

303. *Protein Kinase C-delta Inactivation Inhibits the Proliferation and Survival of Cancer Stem Cells in Culture and in vivo.* Chen, Z.; Forman, L.W.; Williams, R.M.; Faller, D.V. *BMC Cancer*, **2014**, *14*, 90.).

2015

304. **Ecteinascidins. A Review of the Chemistry, Biology and Clinical Utility of Potent Tetrahydroisoquinoline Antitumor Antibiotics.* Le, V.; Inai, M.; Williams, R.M.; Kan, T., *Nat. Prod. Rep.* **2015***, 32*, 328-347. (published on-line October 2, 2014).

305. **Bioconversion of 6-Epi-Notoamide T Produces Metabolites of Unprecedented Structures in a Marine-derived Aspergillus sp.* Kato, H.; Nakahara, T.; Yamaguchi, M.; Kagiyama, I.; Finefield, J.M.; Sunderhaus, J.D.; Sherman, D.H.; Williams, R.M.; Tsukamoto, S. *Tetrahedron Lett.* **2015**, *56*, 247-251.

306. *Isolation of Notoamide S and Enantiomeric 6-epi-Stephacidin A from the Fungus Aspergillus amoenus: Biogenetic Implications.* Kato, H.; Nakahara, T.; Sugimoto, K.; Matsuo, K.; Kagiyama, I.; Frisvad, J.C.; Sherman, D.H.; Williams, R.M.; Tsukamoto, S. *Org. Lett.* **2015**, *17*, 700-703. (published on-line January 23, 2015).

307. *Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs.* Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M. US 2015/0050704 A1, February 19, **2015**.

308. **Comparative Pharmacokinetic Properties and Antitumor Activity of the Marine HDACi Largazole and Largazole Peptide Isostere.* Pilon, J.L.; Clausen, D.J.; Hansen, R.; Lunghofer, P.; Rose, B.; Thamm, D.H.; Gustafson, D.; Bradner, J.E.; Williams, R.M. *Cancer Chemother. Pharmacol.* **2015***, 75,* 671-682. (published on-line January 24, 2015).

309. *Variable Active Site Loop Conformations Accommodate the Binding of Macrocyclic Largazole Analogues to HDAC8.* Decroos, C.; Clausen, D.; Wiest, O.; Williams, R.M.; Christianson, D.W. *Biochemistry* **2015**, *54*, 2126-2135 (published on-line March 20, 2015).

310. **Modular Synthesis and Biological Activity of Pyridyl-based Analogs of the Potent Class I*

Histone Deacetylase Inhibitor Largazole. Clausen, D.J.; Smith, W.B.; Haines, B.E.; Wiest, O.; Bradner, J.E.; Williams, R.M. *Bioorg. Med. Chem.* **2015**, *23*, 5061-5074. (published on-line March 30, 2015).

311. **Comment on "Asymmetric Syntheses of Sceptrin and Massadine and Evidence for Biosynthetic Enantiodivergence" (Technical Comment)* Sherman, D.H.; Tsukamoto, S.; Williams, R.M., *Science* **2015**, *349*, 149b.

312. **Natural Diels-Alderases: Elusive and Irresistible.* Klas, K.; Tsukamoto, S.; Sherman, D.H.; Williams, R.M. *J. Org. Chem.* (invited submission for a special issue "50 Years of the Woodward Hoffman Rules") **2015**, *80*, 0000 (in press).

313. **Hapalindole/Ambiguine Biogenesis is Mediated by a Cope Rearrangement, C-C Bond-forming Cascade.* Li, S.; Lowell, A.N.; Yu, F.; Raveh, A.; Newminster, S.A.; Bair, N.; Schaub, J.M.; Williams, R.M.; Sherman, D.H., *J. Am. Chem. Soc.* **2015***, 137,* 0000 (in press).

314. *Substrate Controlled Flavin Monooxygenases Reveal Strategy for Fungal Indole Alkaloid Structural Diversification.* Tran, H.T.; Ashootosh, T.; Newmister, S.A.; Li, S.; Tsukamoto, S.; Williams, R.M.; Sherman, D.H. *J. Am. Chem. Soc.* **2015**, 0000 (under revision).

315. **Chemical Validation of the Proposed Intramolecular Diels-Alder Construction for the Assembly of the Monooxopiperazine Prenylated Indole Alkaloids of the Malbrancheamide and Paraherquamide Families.* Sunderhaus, J.D.; Finefield, J.M.; McAfoos, T.J.; Tran, H.; Tsukamoto, S.; Sherman, D.H.; Williams, R.M. *Nature* (submitted).

316. **Taichunamides: Unprecedented Prenylated Indole Alkaloids from Aspergillus taichungensis (IBT19404).* Kagiyama, I.; Kato, H.; Nehira, T.; Frisvad, J.C.; Sherman, D.H.; Williams, R.M.; Tsukamoto, S., *Angew. Chem. Int. Ed.* (submitted).

317. **Improved Total Synthesis of Et-743.* Le, V.; Williams, R.M., *J. Org. Chem.* (submitted)

318. **Studies on the Biosynthesis of Prenylated Indole Alkaloids Hapalindoles, Fischerindoles and Welwitindolinones. Synthesis of Hapalindoles C and D.* Bair, N., Sherman, D.H.; Williams, R.M., *Org. Lett.* (submitted)

Books and Monographs:

(1) *Synthesis of Optically Active α-Amino Acids*, Robert M. Williams, Pergamon Press, **1989**, Oxford, (*The Organic Chemistry Series*, J.E. Baldwin, Series Editor). 410 pp.

(2) *Organic & Biological Chemistry* (undergraduate textbook, under contract).

(3) *Biosynthesis of Primary and Secondary Metabolites* (in preparation).

Chapters:

1. *Biomimetic Synthesis of Alkaloids Derived from Tryptophan: Dioxopiperazine Alkaloids.* Welch. T.; Williams, R.M., chapter in *Biomimetic Organic Synthesis*, Wiley-VCH, Poupon, E.; Nay, B., Editors. **2011**, *1*, 117-148.
2. *New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products.* ACS Symposium Book Series. Soloshonok, V.A.; Izawa, K., Eds., American Chemical Society, Washington, D.C., Burnett, C.; Williams, R.M. **2009**, Chapter 26, 420-442.
3. *Diphenyloxazinones: Versatile Templates for the Asymmetric Synthesis of Amino Acids, Peptide Isosteres and Natural Products.* Burnett, C.; Williams, R.M., *Strategies and Tactics in Organic Synthesis* **2008** Vol.7, Chapter 8, pp 268-327.
4. *Biosynthesis of Prenylated Alkaloids Derived from Tryptophan.* Williams, R.M.; Sanz-Cervera, J.F.; Stocking, E., in *Topics in Current Chemistry*, Volume on *Biosynthesis-Terpenes and Alkaloids*, Leeper, F.; Vederas, J.C., Eds., Springer-Verlag, Berlin, **2000**, *209*, 97-173.

5. *Asymmetric Syntheses of Unnatural Amino Acids and Hydroxyethylene Peptide Isosteres,* Williams, R.M., Humana Press, *Peptidomimetics Protocols,* Kazmierski, W., Ed., Totowa, **1999,** Chapter 19, pp. 339-356.

6. *Amino Acid Synthesis,* Williams, R.M., *The Encyclopedia of Molecular Biology and Molecular Medicine,* VCH, Weinheim, Meyers, R.A., Ed., Volume 1, pp 52-58, **1996**.

7. *Asymmetric Synthesis of α-Amino Acids,* Williams, R.M., *Advances in Asymmetric Synthesis*, JAI Press Volume 1 (pp 45-94) A. Hassner, Ed., **1995**.

8. *Amino Acid Synthesis,* Williams, R.M., *Molecular Biology and Biotechnology. A Comprehensive Desk Reference,* VCH, Weinheim, Meyers, R.A., Ed., Volume 1, pp 28-33, **1995**.

9. *Stereoselective Synthetic and Mechanistic Chemistry of Bicyclomycin,* Williams, R.M., *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Editor, Elsevier Pub., Amsterdam, Vol. 12, **1993**.

10. *Design and Synthesis of Biologically Active Peptide Mimics,* Williams, R.M., *The Development and Utilization of Biologically Active Peptides*, Technomic Publishing Co., Lancaster, PA, **1992**, p. 187-215.

11. *Cannizzaro-Based O_2-Dependent Cleavage of DNA by Quinocarcin,* Williams, R.M.; Glinka, T.; Flanagan, M.E.; Gallegos, R.; Coffman, H.; Pei, D. *Advances in New Drug Development,* **1991**, p.38-51, The Pharmaceutical Society of Korea, Seoul.

12. *Synthesis and X-Ray Crystal Structure Determination of 1,3-Bridged β-Lactams: Novel, Anti-Bredt β-Lactams.* in *Recent Advances in the Chemistry of β-Lactam Antibiotics*, Williams, R.M.; Lee, B-H.; Miller, M.M.; Anderson, O.P. Royal Society of Chemistry, London **1988** p. 106.

Patents and Published Patent Applications

(1) *Design of Bioactive Peptides Based on Immunoglobulin Structure*. Greene, M.I.; Kieber-Emmons, T.; Weiner, D.; Williams, R.M.; Williams, W.V., CA 2091258 A1, April 2, **1992**.

(2) *Design of Bioactive Peptides Based on Immunoglobulin Structure.* Greene, M.I.; Kieber-Emmons, T.; Weiner, D.; Williams, R.M.; Williams, W.V., WO 1992004914 A1, April 2, **1992**.

(3) *Vinyl Glycine Derivatives for Memory and Learning Enhancement or Treatment of a Cognitive Disorder*, A.A. Cordi, J.B. Monahan and R.M. Williams; U.S. Patent No. 5,208,260, May 4, **1993**.

(4) *Biologically Active Compounds and Methods of Constructing and Using the Same*; Greene, Mark I.; Williams, William V.; Weiner, David B.; Cohen, Jeffrey A.; Kieber-Emmons, Thomas; Williams, Robert M.; U.S. Patent No. 5,637,677, June 10, **1997**.

(5) *Antibiotic for Methicillin Resistant Bacteria*. R.M. Williams and C. Yuan ; U.S. Patent No. 6,110,925, August 29, **2000**.

(6) *Antibiotic for Methicillin Resistant Bacteria*. R.M. Williams and C. Yuan ; U.S. Patent No. 6,162,925, December 19, **2000**.

(7) *Biologically Active Compounds and Methods of Constructing and Using the Same*; Greene, Mark I.; Williams, William V.; Weiner, David B.; Cohen, Jeffrey A.; Kieber-Emmons, Thomas; Williams, Robert M.; U.S. Patent No. 6,372,884 B1, April 16, **2002**.

(8) *Design of Bioactive Peptides Based on Immunoglobulin Structure*. Williams, William V.; Kieber-Emmons, Thomas; Williams, Robert M.; Weiner, David B.; Greene, Mark I.; EP 0551440B1, December 18, **2002**.

(9) *Design of Bioactive Peptides Based on Immunoglobulin Structure*. Weiner, David B.; Williams, Robert M.; Kieber-Emmons, Thomas; Williams, William V.; Greene, Mark I.; EP 1310557A1, May 14, **2003**.

(10) *Chiral Lactones*. Onishi, T.; Williams, R.M. U.S. Patent No. 7,173,141 B2, February 6, **2007**.

(11) *Method for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., WO 2010009334 A1, January 21, **2010**.

(12) *Methods for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., U.S. Patent No. 8,217,076 B2, July 10, **2012**.

(13) *A Biosynthetic Pathway for Heterologous Expression of an Nonribosomal Peptide Synthetase Drug and Analogs.* Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M., PCT 13/640,815, April 18, **2013**.

(14) *Method for Preparing Largazole Analogs and Uses Thereof.* Williams, R.M.; Bradner, J.E.; Bowers, A.; Newkirk, T., Wiest, O.G., U.S. Patent No. 8,513,290 B2, August 20, **2013**.

(15) *Biosynthetic Systems Producing Fungal Indole Alkaloids.* Li, S.; Anand Srinivasan, K.; Williams, R.M.; Sherman, D.H., PCT/US/2013/03513, October 10, 2013.

(16) *Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs.* Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M. U.S. Patent No. 8,815,562 B2, August 26, **2014**.

(17) *Biosynthetic Pathway for Heterologous Expression of a Nonribosomal Peptide Synthetase Drug and Analogs.* Sherman, D.H.; Ehrlich, G.D.; Janto, B.; Williams, R.M.; Rath, C.M. US 2015/0050704 A1, February 19, **2015**.

News Coverage of Published Work:

1. "Natural Products from Symbionts" Nature Biotechnology, (Kathy Aschheim) Published 9 January, 2012. *Nature Biotechnology* **2012**, *30*, 60 doi:10.1038/nbt.2103 (covering *ACS Chem. Biol.* **2011**, *6*, 1244–1256) (http://www.nature.com/nbt/journal/v30/n1/full/nbt.2103.html)

2. *"Woodward's Unfinished Work" Chemical & Engineering News*, (Bethany Halford) May 30, 2011, Volume 89 (No.22) pages 46-49 (covering *Tetrahedron* **2011**, *67*, 6771-6797)

3. *"Nobel Prize Winner's Unfinished Symphony"* Science Daily, August 1, 2011 (covering *Tetrahedron* **2011**, *67*, 6771-6797) http://www.sciencedaily.com/releases/2011/08/110801111526.htm

4. *"Nobel Prize Winner's Unfinished Symphony. Family Archives Provide Fascinating Insight into R.B. Woodward's Work on Organic Superconductors."* Eureka Alert (Philippe Terheggen) August 1, 2011 (covering *Tetrahedron* **2011**, *67*, 6771-6797)

5. *"Alkaloid Biogenesis: Natural Selection"* (Stephen Davey) *Nature Chemistry,* Published online: 24 April 2008 doi:10.1038/nchem.2008.3 (covering *Angew. Chem. Int. Ed.* **2008**, *47*, 3573-3577).

6. *"Quinine Steps Back in Time"* (Philip Ball) *Nature*, **2008**, *451*, 1065-1066 (History of Science covering *Angew. Chem. Int. Ed.* **2008**, *47*, 1736-1740).

7. *"Quinine Quest", Chemical & Engineering News*, (Bethany Halford) February 4, **2008**, Volume 86 (No. 5) page 8 (News of the Week covering *Angew. Chem. Int. Ed.* **2008**, *47*, 1736-1740). (http://pubs.acs.org/cen/news/86/i05/8605notw4.html)

8. *"Air to the Rescue", Science*, February 22, **2008**, (JSY) Vol. 319, pg. 1013 (Editor's Choice covering *Angew. Chem. Int. Ed.* **2008**, *47*, 1736-1740). (http://www.sciencemag.org/content/vol319/issue5866/twil.dtl#319/5866/1013a)

9. *"A tonic for quinine chemistry. 1940s re-enactment helps to verify old chemical claim."* (Katharine Sanderson) News@Nature (February 4, 2008). (http://www.nature.com/news/2008/080204/full/news.2008.554.html)

10. "Quinine synthesis mystery solved". Chemistry World, RSC, February 5, 2008, Henry Nicholls (http://www.rsc.org/chemistryworld/News/2008/February/05020801.asp)

11. *"Disputed total synthesis of quinine by Woodward and Doering confirmed."* Physorg.com, January 31, 2008 (http://www.physorg.com/news120993858.html)

12. *"Not A Myth: Quinine Synthesis In The Lab Using Old Methods Confirmed"* Scientific Blogging, February 3, 2008. (http://www.scientificblogging.com/news_releases/not_a_myth_quinine_synthesis_in_the_lab_using_old_methods_confirmed)

13. *"They Were Right After All: Disputed Total Synthesis of Quinine by Woodward and Doering Confirmed."* Eureka Alert, January 31, 2008 (http://www.eurekalert.org/pub_releases/2008-01/w-twr013108.php)

14. *"Directions For Quinine Synthesis From 1945 Work When Slightly Aged Aluminum Powder Is Used."* ScienceDaily, January 31, 2008. (http://www.sciencedaily.com/releases/2008/01/080131111817.htm)

15. *"Quinine, Definitely…"* Totallysynthetic.com. (http://totallysynthetic.com/blog/?p=838)

16. *"New Routes to Antitumor Antibiotics", Chemical & Engineering News*, December 16, **2002**, page 32 (Highlights of *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 4683-4685).

17. *"Key Step in Taxol Biosynthesis Revealed", Chemical & Engineering News*, December 11, **2000**, Stu Borman (Highlights of *Chem. Biol.* **2000**, *7*, 969-977).

18. *"Researchers Probe Steps of Taxol Biosynthesis" Chemical & Engineering News*, July 1, **1996**, Stu Borman, (Highlights of *Chem. Biol.,* **1996**, *3*, 479-489).

INVITED LECTURES

Robert M. Williams, Professor of Chemistry, Colorado State University

1982

Purdue University; July 6, 1982. Title: "*Bicyclomycin Chemistry*"

Merrell-Dow Pharmaceuticals, Indianapolis, Indiana; July 7, 1982.Title:"*Bicyclomycin Chemistry*"

Syntex Pharmaceutical Co., Palo Alto, California; August 20,1982.Title:"*Bicyclomycin Synthesis*"

*Hokkaido University, Post ICOS IV Symposium, "Highlights in Organic Synthesis", Sapporo, Japan; August 30-31,1982. Title: "*A New and Efficient Cyclization Reaction to Construct the Bicyclomycin Ring System*" (Plenary Lecture)

Fujisawa Pharmaceutical Co., Osaka, Japan; September 1, 1982. Title: "*Bicyclomycin Chemistry*"

University of Wyoming; October 5, 1982. Title: "*Bicyclomycin Chemistry*"

1983

University of Idaho; April 21, 1983. Title: "*Bicyclomycin Synthesis*"

University of Hawaii; November 23, 1983. Title: "*Bicyclomycin Chemistry*"

Symposium on C-Glycosides, St. Louis, Missouri; April 11, 1983 (National ACS Meeting) Title: "*C-Glycosidation of Pyridyl Thioglycosides*"

1984

Pfizer Pharmaceutical Co.; September 4, 1984.Title:"*Bicyclomycin: Stereocontrolled Total Synthesis, Mechanistic and Biological Studies*"

Harvard University; September 5, 1984. Title: "*Stereocontrolled Total Synthesis of Bicyclomycin*"

Merck, Sharp and Dohme Research Laboratories; September 6, 1984.Title:"*Bicyclomycin: Stereocontrolled Total Synthesis, Mechanistic and Biological Studies*"

Hoffmann-La Roche, Inc.; September 7, 1984.Title: "*Bicyclomycin: Stereocontrolled Total Synthesis, Mechanistic and Biological Studies*"

University of Colorado, Boulder; October 3, 1984. Title: "*Stereocontrolled Total Synthesis of Bicyclomycin*"

1985

Sterling-Winthrop Research Institute; February 20, 1985. Title: "*Electrophilic Glycinates: New Templates for the Asymmetric Synthesis of Amino Acids*"

Merck-Frostt Pharmaceutical Co., Canada; February 21, 1985. Title: "*Stereocontrolled Total Synthesis of Bicyclomycin*"

California Institute of Technology; March 27, 1985. Title: "*Nucleophilic Additions to Bicyclomycin and Analogs*"

University of California, Irvine; March 28, 1985. Title: "*Electrophilic Glycinates: New Templates for the Asymmetric Synthesis of Amino Acids*"

Syntex Pharmaceutical Co., Palo Alto, California; April 2, 1985. Title: "*Electrophilic Glycinates: New Templates for the /Asymmetric Synthesis of Amino Acids*"

Stanford University; April 3, 1985. Title: "*Nucleophilic Additions to Bicyclomycin and Analogs: Is Bicyclomycin a Latent Michael Acceptor?*"

Gordon Research Conference, "Organic Reactions and Processes", New Hampshire; July 17, 1985. Title: "*New Carbon-Carbon Bond-Forming Reactions via Electrophilic Glycine Derivatives*"

Gordon Research Conference, "Natural Products", New Hampshire; July 24, 1985. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

NSF-Sponsored Synthesis Workshop in Pingree Park, Colorado; July 10, 1985. Title: "*Bicyclomycin Chemistry*"

The Upjohn Company, Inc.; September 25, 1985. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

The University of Chicago; October 4, 1985. Title: "*Bicyclomycin: New Synthetic and Mechanistic Insights*"

Monsanto Co., Inc.; October 18, 1985. Title: "*Electrophilic Glycinates: Versatile Templates for Asymmetric Amino Acid Synthesis*"

The University of Rochester; October 16, 1985. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

Ajinomoto Company, Tokyo, Japan; November 13, 1985. Title: "*Electrophilic Glycinates: Versatile Templates for Asymmetric Amino Acid Synthesis*"

Tokyo Institute of Technology, Tokyo, Japan; November 14, 1985. Title: "*Bicyclomycin: New Synthetic and Mechanistic Insights*"

The University of Tokyo, Tokyo, Japan; November 15, 1985. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

Fujisawa Pharmaceutical Company, Osaka, Japan; November 25, 1985. Title: "*Bicyclomycin: New Mechanistic and Synthetic Insights*"

Riken Institute of Physical and Chemical Research, Saitama, Japan; November 26, 1985. Title: "*C-C Bond Forming Reactions of Activated Acetals and Animals: Syntheses of C-Glycosides and Amino Acids*"

The University of Oregon, Eugene; December 6, 1985. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

Oregon State University, Corvallis; December 9, 1985. Title: "*Bicyclomycin: New Synthetic and Mechanistic Insights*"

1986

Schering Corporation, Bloomfield, New Jersey; January 24, 1986. Title: "*Bicyclomycin: New Synthetic and Mechanistic Insights*"

Northern New Jersey Local Section ACS Meeting; January 24, 1986. Title: "*Asymmetric Amino Acid Synthesis via Electrophilic Glycinates*"

Squibb Pharmaceutical Company, New Brunswick, New Jersey; January 28, 1986. Title: "*Asymmetric Synthesis of Amino Acids via Electrophilic Glycinates*"

Squibb Institute for Medical Research, Princeton, New Jersey; January 29, 1986. Title: "*Progress Toward Understanding the Mechanism of Action of Bicyclomycin*"

Eli Lilly Pharmaceutical Company, Indianapolis, Indiana; May 14, 1986. Title: "*Bicyclomycin: Evolution of New Concepts and Strategies in Amino Acid Chemistry*"

W.R.Grace Company, Columbia, Maryland; June 20, 1986. Title: "*Asymmetric Synthesis of Amino Acids via Electrophilic Glycine Templates*"

Gordon Research Conference, "Heterocycles", New Hampshire; July 9, 1986. Title: "*Electrophilic Glycinates: Versatile Templates for Amino Acid Synthesis*"

The University of Michigan, Ann Arbor; September 24, 1986. Title: "*Synthetic and Mechanistic Adventures in Unusual Amino Acid Chemistry*"

*The Brook Lodge Conference on "Peptide Based Therapeutics" sponsored by the Upjohn Company, Kalamazoo, Michigan; September 26, 1986. Title: "*Synthetic and Mechanistic Adventures in Unusual Amino Acid Chemistry*" (Plenary Lecture)

The University of Delaware, Newark, Delaware; October 1, 1986. Title: "*Recent Advances in Amino Acid Chemistry*"

The State University of New York at Stony Brook; October 23, 1986. Title: "*Recent Synthetic and Mechanistic Explorations in Novel Amino Acid Chemistry*"

The University of Virginia; November 3, 1986. Title: "*Recent Synthetic and Mechanistic Explorations in Novel Amino Acid Chemistry*"

1987

The University of Wyoming, April 2, 1987. Title: "*A Synthetic Attack on the Brevianamides*"

Merck, Sharp & Dohme Research Laboratories, West Point, Penn., April 16, 1987. Title: "*Asymmetric Synthesis of Amino Acids*"

Texas Tech University, Lubbock, Texas; April 29, 1987. Title: "*A Synthetic Attack on the Brevianamides*"

The University of California, Riverside; May 6, 1987. Title: "*A Synthetic Attack on the Brevianamides*"

The University of California, Los Angeles; May 7, 1987. Title: "*A Synthetic Attack on the Brevianamides*"

New Mexico State University, Las Cruces, New Mexico; November 12, 1987. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Brevianamide B*"

Searle Pharmaceutical, Skokie, Illinois; November 9, 1987. Title: "*Asymmetric Synthesis of α-Amino Acids*"

Northwestern University, Evanston, Illinois; November 20, 1987. Title: "*Bicyclomycin: Mechanistic, Biological and Synthetic Investigations*"

Smith, Kline & French Laboratories, King of Prussia, Pennsylvania; November 30, 1987. Title: "*Asymmetric Synthesis of α-Amino Acids*"

Stuart Pharmaceuticals (ICI), Wilmington, Delaware; December 1, 1987. Title: "*Asymmetric Synthesis of α-Amino Acids*"

1988

The University of Alberta, Edmonton, Canada, January 25, 1988. Title: "*Asymmetric Total Synthesis of Brevianamide B*"

G. D. Searle Research Division of Monsanto, St. Louis, Missouri, February 1988. Title: "*Asymmetric Synthesis of α-Amino Acids*"

W. R. Grace & Co., Columbia, Maryland, February, 4, 1988. Title: "*Recent Advances in Synthetic, Mechanistic and Bio-organic Chemistry*"

Pennsylvania State University, State College, Penn., March 1, 1988. Title: "*Asymmetric Total Synthesis of Brevianamide B*"

The University of Michigan, Ann Arbor, Michigan, March 2, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Total Synthesis of Brevianamide B*"

*The Third Biennial Lilly Grantee Symposium, Eli Lilly, Indianapolis, Indiana, March 8, 1988. Title: "*Approach to Understanding the Molecular Mechanism of Action of Bicyclomycin*" (Plenary Lecture)

The 9th Rocky Mountain Regional ACS Meeting, Symposium on Natural Products, Las Vegas, Nevada, March 29, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Brevianamide B*"

Ciba-Geigy Pharmaceuticals, Basel Switzerland; April 27, 1988. Title: "*Bicyclomycin: Mechanistic and Synthetic Investigations*"

University of Geneva, Geneva Switzerland; April 28, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

**Burgenstock Conference on Stereochemistry; Burgenstock Switzerland; May 1-7, 1988. Title: "*Bicyclomycin: A Mechanistic, Biological and Synthetic Pandoras' Box*" (Plenary Lecture)

ETH (Eidgenössischen Technischen Hochschule), Zurich Switzerland; May 9, 1988. Title: "*Bicyclomycin: A Mechanistic, Biological and Synthetic Pandoras' Box*"

Chengdu Institute of Organic Chemistry, Chengdu, People's Republic of China; May 18-20 1988. Title: "*Recent Advances in Stereocontrolled Synthesis*"

IUPAC 88 Kyoto 16th International Symposium on the Chemistry of Natural Products, Kyoto Japan; May 31, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Fujisawa Pharmaceutical Co., Osaka Japan; June 3, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Nagoya Post-Symposium "New Topics on Natural Products", Toba Japan; June 7, 1988. Title: *"Facial Selectivity of the Intramolecular SN2' Reaction: Asymmetric Total Synthesis of Brevianamide B"*

Takeda Pharmaceutical Co., Osaka Japan; June 5, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Sumitomo Chemical Co., Osaka Japan; June 9, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Sankyo Pharmaceutical Co., Tokyo Japan; June 10, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Hoffmann-LaRoche, Nutley, New Jersey, June 24, 1988: Title: *"Asymmetric Synthesis of α-Amino Acids"*

The University of Gottingen, Gottingen West Germany, June 27, 1988. Title: *"Novel Syntheses of Chiral Amino Acids"*

Cambridge University, Cambridge, England, June 29, 1988. Title: "*Bicyclomycin: Synthetic, Mechanistic and Biological Studies*"

Oxford University, Oxford, England, June 30, 1988. Title: "*Practical Asymmetric Synthesis of α-Amino Acids"*

Imperial Chemical Industries, Alderly Edge, England, July 1, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Churchill College, Cambridge, England, July 4, 1988. Title: "*Synthesis and Properties of 1,3-Bridged β-Lactams: Novel Anti-Bredt β-Lactams*"

Imperial College, London, England, July 7, 1988. Title: "*Bicyclomycin: Recent Mechanistic, Biological and Synthetic Investigations*"

Pfizer Pharmaceutical Co., Sandwich, England, July 8, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Texas A & M University, College Station, Texas October 13, 1988. Title: "*Studies on the Chemistry of Amino Acids and Selected Derivatives*"

The University of Texas, Austin, Texas, October 14, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Ciba-Geigy Pharmaceutical, Summit, New Jersey, October 19, 1988. Title: "*Asymmetric Synthesis of Biologically Important Non-Proteinogenic α-Amino Acids*"

Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey, October 20, 1988. Title: "*Asymmetric Synthesis of Biologically Important Non-Proteinogenic α-Amino Acids"*

1989

*****The Carl Marvel Symposium, Tucson, Arizona, March 7, 1989. Title: "*Total Synthesis and Biogenetic Intrigue of the Brevianamides*" (Plenary Lecture)

Abbott Laboratories, Abbott Park, Illinois, May 4, 1989. Title: *"Synthetic and Mechanistic Investigations on the Anti-Tumor Antibiotic Quinocarcin"*

Lederle Laboratories, Pearl River, New York, May 23, 1989. Title: *"Synthetic and Mechanistic Investigations on the Anti-Tumor Antibiotic Quinocarcin"*

The UpJohn Company, Kalamazoo, Michigan, June 5-7, 1989. 3-Day Short course on the "*Synthesis of Optically Active α-Amino Acids*"

Eli Lilly Company, Indianapolis, Indiana, June 23, 1989. Title: *"Probing the Mode of Action Bicyclomycin and Anti-tumor Action of Quinocarcin"*

W.R. Grace and Company, Columbia, Maryland, June 29, 1989. Title: "*Synthesis of Optically Active α-Amino Acids*"

Hoffman-LaRoche, Inc., Nutley, New Jersey, July 21, 1989. Title: "*Synthesis of Optically Active α-Amino Acids*"

Yale University, New Haven, Connecticut, November 15, 1989. Title: *"Oxazolidines, Carbinolamines and Amides: Intrigue at Nitrogen"*

1990

Nutrasweet Co., Mt. Prospect, Illinois, March 20, 1990. Title: *"Synthesis of Optically Active α-Amino Acids"*

Syntex Research, Palo Alto, California, May 11, 1990. Title: *"Studies on the Mechanism of DNA Cleavage by Quinocarmycin"*

*CU-SYNTEX Synthetic Chemistry Symposium, June 7, 1990. Title: *"Mechanistic and Synthetic Studies on the Mode of Cleavage of Superhelical DNA by Quinocarcin"*

Special Bilateral U.S.-Britain Workshop on "Asymmetric Synthesis," July 3-8, 1990, Pingree Park, Colorado (RMW Co-organizer)

Gordon Research Conference on "Organic Reactions and Processes, New Hampton, New Hampshire, July 15-20, 1990. Title: *"The Mechanism of Oxygen Reduction by Quinocarcin"*

Gordon Research Conference on "Natural Products," New Hampton, New Hampshire, July 22-27, 1990. Title: *"Bioorganic, Mechanistic and Synthetic Chemistry of Biologically Significant Nitrogenous Substances"*

Hoffman-LaRoche, Inc., Nutley, N.J., August 24, 1990. Title: *"Probing the Mechanism of DNA Cleavage by the Antitumor Drug Quinocarcin"*

Bio Mega, Inc., Montreal, Canada, September 18, 1990. Title: *"Asymmetric Synthesis of α-Amino Acids"*

The University of California, Santa Barbara, California, November 9, 1990. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

Stanford University, Palo Alto, California, November 28, 1990. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

1991

Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, May 27, 1991. Title: *"Mechanistic and Synthetic Studies on Anti-Tumor Antibiotics"*

SunBor (Suntory Institute of Bio-Organic Research) Osaka, Japan, May 28, 1991. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

Sagami Research Institute, Kanagawa, Japan, May 29, 1991. Title: *"Mechanistic and Synthetic Studies on Quinocarcin: Superoxide-Mediated DNA Cleavage"*

Ajinomoto Co., Kawasaki, Japan, May 30, 1991. Title: *"Recent Studies on the Asymmetric Synthesis of α-Amino Acids"*

Shionogi Pharmaceutical Co., Tokyo, Japan, May 31, 1991. Title:*" Recent Studies on the Asymmetric Synthesis of α-Amino Acids"*

Tokyo Institute of Technology, Tokyo, Japan, June 1, 1991. Title: *"Facial Selectivity of the [2,3] Wittig Rearrangement and S_N2' Cyclization Reactions in Total Synthesis"*

NSF-JSPS Bilateral Seminar on "Selectivity in Synthetic and Bio-Organic Chemistry", Tokyo, Japan, June 3-7, 1991. Title: *"Unusual Facial Selectivity in the Biosynthesis and Synthesis of*

the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"

*Otsuka Pharmaceutical Co., Tokushima, Japan. June 11, 1991. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids"*

*International Congress of New Drug Development, Seoul, Korea, August 21, 1991. Title: *"Synthetic and Mechanistic Studies on Anti-Tumor Antibiotics"*

*4th Chemical Congress of North America, Division of Organic Chemistry, New York, August 29, 1991. Title: *"Unusual Stereofacial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*

DowElanco Co., Walnut Creek, California, November 8, 1991. Title: *"Design and Synthesis of Lysine Biosynthesis Inhibitors"*

The University of California, Santa Cruz, California, November 11, 1991. Title: *"Synthetic and Biosynthetic Studies on the Brevianamides and Paraherquamides"*

The University of California, Davis, California, November 12, 1991. Title: *"Cannizzarro-Based Oxygen-Dependent Cleavage of DNA by Quinocarcin and Tetrazomine"*

1992

Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn., March 18, 1992. Title: *"Asymmetric Synthesis of α-Amino Acids and What's Next?"*

24th Central Regional ACS Meeting (CMACS-92), Cincinnati, Ohio, Symposium on Amino Acids and Peptides, May 28, 1992. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids"*

Hoffman-La Roche, Inc., Workshop on Prospects for Antibacterial Agents Based on Diaminopimelic Acid, May 11, 1992. Title: *"Stereochemistry and DAP Inhibitors"*

Amgen Boulder, Colorado, August 14, 1992. Title: *"A Combinatorial Approach to Vancomycin Resistance"*

Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan, September 25, 1992, Title: *" Chemical, Biological and Combinatorial Adventures with Amino Acids"*

*48th Southwest Regional ACS Meeting, Lubbock, Texas, October 21-23, 1992, A.I. Meyers Symposium. Title: *"Recent Studies in Asymmetric and Bioorganic Methods"*

Purdue University, November 10, 1992, West Lafayette, Indiana, Title: *"Chemical, Biological and Combinatorial Adventures with Amino Acids"*

Monsanto Corporate Research, St. Louis, Missouri, November 11, 1992, Title: *" Recent Adventures in the Synthesis of Amino Acids and Complex Natural Products"*

Pfizer Central Research, Groton, Conn., November 12, 1992, Title: *"A Combinatorial Approach to Multiple Drug-Resistant Bacterial Infections"*

R.W. Johnson Pharmaceutical Research Co., Raritan, New Jersey, November 13, 1992, Title: *"Chemical and Biological Adventures with Amino Acids"*

1993

* BioEast '93, Washington, D.C., January 27, 1993, Title: *"New Approaches to Antibiotic Design and Synthesis"*

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, N.J., February 22, 1993, Title: *"Utility of Natural Products Synthesis in Studying Biosynthesis and Biomechanism"*

Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, N.J., February 23, 1993, Title: *"Utility of Natural Products Synthesis in Studying Biosynthesis and Biomechanism"*

Scripps Research Institute, Department of Chemistry, La Jolla, California, April 12, 1993. Title: *"Chemical, Biological and Combinatorial Adventures with Amino Acids"*

Microcide Pharmaceutical Co., Menlo Park, California, April 18, 1993. Title: *"Combinatorial Approaches to Treating Drug-Resistant Mycobacterial Infections"*

Hoffman-La Roche, Inc., Basel Switzerland, April 26, 1993. Title: *"Unnatural α-Amino Acids as a Vehicle for Biological, Mechanistic and Synthetic Inquiries"*

Sandoz Pharmaceutical Co., Inc., Basel Switzerland, April 27, 1993. Title: *"Recent Studies in Asymmetric and Bioorganic Methods"*

Pasteur Institute, Paris, France, April 29, 1993. Title: *"New Approaches to Antibiotic Design and Synthesis"*

3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic α-Amino Acids Acids via Chiral Glycinates"*

Burroughs-Wellcome Pharmaceutical Co., Research Triangle, October 11, 1993. Title: *"Studies on the Total Synthesis and Biogenesis of the Paraherquamide / Brevianamide Class of Alkaloids"*

***** 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Symposium on "Glycopeptide Resistance and the Cell Wall", New Orleans, Louisiana, October 18, 1993. Title: *"Implications for Drug Discovery"*

1994

***** Sino-American Symposium on Asymmetric Synthesis, Taichung, Taiwan, April 8-9, 1994. Title:*"The Asymmetric Synthesis of α-Amino Acids"*

E.I. Dupont De Nemours & Co. / Dupont-Merck Pharmaceutical Co., Newark, Delaware, May 16, 1994. Title: *"The Use of Total Synthesis of Indole Alkaloids as a Biosynthetic Periscope"*

Hoechst-Roussel Pharmaceuticals, Inc., Sommerville, N.J., July 13, 1994. Title: *"Bioxalomycins, Tetrazomine and Quinocarcin: Synthetic, Mechanistic and Biological Studies"*

*****The 4th International Conference on Chemical Synthesis of Antibiotics and Related Microbial Products, Nashville, Indiana, September 11-16, 1994. Title: *" Studies on the Mechanism of Action of Bioactive Nitrogenous Substances"*

Brandeis University, Waltham, Mass. October 27, 1994. Title: *"Synthesis and Biosynthesis of the Paraherquamides and Brevianamides"*

Eisai Pharmaceutical Co., Andover, Mass., December 1, 1994. Title: *"Asymmetric Synthesis of α-Amino Acids, Peptidomimetics and Other Nitrogenous Substances"*

Harvard University, Cambridge, Mass., December 12, 1994. Title: *"Chemical Probes and Microbes"*

1995

Rice University, Houston, Texas, February 22, 1995. Title: *"Total Synthesis of Natural Products: A Useful Periscope for Exploring Biosynthesis and Biomechanism"*

Texas A & M University, College Station, Texas, February 23, 1995. Title: *"Synthetic and Biosynthetic Studies on the Paraherquamides and Brevianamides"*

The University of Texas at Austin, Austin, Texas, February 24, 1995. Title: *"O_2-Dependent Cleavage of DNA by Tetrazomine, Quinocarcin and Synthetic Analogs"*

Imperial College, London England, May 15, 1995. Title: *"Recent Adventures in the Total Synthesis of Biosynthetically and Biomechanistically Intriguing Natural Products"*

*The SCI Meeting on Amino Acids, London, England, May 16, 1995. Title: *"Overview of the Major Conceptual Approaches to the Construction of Amino Acids"*

Rhone Poulenc Rorer Pharmaceutical Co., England, May 17, 1995. Title: *"Studies on the Biosynthesis of Taxol and the Brevianamide / Paraherquamide Class of Alkaloids"*

Eli Lilly Pharmaceuticals, England, May 18, 1995. Title: *"The Asymmetric Synthesis of α-Amino Acids"*

Astra-Zeneca Agrochemicals, Jealott's Hill, Bracknell, England, May 19, 1995. Title: *"Studies on the Total Synthesis and Mechanism of Action of Anti-tumor Antibiotics FR-900482 and Quinocarcin"*

Darwin Molecular Corp., Bothell, Washington, October 16, 1995. Title: *"Organic Synthesis: An Important Vehicle to Probe Biomechanism and Biosynthesis"*

The University of Washington, Seattle, Washington, October 17, 1995. Title: *"Organic Synthesis: An Important Vehicle to Probe Biomechanism and Biosynthesis"*

Hokkaido University, Sapporo, Japan, November 20, 1995. Title: *"Biosynthetic Studies on the Brevianamides and Paraherquamides: A Quest for the Biosynthetic Diels-Alder Construction"*

Takeda Pharmaceutical Company, Osaka, Japan, November 21, 1995. Title: *"Studies on the Total Synthesis of TAN-1057 and Novel Approaches to MRSA"*

Osaka University, Osaka, Japan, November 22, 1995. Title: *"Organic Synthesis as a Vehicle for Probing Biomechanism and Biosynthesis"*

Kyowa Hakko Pharmaceutical Co., Tokyo, Japan, November 24, 1995. Title: *"Synthetic and Bio-Mechanistic Studies on Quinocarcin, Tetrazomine and the Bioxalomycins"*

Tokyo University, Tokyo, Japan, November 24, 1995. Title: *"Short Stories in Natural Products Chemistry"*

Tokyo Institute of Technology, Tokyo, Japan, November 25, 1995. Title: *"Natural Products Synthesis as a Vehicle for Probing Biosynthesis and Discovering New Reactions"*

1996

Case Western Reserve University, Cleveland, Ohio, February 15, 1996. Title: *"Probing Biosynthesis and Biomechanism through Natural Products Synthesis"*

1997

The Upjohn Company, Kalamazoo, Michigan. April 2, 1997. Title: *"Studies on the Asymmetric Total Synthesis and Biosynthesis of the Paraherquamide/Marcfortine/Brevianamide Class of Alkaloids"*

Michigan State University, April 3, 1997. Title: *"Recent Studies on the Synthesis of Antitumor Antibiotics: Exploiting Drug-DNA Interactions"*

Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut. April 11, 1997. Title: *"Recent Studies on the Synthesis of Antitumor Antibiotics: Drug-DNA Interactions"*

*CU Hauser Symposium, May 29 & 30, 1997, Boulder, Colorado. Title: *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis"*

*Hokkaido University, Sapporo, Japan, June 24 & 25, 1997. Title: *"In Search of the Enzyme-Catalyzed Diels-Alder Construction: The Paraherquamide/Brevianamide Paradox"*

Dai-ichi Pharmaceutical Co., Tokyo, Japan, June 27, 1997. Title: *"Recent Studies in Natural Products Synthesis and Biomechanism"*

The University of Alberta, Edmonton, Canada. October 1, 1997. Title: *"Synthetic and Biomechanistic Studies on the Antitumor Antibiotics Bioxalomycin, Quinocarcin and FR900482"*

NexStar Pharmaceutical Co., Boulder, Colorado, December 2, 1997. Title: *"New and Old Approaches to Scaling the Bacterial Cell Wall: Design and Synthesis of Antibiotics".*

Tularik Co., San Francisco, California, December 4, 1997. Title: *"Organic Synthesis: A Probe for Studying Biomechanism and Biosynthesis".*

1998

Ligand Pharmaceutical Co., LaJolla, California, February 19, 1998. Title: *"The Asymmetric Synthesis of Amino Acids and Peptide Isosteres".*

The Scripps Research Institute, La Jolla California, February 20, 1998. Title: *"Natural Products Synthesis: An Important Probe for Studying Biosynthesis and Biomechanism".*

The University of Arizona, Tucson, Arizona, Department of Pharmaceutical Sciences April 20, 1998. Title: *"The Oxazolidine Family of Antitumor Antibiotics: Deconvoluting Multiple Modes of Action".*

The University of Arizona, Tucson, Arizona, Department of Chemistry April 21, 1998. Title: *"The Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Gottingen, Gottingen, Germany, June 19, 1998. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Konstanz, Konstanz, Germany, June 23, 1998. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Geneva, Geneva Switzerland, June 29, 1998. Title: *"Total synthesis of Natural Alkaloids of Biological and Biosynthetic Interest".*

*The Gordon Research Conference on Natural Products, July 5-9, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*

The University of Illinois at Urbana Champaign, October14, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*

1999

Array BioPharma, Boulder, Colorado, January 14, 1999. Title: *"Recent Studies in the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

The University of Tokyo, Tokyo, Japan, May 11, 1999. Title: *"Adventures in the Total Synthesis of Natural Products: Discovery and Surprises".*

Riken Institute, Tokyo, Japan. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Waseda University, Tokyo, Japan May 13, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Tohoku University, Sendai, Japan. May 14, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Tokyo Institute of Technology, Tokyo, Japan. May 17, 1999. Title: *"Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Studies on the Biosynthesis of Taxol: Synthesis and Labeling of Biosynthetically Relevant Taxoids".*

Keio University, Tokyo, Japan. May 18, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Tokyo University School of Pharmacy and Life Science, Hachioji, Japan. May 19, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Teikyo University, Sagamiko, Kanagawa, Japan. May 20, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Ajinomoto Co., Tokyo, Japan. May 21, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Sankyo Co., Tokyo, Japan. May 24, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Yamanouchi Pharmaceutical Co., Tsukuba, Japan. June 3, 1999. Title: "*The Oxazolidine Family of Antitumor Antibiotics: Total Synthesis and Deconvoluting Multiple Modes of Nucleic Acid Damage"*

Eisai Pharmaceutical Co., Tsukuba, Japan. June 4, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Pfizer Pharmaceutical Co., Nagoya, Japan. June 7, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Nagoya University, Nagoya, Japan. June 8, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Nagoya City University, Nagoya, Japan. June 9, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of Natural Products Synthesis and Studies on the Biosynthesis of Taxol: Synthesis and Labeling of Biosynthetically Relevant Taxoids".*

Nara Institute of Science and Technology, Nara, Japan. June 10, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Osaka City University, Osaka, Japan. June 11, 1999. Title: "*The Oxazolidine Family of Antitumor Antibiotics: Total Synthesis and Deconvoluting Multiple Modes of Nucleic Acid Damage".*

Osaka University, Osaka, Japan. June 14, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Kyowa Hakko Kogyo Co., Osaka, Japan. June 15, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Fujisawa Pharmaceutical Co., Osaka, Japan. June 16, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Studies on the Total Synthesis and Mechanism of Action of FR900482".*

Kyoto University, Kyoto, Japan. June 17, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Mitsubishi Corporation, Tokyo, Japan. June 18, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Sagami Chemical Research Institute, Tokyo, Japan. June 21, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Meiji Pharmacy College, Tokyo, Japan. June 22, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Monsanto Company, St. Louis, Missouri, September 28, 1999. Title: *"Recent Studies on the Synthesis of Amino Acid Derivatives, Peptide Isosteres and Natural Products".*

The State University of New York at Buffalo, Buffalo, N.Y., Oct. 15, 1999. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

2000

Cubist Pharmaceutical Co., Cambridge, Mass., January 18, 2000. Title: *"Total Synthesis of the Anti-MRSA Peptide Antibiotic TAN 1057 and Analogs".*

SmithKline Beecham Pharmaceutical Co., Philadelphia, Penn. April 17, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Schering-Plough Pharmaceutical Co., April 18, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products; Tools for Exploring and Exploiting Biomechanisms".*

The University of California at San Diego, May 22, 2000. Title: *"Asymmetric Stereocontrolled Total Synthesis of the Spirooxindole Alkaloids Spirotryprostatin B and Paraherquamide A".*

*The 83rd Canadian Society for Chemistry Conference, Calgary, Canada, May 28-31, 2000. Title: *"Asymmetric Total Synthesis of the Oxindole Alkaloids Paraherquamide A and Spirotryprostatin B".*

*Herbert C. Brown Distinguished Professorship Symposium, Purdue University, Sept. 28-29, 2000. Title: "*Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis".*

Gilead Sciences, San Francisco, California, October 27, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products; Tools for Exploring and Exploiting Biomechanisms".*

SmithKline Beecham Pharmaceutical Co., Philadelphia, Pennsylvania, November 1, 2000. Title: *"Studies on the Asymmetric Total Synthesis of FR900482, Tetrazomine and Spirooxindole Alkaloids".*

The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Title: "*Synthesis and Antimicrobial Evaluation of TAN1057A/B Analogs".*

2001

*University of California Irvine, UCI Synthesis Symposium on *Biological Tools and Targets*. January 20, 2001. Title: "*DNA Crosslinking Agents: Synthesis, New Methods for Activation and the Elucidation of New Targets in the Nucleus in vivo".*

*Michigan State University, Pfizer Lecturer (May 7-9, 2001). Titles: (1) *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids"*; (2) *"Antitumor Antibiotics: Mechanistic Discoveries, Synthesis and Exploitation"*; (3) *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis".*

*The 2001 CU-Array Biopharma Symposium on Medicinal and Synthetic Organic Chemistry, June 6-8, 2001, Boulder, Colorado. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

The 18th International Congress of Heterocyclic Chemistry, Yokohama, Japan, July 29 - August 3, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

The Kyowa Hakko Kogyo Co., Osaka, Japan. August 1, 2001. Title: *"Studies on the Total Synthesis of Tetrazomine, Bioxalomycin and Et743".*

57th Southwest Regional ACS Meeting, Symposium Honoring Al Meyers Retirement. San Antonio, Texas. October 18-19, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

2002

Ohio State University, Columbus, Ohio, March 7, 2002 Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Montana State University, Bozeman, Montana, March 22, 2002. Abbott Distinguished Lecturer. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Reaction Mechanisms Conference June 28 - July 1, 2002. Ohio State University. Title: *"Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids"*

*University of Wyoming Distinguished Summer Lecture Series. Laramie, Wyoming.

June 25 lecture #1: Title: *"Studies on the Asymmetric Synthesis of Amino Acids"*

June 26 lecture #2: Title: *"Elucidating the Biosynthesis of Taxol"*

July 17 lecture #3: Title: *"Total Synthesis and Biosynthesis of the Paraherquamides"*

July 18 lecture #4: Title: *"Synthesis and Mechanism of Action of FR900482"*

July 19 lecture #5: Title: *"Antitumor Agents Armed with an Oxazolidine: Synthetic and Biomechanistic Studies"*

*Belgian Organic Synthesis Symposium (BOSS IX), Namur, Belgium, July 8-12, 2002. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids"*

Karolinska Institute, Dept. of Biosciences at Novum, Huddinge, Sweden. August 16, 2002. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*American Chemical Society Meeting, Boston, Massachusetts, August 20, 2002, Arthur C. Cope Scholar Awardee lecture. Title: *"Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis"*

Western Washington University, Bellingham, Washington. October 15, 2002. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*November 4, 2002, College Colloquium Series, Colorado State University. Title: *"Vitalism in Natural Products: Finessing Molecular Secrets from Nature"*

November 6, 2002, Emory University, Atlanta, Georgia. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

December 2, The University of Minnesota, Minneapolis, Minnesota. Title: *"Studies on the Synthesis and Biology of FR900482 and the Paraherquamides"*

2003

January 24, 2003, Brigham Young University, Provo, Utah. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

March 17, 2003, Amgen, Inc., Thousand Oaks, California. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

April 17, 2003, The University of Florida, Gainseville, Florida. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

April 28, 2003. Colorado State University Department of Biochemistry. Title: *"Utilizing Organic Synthesis to Penetrate Drug-Nucleic Acid Interactions"*

October 10, 2003. Pennsylvania State University, State College, Pennsylvania. Title: *"Total Synthesis of Natural Products as a Vehicle for Penetrating Secondary Metabolism"*

*October 22, 2003, The 8th Loughborough Synthesis Symposium Sponsored by Astra Zeneca, Loughborough, Leicestershire, UK. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*November 4, 2003, The Barton Symposium. Heron Island, Australia. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*The University of Colorado, Boulder, Colorado. December 2, 2003. Roche Distinguished Lecture. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

2004

Celera Co., South San Francisco, CA January 13, 2004. Title: *"Synthesis and Mechanistic Studies on Antitumor Antibiotics"*

*March 1,2, Eli Lilly Grantee Symposium, Indianapolis, Indiana. Title: *"From Bicyclomycin to Biosynthesis"*

March 17, 2004. Merck & Co. West Point, Pennsylvania. Title: *"Synthesis of Biologically Intriguing Alkaloids"*

March 18, 2004. Aventis Pharmaceutical Co., New Jersey. Title: *"Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids"*

*March 19, 2004. *Chemistry as a Life Science Symposium XII*. Rutgers University, New Jersey. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*March 29, 30, 2004. ACS Meeting in Anaheim California. Tohru Fukuyama ACS Award Symposium. Title: *"Total Synthesis of Biologically Intriguing Natural Products"*

April 9, 2004. The University of Nebraska, Lincoln, Nebraska. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

April 27, 2004. The University of Iowa. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

April 28, 2004. Wayne State University. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

November 1, 2004. Colorado State University Veterinary Cancer Center. Title: "*Studies on Antitumor Antibiotics."*

*December 13, 2004. University of California, SanDiego *UCSD/Merck Symposium, Perspectives in Organic Synthesis*. Title: "*Studies on Biologically Intriguing Natural Products."*

2005

*January 28, 2005. Yale University, Connecticut Organic Chemistry Symposium, New Haven, Connecticut. Title: "*Exploiting Total Synthesis for Biological Intrigue."*

April 22, 2005. The University of Chicago. Title: "*Exploiting Total Synthesis for Biological Intrigue."*

April 25, 2005. Abbott Laboratories, Chicago, Illinois. Title: *"Total Synthesis of Biomedically Significant Alkaloids"*

April 26, 2005. The University of Illinois at Chicago. Title: "*Total Synthesis of Biologically Intriguing Natural Products."*

May 5, 2005. The University of Kansas. Title: *"Total Synthesis of Biologically Significant Alkaloids"*

*May 24, 2005. Imperial College, London, UK. Eli Lilly Lecture. Title: *"Vitalism in Natural Products: Finessing Molecular Secrets from Nature"*

May 25, 2005. Eli Lilly, England. Title: *"Total Synthesis of Biologically Significant Natural Alkaloids"*

*July 21, 2005 ***"****Synthesis in Organic Chemistry"* sponsored by the Perkin Division of the Royal Society of Chemistry, Oxford University, Oxford, England. Title: *"Total Synthesis of Biologically Significant Nitrogenous Substances"*

December 15-20, 2005. Pacifichem Congress Symposium on *"New Aspects of Heterocyclic Chemistry"* Title: *"Total Synthesis of Biologically Intriguing Natural Products"*

2006

*February 26 – March 2, 2006. 7th Winter Conference on Medicinal and Bioorganic Chemistry (WCMBC), Clearwater, Florida. Title: *Total Synthesis of Biomedically Significant Nitrogenous Substances*

*April 27, 2006. University of Oklahoma, *"Frontiers in Chemical Research"* Distinguished J. Clarence Karcher Lecturer. Title: *Total Synthesis of Biologically Intriguing Alkaloids*

May 1, 2006. Amgen, Inc., Cambridge, Mass. Title: "*New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

*May 2, 2006. Boston College, Boston, Mass. *Bristol-Myers Squibb Organic Chemistry Symposium*. Title: "*Total Synthesis as a Vehicle to Penetrate Biosynthesis and Biomechanism"*

May 17, 2006. Abbott Bioresearch Center, Worcester, Mass. Title: "*New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

*May 18, 2006. MIT, Organic Syntheses Lecture. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

May 19, 2006. Eisai Research Institute, Andover, Mass. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

*July 21-23, Tokushima 2006 Presymposium-Natural Product Chemistry, Tokushima, Japan. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*July 23-28, 2006 Kyoto, Japan. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products Chemistry. Title: *Total Synthesis of Biologically and Structurally Intriguing Alkaloids*

*July 30,31, Post-Symposium at Hokkaido University, Sapporo, Japan. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

*September 15-16, 2006. Inaugural Negishi-Brown Lectures. Purdue University, West Lafayette, Indiana. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids"*

September 25, 2006. Mexican Chemical Society 50th Anniversary Symposium. Mexico City, Mexico. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

2007

March 25-29, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Biomimetic Natural and Unnatural Product Synthesis".* Title: *"Biomimetic Total Syntheses of Prenylated Indole Alkaloids"*

March 26, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Asymmetric Synthesis of α-Amino Acids. Novel Developments and Future Directions".* Title: *"New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

April 27, 2007. University of Missouri-Columbia. Title*: "Harnessing Total Synthesis of Natural Products to Probe Their Biosynthesis"*

April 28, 2007. University of Missouri-Columbia. Organic Chemistry Day. Title: "New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

May 27 – June 2, 2007. Barton Symposium. *Organic Chemistry: Perspectives on the 21st Century IV.* Anse Chatanet Resort, St. Lucia, Caribbean Islands. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

September 19, Pfizer La Jolla Labs, San Diego, CA. Title: *"The Grubbs Methodological Series and Profound Implications for the Future of Natural Product Synthesis"*

September 20, 2007. Johnson & Johnson Pharmaceutical Research & Development, LaJolla, CA. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

*September 21, 2007. Scripps Research Institute. Roche Lecture. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles: Challenges in Natural Products Chemistry and the Exploitation of Synthesis"*

October 2, 2007. The University of Wisconsin, Madison, Wisconsin. Title: *"Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis"*

October 4, 2007. The University of Missouri, Kansas City, Missouri. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

November 8, 2007.Queen's University, Kingston, Ontario. Title*: "Total Synthesis as a Vehicle for Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

*November 9-11, 2007. *Quebec-Ontario Mini-symposium in Bio-Organic and Organic Chemistry* (QOMSBOC), University of Montreal, Quebec, Canada. Title: "*Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis"*

2008

*May 24-28, 2008. 91st Canadian Society for Chemistry Conference, Edmonton, Alberta, Canada. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

June 15-18, 2008. Regional ACS meeting, Park City, Utah. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

*July 22-25, 2008. The 9th Tetrahedron Symposium, Berkeley, California. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

July 24, 2008. Genentech. South SanFrancisco, CA. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles; Challenges in Natural Products Chemistry"*

October 13, 2008. Bristol-Myers Squibb Pharmaceutical Co., Princeton, N.J. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

October 14, 2008. Princeton University, Princeton, N.J. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

October 23, 2008. Symposium in Honor of Dr. Robin D.G. Cooper's 70th Birthday, Rigel Pharmaceutical Co., SanFrancisco, CA. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

2009

*January 25-29, 2009. Steamboat Winter Medicinal Chemistry Conference. Plenary Lecture Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

May 11, 2009. Indiana University, Bloomington, Indiana. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

June 28-July 3, 2009. Gordon Research Conference on Heterocyclic Chemistry, Newport, Rhode Island. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

*August 2-7, 2009. 22nd International Congress of Heterocyclic Chemistry, St. Johns, Newfoundland, Labrador, Canada. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

September 11, 2009. University of Notre Dame, South Bend, Indiana. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

2010

*August 22-26, 2010. Boston, Massachusetts. Symposium in Honor of Robert Burns Woodward. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

Honolulu, Hawaii, December 2010. Pacifichem Symposium #148: *Design and Synthesis of Biologically Active Compounds for Elucidating Mode of-Action*. Title: *Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors*

2011

*March 28, 2011. American Chemical Society 241st National Meeting, Anaheim, California. Ernest Guenther Award Symposium in Honor of Robert M. Williams, Award address. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

*June 26-30. Conference on Advances in Organic Synthesis. Hradec, Kralov, Czech Republic. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

October 3, 2011. Givaudan Co., Cincinnati, Ohio. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

October 28, 2011. University of Michigan, Ann Arbor, Michigan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

2012

*February 21, 2012 Texas Tech University. Henry J. Shine Endowment Lectureship. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

February 22, 2012 Texas Tech University. Public Lecture: Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

May 9, 2012. Merck & Co., Rahway, New Jersey. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

*June 4, 2012. Oregon State University, Corvalis, Oregon. James D. White Honorary Lecture. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

*June 26-29, 2012. 13th Tetrahedron Symposium, Amsterdam, the Netherlands. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

July 22-27, 2012. Proctor Academy, Andover, New Hampshire. Natural Products Gordon Research Conference. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

August 20, 2012. University of Alberta, Edmonton, Canada. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

October 18, 2012. ACS Rocky Mountain Regional Meeting, Cope Scholars Symposium. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

*November, 27-30, 2012. 13th Tetrahedron Symposium, Taiwan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

2013

January 11, 2013. University of Shizuoka, Shizuoka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 15, 2013. Kyoto University, Kyoto, Japan. Title: "*Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

January 16, 2013. Osaka City University, Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Alkaloids and Peptides"*

January 17, 2013. Osaka University, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 18, 2013. Kwansei, Gakuin University, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 21, 2013. Keio University, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 22, 2013. Tokyo Institute of Technology, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 23, 2013. Waseda University, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

January 31, 2013. Kumamoto University, Kumamoto, Japan. Title: *"Total Synthesis and Biosynthesis of Prenylated Indole Alkaloids of the Notoamide and Paraherquamide Families".*

February 5, 2013. Tokyo University of Science, Chiba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 6, 2013. Tsukuba University, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 6, 2013. Eisai Pharmaceutical Company, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 7, 2013. Toray Company, Kamakura, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 12, 2013. Dainippon-Sumitomo Company, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 13, 2013. Kaneka Corporation, Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

February 14, 2013. Shionogi Pharmaceutical Company, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 20, 2013. Kobe Pharmaceutical University, Kobe, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 21, 2013. Asubio Pharma, Co., Ltd., Kobe, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 22, 2013. Kyoto Pharmaceutical University, Kyoto, Japan. Title: *"Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

February 26, 2013. Institute of Microbial Chemistry, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 28, 2013. Otsuka Pharmaceutical Co., Tokushima, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 1, 2013. Tokushima University, Tokushima, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

March 6, 2013. Tokyo University of Science, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

March 12, 2013. Keio University, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 13, 2013. Chugai, Co., Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors and the Asymmetric Total Synthesis of Capreomycin"*

March 19, 2013. Mitsubishi Tanabe Pharma, Inc., Yokohama, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 21, 2013. Astellas Pharma, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 2, 2013. Daiichi Fine Chemicals, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 3, 2013. Gifu University, Gifu, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 4, 2013. Meiji Pharmaceutical University, Tokyo, Japan. Title: *"Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

April 9, 2013. ACS Meeting, New Orleans, Louisiana. Special Award Symposium in Honor of Professor Dale L. Boger, recipient of the 2013 Ralph Hirschmann Award, Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Alkaloids and Peptides"*

April 15, 2013. Kyowa-Kirin Co., Shizuoka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 17, 2013. Takasago International, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 18, 2013. Kyoto University, Faculty of Science, Kyoto, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 22, 2013. Tokyo University of Pharmacy and Life Sciences. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 23, 2013. Kitasato Institute, Tokyo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

April 24, 2013. Tokyo University, Tokyo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 20, 2013. Tohoku University, Faculty of Science, Sendai, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 21, 2013. Tohoku University, Faculty of Pharmaceutical Sciences, Sendai, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 22 & 23, 2013. Tokyo University, Faculty of Pharmaceutical Sciences, Tokyo, Japan.

Lecture 1, Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

Lecture 2, Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

Lecture 3, Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

Lecture 4, Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 24, 2013. Hokkaido University, Sapporo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 27, 2013. Teijin Pharma, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 29, 2013. Nagoya City University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 30, 2013. Nagoya University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 31, 2013. Kinjo Gakuin University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 6, 2013. Kyushu University, Kyushu, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 7, 2013. Nagasaki University, Nagasaki, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 10, 2013. Taisho Company, Tokyo, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

July 12, 2013. Daiichi Sankyo Co., Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

July 16, 2013. Astellas Pharma, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

July 17, 2013. Ajinomoto Company, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

July 18 & 19, 2013. Japanese Society for Process Chemistry, Tsukuba, Japan. Title: *"The Evolution of Synthetic Strategies: Lessons from Nature"*

July 22, 2013. Hamari Chemical Co., Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

October 7, 2013. Richmond University, Richmond, Virginia. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

October 8, 2013. College of William and Mary, Williamsburg, Virginia. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

October 21, 2013. Colorado State University, Department of Biochemistry & Molecular Biology. Title: "*Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

November 16, 2013. Charles R. Martin 60th Birthday Symposium, University of Florida, Gainseville, Florida. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

December 2, University of Pennsylvania, Philadelphia, Pennsylvania. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

2014

*June 30, 2014. The Barton Lecture, Imperial College, London. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

July 3, 2014. University of St. Andrews, St. Andrews, Scotland. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

July 4, 2014. Edinburgh University, Edinburgh, Scotland. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

2015

June 1-5, 2015. TERPNET 2015, Vancouver, Canada. Title: *"Prenylated Indole Alkaloid Biosynthesis: Reverse and Normal Prenyl Transferases Direct Rich Molecular Diversity"*

June 21-26, 2015. Salve Regina University, Rhode Island. Gordon Research Conference on Heterocyclic Compounds. Title: *"Synthesis and Biosynthesis of Heterocyclic Ring Systems in Natural Products Derived from Marine and Terrestrial Fungi"*

*November 15-19, 2015, 16th Brazilian Meeting on Organic Synthesis, Rio de Janeiro, Brazil. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

(*Plenary Lecture or Distinguished Named Lecture)

Conferences Attended

1. 183rd National ACS Meeting, Las Vegas, Nevada; March 1982 (Abstract #17), Division of Organic Chemistry.
2. Fourth International Conference on Organic Synthesis (IUPAC), Tokyo, Japan; August 1982 (Abstract #A-I-4301).
3. Post ICOS IV Symposium, "Highlights in Organic Synthesis", Hokkaido University, Sapporo, Japan; August 30-31, 1982. Title: *"A New and Efficient Cyclization Reaction to Construct the Bicyclomycin Ring System".*
4. 185th National ACS Meeting, Seattle, Washington; March 1983 (Abstract #10), Division of Organic Chemistry.
5. 187th National ACS Meeting, St. Louis, Missouri; April 1984 (Abstract #24), Division of Carbohydrate Chemistry.
6. 188th National ACS Meeting, Philadelphia, Pennsylvania; August 1984 (Abstract #92), Division of Organic Chemistry.
7. The 1984 International Chemical Congress of Pacific Basin Societies; December 1984 (Abstract #10E57), Division of Organic Chemistry.
8. The 1985 NSF Synthesis Workshop, Pingree Park, Colorado.
9. The 1985 Gordon Research Conference on "Organic Reactions and Processes", New Hampshire; July 17, 1985. Title: *"New Carbon-Carbon Bond-Forming Reactions via Electrophilic Glycine Derivatives".*
10. The 1985 Gordon Research Conference on "Natural Products", New Hampshire; July 24, 1985. Title: *"Progress Toward Understanding the Mechanism of Action of Bicyclomycin".*
11. The Third International Kyoto Conference on New Aspects of Organic Chemistry; November 1985.
12. The 1986 Gordon Research Conference on "Heterocycles"; July 9, 1986. Title: *"Electrophilic Glycinates: Versatile Templates for Amino Acid Synthesis".*
13. The 192nd National ACS Meeting, Anaheim, California; September 1986 (Abstract #152 and #272), Division of Organic Chemistry.
14. The 193rd National ACS Meeting, Denver, Colorado, April 1987 (Abstracts #22 and #81), Division of Organic Chemistry.
15. The 1987 Gordon Research Conference on "Heterocycles" July 6-10, 1987. Title: *"Electrophilic Glycinates: Versatile Templates for Amino Acid Synthesis".*
16. The 1987 NSF Workshop on Environmental Chemistry, Stanford Sierra Camp, Lake Tahoe, California, Sept. 25-27, 1987.
17. *Eli Lilly Grantee Symposium, Indianapolis, Indiana, March 7-8, 1988. Title: *"Approach to Understanding the Molecular Mechanism of Action of Bicyclomycin".*
18. The 9th Rocky Mountain Regional ACS Meeting, Symposium on Natural Products, Las Vegas, Nevada, March 28-30, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Brevianamide B".*
19. *Burgenstock Stereochemistry Conference, Burgenstock, Switzerland; April 28, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B".*
20. IUPAC 88 Kyoto 16th International Symposium on the Chemistry of Natural Products, Kyoto, Japan; May 31, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B".*
21. Nagoya Post-Symposium "New Topics on Natural Products", Toba, Japan; June 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B".*

22. Fourth International Symposium on "Recent Advances in the Chemistry of β-Lactam Antibiotics", Churchill College, Cambridge, England, July 4, 1988. Title: *"Synthesis and Properties of 1,3-Bridged β-Lactams: Novel Anti-Bredt β-Lactams"*.
23. *Eighth Biennial Carl S. Marvel Symposium, Tucson, Arizona, March 7, 1989. Title: *"Total Synthesis and Biogenetic Intrigue of the Brevianamide"* (Plenary Lecture).
24. The 1989 Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, December 17-22, 1989 (co-organizer of a Symposium on Amino Acids).
25. *The 1990 CU-Syntex Symposium, Boulder, Colorado, June 5-8, 1990. Title: *"Mechanistic and Synthetic Studies on the Mode of Cleavage of Superhelical DNA by Quinocarcin"*. (Plenary Lecture).
26. Special Bilateral U.S.-Britain Workshop on "Asymmetric Synthesis." July 3-8, 1990. Pingree Park, Colorado (RMW Co-organizer).
27. The 1990 Gordon Research Conference on "Organic Reactions and Processes," July 15-20, New Hampton, New Hampshire. Title: *"The Mechanism of Oxygen Reduction by Quinocarcin"*.
28. The 1990 Gordon Research Conference on "National Products," July 22-27, New Hampton, New Hampshire. Title: *"Bioorganic, Mechanistic and Synthetic Chemistry of Biologically Significant Nitrogenous Substances"*.
29. The 200th National ACS Meeting, August 27-31, 1990, Washington, DC.
30. NSF-JSPS Bilateral Seminar on "Selectivity in Synthetic and Bio-Organic Chemistry", Tokyo, Japan, June 3-7, 1991. Title: *"Unusual Facial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*.
31. *International Congress of New Drug Development, Seoul, Korea, August 21, 1991. Title: *"Synthetic and Mechanistic Studies on Anti-Tumor Antibiotics"*. (Plenary Lecture).
32. *4th Chemical Congress of North America, Division of Organic Chemistry, New York, August 29, 1991. Title: *"Unusual Stereofacial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*.
33. 24th Central Regional ACS Meeting (CMACS-92), Cincinnati, Ohio, Symposium on Amino Acids and Peptides, May 28, 1992. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids".*
34. Hoffman-La Roche, Inc., Workshop on Prospects for Antibacterial Agents Based on Diaminopimelic Acid, May 11, 1992. Title: *"Stereochemistry and DAP Inhibitors".*
35. *48th Southwest Regional ACS Meeting, Lubbock, Texas, October 21-23, 1992, A.I. Meyers Symposium. Title: *"Recent Studies in Asymmetric and Bioorganic Methods".* (Plenary Lecture).
36. * BioEast '93, Washington, D.C., January 27, 1993, Title: *"New Approaches to Antibiotic Design and Synthesis"*. (Plenary Lecture).
37. 3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic α-Amino Acids via Chiral Glycinates".*
38. *33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Symposium on *"Glycopeptide Resistance and the Cell Wall",* New Orleans, Louisiana, October 18, 1993. Title: *"Implications for Drug Discovery".* (Plenary Lecture).
39. *Sino-American Symposium on Asymmetric Synthesis, Taichung, Taiwan, April 8-9, 1994. Title: *"The Asymmetric Synthesis of α-Amino Acids".* (Plenary Lecture).
40. *The 4th International Conference on Chemical Synthesis of Antibiotics and Related Microbial Products, Nashville, Indiana, September 11-16, 1994. Title: *"Studies on the Mechanism of Action of Bioactive Nitrogenous Substances".* (Plenary Lecture).
41. *The SCI Meeting on Amino Acids, London, England, May 16, 1995. Title: *"Overview of the Major Conceptual Approaches to the Construction of Amino Acids"*. (Plenary Lecture).

42. *CU Hauser Symposium, May 29 & 30, 1997, Boulder, Colorado. Title: *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis".*
43. *The Gordon Research Conference on Natural Products, July 5-9, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*
44. *The 83rd Canadian Society for Chemistry Conference, Calgary, Canada, May 28-31, 2000. Title: *"Asymmetric Total Synthesis of the Oxindole Alkaloids Paraherquamide A and Spirotryprostatin B".*
45. *Herbert C. Brown Distinguished Professorship Symposium, Purdue University, Sept. 28-29, 2000. Title: "*Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis".* (Plenary Lecture).
46. The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Title: "*Synthesis and Antimicrobial Evaluation of TAN1057A/B Analogs".*
47. *University of California Irvine, UCI Synthesis Symposium on *Biological Tools and Targets*. January 20, 2001. Title: "*DNA Crosslinking Agents: Synthesis, New Methods for Activation and the Elucidation of New Targets in the Nucleus in vivo".* (Plenary Lecture).
48. *The 2001 CU-Array Biopharma Symposium on Medicinal and Synthetic Organic Chemistry, June 6-8, 2001, Boulder, Colorado. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".* (Plenary Lecture).
49. The 18th International Congress of Heterocyclic Chemistry, Yokohama, Japan, July 29 - August 3, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*
50. 57th Southwest Regional ACS Meeting, Symposium Honoring Al Meyers Retirement. San Antonio, Texas. October 18-19, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*
51. *Reaction Mechanisms Conference June 28 - July 1, 2002. Ohio State University. Title: *Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids.* (Plenary Lecture).
52. *Belgian Organic Synthesis Symposium (BOSS IX), Namur, Belgium, July 8-12, 2002. Title: *Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids.* (Plenary Lecture).
53. *American Chemical Society Meeting, Boston, Massachusetts, August 20, 2002, Arthur C. Cope Scholar Awardee lecture. Title: *"Total Synthesis of Natural Products: Tools for Probing and Expoliting Biomechanism and Biosynthesis"*
54. *The 8th Loughborough Synthesis Symposium Sponsored by Astra Zeneca, Loughborough, Leicestershire, UK, October 22, 2003. Title: *Total Synthesis of Natural Products of Biological Intrigue.* (Plenary Lecture).
55. *The Barton Symposium. Heron Island, Australia, November 4, 2003. Title: *"Total Synthesis of Natural Products of Biological Intrigue".* (Plenary Lecture).
56. *March 1,2, 2004. Eli Lilly Grantee Symposium, Indianapolis, Indiana. Title: *"From Bicyclomycin to Biosynthesis"*
57. *March 19, 2004. *Chemistry as a Life Science Symposium XII*. Rutgers University, New Jersey. Title: *"Total Synthesis of Natural Products of Biological Intrigue"* (Plenary Lecture).
58. *March 29, 30, 2004. ACS Meeting in Anaheim California. Tohru Fukuyama ACS Award Symposium. Title: *"Total Synthesis of Biologically Intriguing Natural Products"*
59. *December 13, 2004. University of California, SanDiego *UCSD/Merck Symposium, Perspectives in Organic Synthesis*. Title: "*Studies on Biologically Intriguing Natural Products."*
60. *January 28, 2005. Yale University, Connecticut Organic Chemistry Symposium, New Haven, Connecticut. Title: "*Exploiting Total Synthesis for Biological Intrigue."*
61. *July 21, 2005 *"Synthesis in Organic Chemistry"* sponsored by the Perkin Division of the Royal Society of Chemistry, Oxford University, Oxford, England. Title: *"Total Synthesis of Biologically Significant Nitrogenous Substances."*

62. December 15-20, 2005. Pacifichem Congress Symposium on *"New Aspects of Heterocyclic Chemistry"* Title: *"Total Synthesis of Biologically Intriguing Natural Products."*

63. February 26 – March 2, 2006. 7th Winter Conference on Medicinal and Bioorganic Chemistry (WCMBC), Clearwater, Florida. Title: "*Total Synthesis of Biomedically Significant Nitrogenous Substances."*

64. July 21-23, Tokushima 2006 Presymposium-Natural Product Chemistry, Tokushima, Japan. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

65. July 23-28, 2006 Kyoto, Japan. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products Chemistry. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids."*

66. July 30,31, Post-Symposium at Hokkaido University, Sapporo, Japan. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

67. *September 15-16, 2006. Inaugural Negishi-Brown Lectures. Purdue University, West Lafayette, Indiana. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids"* (Plenary Lecture).

68. September 25, 2006. Mexican Chemical Society 50th Anniversary Symposium. Mexico City, Mexico. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

69. March 25-19, 2007. The 233rd American Chemical Society National Meeting, Chicago, Illinois. Symposium on *"Biomimetic Natural and Unnatural Product Synthesis".* Title: "*Biomimetic Total Syntheses of Prenylated Indole Alkaloids"*

70. March 26, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Asymmetric Synthesis of α-Amino Acids. Novel Developments and Future Directions".* Title: *"New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

71. May 27 – June 2, 2007. Barton Symposium. *Organic Chemistry: Perspectives on the 21st Century IV.* Anse Chatanet Resort, St. Lucia, Caribbean Islands. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

72. *November 9-11, 2007. *Quebec-Ontario Mini-symposium in Bio-Organic and Organic Chemistry* (QOMSBOC), University of Montreal, Quebec, Canada. Title: "*Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis".* (Plenary Lecture).

73. *May 24-28, 2008. 91st Canadian Society for Chemistry Conference, Edmonton, Alberta, Canada. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

74. June 15-18, 2008. Regional ACS meeting, Park City, Utah. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

75. *July 22-25, 2008. The 9th Tetrahedron Symposium, Berkeley, California. Title: "*Total Synthesis of Natural Products of Biological Intrigue".* (Plenary Lecture).

76. October 23, 2008. Symposium in Honor of Dr. Robin D.G. Cooper's 70th Birthday, Rigel Pharmaceutical Co., SanFrancisco, CA. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

77. October 25, 2008. The Albert I. and Joan Meyers Symposium, Fort Collins, Colorado. (Symposium Organizer)

78. *January 25-29. Steamboat Winter Medicinal Chemistry Conference. Plenary Lecture Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

79. June 28-July 3, 2009. Gordon Research Conference on Heterocyclic Chemistry, Newport, Rhode Island. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

80. *August 22-26, 2010. 240th National American Chemical Society Meeting, Boston, Massachusetts. Robert Burns Woodward Memorial Symposium. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

81. *March 28, 2011. American Chemical Society 241st National Meeting, Anaheim, California. Ernest Guenther Award Symposium in Honor of Robert M. Williams, Award address. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

82. *June 26-30. Conference on Advances in Organic Synthesis. Hradec, Kralov, Czech Republic. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

83. *June 26-29, 2012. 13th Tetrahedron Symposium, Amsterdam, the Netherlands. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles"*

84. July 22-27, 2012. Natural Products Gordon Research Conference. Proctor Academy, Andover, New Hampshire. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

85. *October 18, 2012. ACS Rocky Mountain Regional Meeting, Cope Scholars Symposium. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

86. *November, 27-30, 2012. 13th Tetrahedron Symposium, Taiwan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

87. *July 18 & 19, 2013. Japanese Society for Process Chemistry, Tsukuba, Japan. Title: *"The Evolution of Synthetic Strategies: Lessons from Nature"*

88. July 28 – August 1, 2013. Natural Products Gordon Research Conference. Proctor Academy, Andover, New Hampshire.

89. November 16, 2013. Charles R. Martin 60th Birthday Symposium, University of Florida, Gainseville, Florida. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

90. June 1-5, 2015. TERPNET 2015, Vancouver, Canada. Title: *"Prenylated Indole Alkaloid Biosynthesis: Reverse and Normal Prenyl Transferases Direct Rich Molecular Diversity"*

91. June 21-26, 2015. Salve Regina University, Rhode Island. Gordon Research Conference on Heterocyclic Compounds. Title: *"Synthesis and Biosynthesis of Heterocyclic Ring Systems in Natural Products Derived from Marine and Terrestrial Fungi"*

92. November 15-19, 2015, 16th Brazilian Meeting on Organic Synthesis, Búzios, Brazil. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

(*Plenary Lecture or Distinguished Named Lecture)

Former Co-workers of Robert M. Williams

Department of Chemistry, Colorado State University

Former Graduate Students:

1. Robert W. Armstrong (Ph.D., August 1984)
Thesis entitled: *Total Synthesis of (±)- and (+)-Bicyclomycin*
NIH Postdoctoral Fellow with Y.Kishi, Harvard University (1984-86).
Professor of Chemistry, UCLA, 405 Hilgard Ave., Los Angeles, CA. August 1986-present
Director of Drug Discovery, Amgen Co., Thousand Oaks, California (1995-1999); Vice President of Discovery Research, Eli Lilly Pharmaceutical Co., Indianapolis, IN. (1999-2011). **Current position:** Independent Venture Capitalist.

2. Andrew O. Stewart (Ph.D., December 1985)
Thesis entitled: *Carbohydrates as Chiral Templates*
Current position: Volweiler Research Scientist at Abbott Laboratories, Abbott Park, IL.

3. Peter J. Sinclair (Ph.D., April 1987)
Thesis entitled: *Asymmetric Synthesis of Amino Acids via Electrophilic Glycinates*
Current position: Research Scientist in Drug Discovery at Merck & Co. Research Laboratories, Rahway, N.J.

4. Lynn K. Maruyama (Ph.D., September 1987)
Thesis entitled: *Synthesis and Study of Bicyclomycin Analogs*
Current position: Professor at Southern Oregon University, Ashland, Oregon

5. Paul P. Ehrlich (Ph.D., Spring 1989)
Thesis entitled: *Synthetic and Pharmacophoric Studies of Quinocarcin"*
Current position: Research Scientist at Bayer, AG, Germany

6. Myeong-Nyeo Im (Ph.D. Spring 1991)
Thesis entitled: *The Asymmetric Synthesis of Amino Acids via Glycine Enolate Alkylation*
Current position: Instructor in Seoul, Korea.

7. James A. Hendrix (Ph.D., Spring 1992)
Thesis Entitled: *The Asymmetric Synthesis of Arylglycines*
Postdoctoral Fellow with Prof. A.G.M. Barrett, Colorado State University, (1991-1992)
Current position: Head, Medicinal Chemistry at Sanofi Aventis Pharma, Bridgewater, New Jersey

8. Christopher Sean Esslinger (Ph.D., Fall 1996)
Thesis Entitled: *Studies Toward the Total Synthesis of Fusarin C*
Postdoctoral Fellow with Prof. Richard A. Chamberlin, The University of California , Irvine, September (1992-1995).
Current position: Deceased; formerly Associate Professor at the University of Montana, Missoula, Montana

9. Glenn J. Fegley (Ph.D., Fall 1994)
Thesis Entitled: *Asymmetric Synthesis of 1-Aminocyclopropane Carboxylic Acids*
Postdoctoral Fellow with Prof. William R. Roush, Indiana University, September 1993- August 1995.
Current position: Research Scientist at Onconova Therapeutics, Wynnewood Pennsylvania

10. Timothy D. Cushing (Ph.D., Fall 1993)
Thesis Entitled: *Total Synthesis of (+)-Paraherquamide B*
NIH Postdoctoral Fellow with Prof. Gregory L. Verdine, Harvard University, September 1993- June 1995.
Current position: Chemistry Principal Investigator at Amgen Co., SanFrancisco, California

11. Gregory F. Miknis (Ph.D., Fall 1993)
Thesis Entitled: *The Total Synthesis of Aspirochlorine*
NIH Postdoctoral Fellow with Prof. Phil Magnus, The University of Texas at Austin, (1993- 1995)
Current position: Associate Director, Colorado Center for Drug Discovery (C2D2), Fort Collins, Colorado

12. Tracy N. Tippie (MS., Summer 1995)
Thesis entitled: *Preparation of Azidobrevianamide A and Synthetic and Biological Studies of Tetrazomine*
Current position: physician

13. Mark E. Flanagan (Ph.D., Spring 1995)
Thesis entitled: *Synthesis and Biomechanistic Studies of Quinocarcin and Structural Analogs*
NIH Postdoctoral Fellow with Prof. Peter G. Schultz, The University of California at Berkeley, (1995-1997).
Current position: Research Scientist in Drug Discovery at Pfizer, Inc., Groton, Connecticut

Former Graduate Students of Robert M. Williams (continued)

14. Steven M. Rubenstein (Ph.D. Spring 1996)
Thesis entitled: *Elucidating the Biosynthetic Pathway of Taxol*
Postdoctoral Fellow with Prof. David A. Evans, Harvard University (1996-1998).
Current position: Senior Chemistry Scientist in Drug Discovery at Albany Molecular, Albany, New York.

15. Jennifer Travers (Ph.D., Spring 1996)
Thesis entitled: *Panning Peptide Libraries on Filamentous Phage*
Current position: Instructor at Oregon State University

16. Chester C. Yuan (Ph.D. Spring 1997)
Thesis entitled: *Part I. Asymmetric Synthesis of 2,6-Diaminopimelic Acids (DAP) and g-D(L)-Glutamyl-L-meso-Diaminopimelic Acid Dipeptide. Part II. Total Synthesis of TAN-1057 and Analogues*
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California.

17.Scott R. Rajski (Ph.D., Spring 1997)
Thesis entitled: *Mechanism of Action Studies on the FR900482 Class of Antitumor Antibiotics*
ACS Postdoctoral Fellow with Prof. Jacqueline Barton, Cal.Tech., Pasadena, California (1997-2000)
Current position: Research Associate at the University of Wisconsin, Madison, Department of Pharmacy

18. Samuel B. Rollins (Ph.D., Fall 1997)
Thesis entitled: *Synthesis of a Photoactivated Analog of the Antitumor Antibiotic FR900482*
Current position: Patent Attorney for Kilpatrick Stockton LLP, Winston-Salem, North Carolina

19. Paul B. Gansle (M.S., Fall, 1997)
Thesis Entitled: *Synthesis of D-Alanyl-D-Alanyl Dipeptide Isosteres and Cephalosporin Prodrugs*
Current position: Local High School Science Teacher.

20. David M. Bender (M.S., Spring 1998)
Thesis entitled: *Design and Synthesis of Analogs of the Peptidylnucleoside Antibiotics the Mureidomycins*
Current position: Research Associate in Drug Discovery at Eli Lilly Co., Indianapolis, Indiana

21. Brad Herberich (Ph.D., Fall 1999)
Thesis entitled: *Synthetic and DNA Cross-Linking Studies of Bioxalomycin a2*
NIH Postdoctoral Fellow with Prof. Peter G. Scultz, at the Scripps Institute. (1999-2001)
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California

22. Jeffrey Cao (Ph.D., Spring 2000)
Thesis entitled: *The Total Synthesis of (-)-Paraherquamide A*
Current position: Research Scientist in Drug Discovery at Merck & Co., New Jersey

23. Kathleen M. Halligan (Ph.D. Fall 2000)
Thesis entitled: *Synthetic and Biosynthetic Studies of the Brevianamides*
Current position: Assistant Professor, York College, York, Pennsylvania

24. Jack D. Scott (Ph.D. 2001)
Thesis entitled: *Total Synthesis of (-)-Tetrazomine and Biochemical Studies*
Current position: Research Scientist in Drug Discovery at Merck & Co., Rahway, New Jersey

25. Emily M. Stocking (Ph.D. 2001)
Thesis entitled: *Studies on the Biosynthesis of Paraherquamide A and a Total Synthesis of (+)-VM55599*
Current position: Research Scientist at R.W. Johnson Pharmaceutical Co., San Diego, California.

26. Christi Kosogof (M.S. 2001)
Thesis entitled: *Synthesis and Biochemical Activity of Pyrrolizidine Alkaloid Derivatives*
Current position: Research Associate at Abbott Laboratories, Chicago, Illinois.

27. Paul R. Sebahar (Ph.D. 2002)
Thesis entitled: *Asymmetric, Stereocontrolled Total Synthesis of (+)- and (-)-Spirotryprostatin B.*
Current position: Research Scientist at University of Utah, Salt Lake City, Utah.

28. Ted C. Judd (Ph.D. 2003)
Thesis entitled: *Asymmetric Total Synthesis of FR900482*
NIH Postdoctoral Fellow with Professor Yoshito Kishi, Harvard University, Cambridge, Mass. 2003-2005
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California

Former Graduate Students of Robert M. Williams (continued)

29. Steve Lenger (Ph.D. 2003)
Thesis entitled: *Studies on the total synthesis of putative intermediates in the biosynthesis of Taxol*
Current position: Research Scientist in Process Research at Array BioPharma, Boulder, Colorado.

30. Duane E. DeMong (Ph.D. 2003)
Thesis entitled: *Asymmetric Total Synthesis of Capreomycidine and Capreomycin IB*
Current position: Research Scientist at Merck & Co., Rahway, New Jersey

31. Brian K. Albrecht (Ph.D. 2003)
Thesis Entitled: *A Concise Total Synthesis of the TMC-95A and TMC-95B Proteasome Inhibitors*
Current position: Consulting Scientist, Third Rock Ventures, Cambridge, Mass.

32. Ryan E. Looper (Ph.D. 2004)
Thesis Entitled: *Concise Asymmetric Synthesis of the Cylindrospermopsin Alkaloids*
Current position: Associate Professor at The University of Utah, Salt Lake City, Utah

33. Chandele Gray (Ph.D. 2006)
Thesis Entitled: *Asperparaline A: Biosynthetic Studies and Synthetic Efforts*
Current position: High School teacher, Sumner, Maine

34. Yuyin Chen (Ph.D. 2006)
Thesis Entitled: *Approaches to the Total Synthesis of Lemonomycin*
Current position: Project Manager at BioDuro, China

35. Meriah W.N. Valente (M.S. 2006)
Thesis Entitled: *Studies Towards the Biomimetic Synthesis of the Stephacidin Family of Natural Products and the Concise and Versatile Synthesis of D,L-Brevianamide B, C-12A-epi-Malbrancheamide and Structurally Related Analogs*
Current position: Associate Research Scientist at Bristol-Myers Squibb Pharmaceutical Co., New Jersey

36. Alan R. Grubbs (Ph.D. 2007)
Thesis entitled: *"Concise Synthesis of Notoamides B-E and Stephacidin A"*
Current position: Research Scientist at Ardea Biosciences, San Diego, California.

37. Siyuan Chen (Ph.D. 2007)
Thesis entitled: *Studies Toward the Total Synthesis of Spiroquinazoline*
Current position: Research Scientist at Anichem, Inc.

38. Nick Gearhart (M.S. 2008)
Thesis entitled: *Studies Towards the Synthesis of a Bicyclo[2.2.2]diazaoctane Ring System and Efforts Towards the Synthesis of SB-219383*
Current position: Research Associate Scientist at Eisai Co.

39. Andrea Geiser (M.S. 2009)
Thesis entitled: *Progress Towards Proposed Biosynthetic Intermediates of Stephacidin A*
Current position: Research Associate Scientist at Merck & Co.

40. Ann E. Troutman (M.S. 2009)
Thesis entitled: *Progress Towards the Improved Synthesis of FK228 and Analogs; and the Total Synthesis of Largazole-Azumamide Hybrid*
Current position: Research Associate Scientist at Merck & Co.

41. Daniel A. Gubler (Ph.D. 2009)
Thesis entitled: *Mitomycin Alkaloids: Synthetic Studies*
Current position: Director of Unicity International, Orem, Utah.

42. Cameron Burnett (Ph.D. 2009)
Thesis entitled: *Studies Towards the Total Synthesis of Microsclerodermin G*
Current position: Ensign and Instructor for the U.S. Navy, Goose Creek, South Carolina.

43. Xiangna Jia (Ph.D. 2009)
Thesis entitled: *Progress Toward an Asymmetric Total Synthesis of the Stemona Alkaloid Tuberostemoninol.*
Current position: unknown (in China)

44. Tenaya L. Newkirk (Ph.D. 2009)
Thesis entitled: *Towards the Total Synthesis of 14-Acetoxygelsenicine and Synthesis of Largazole Analogs*
Current position: Instructor at Colorado State University

Former Graduate Students of Robert M. Williams (continued)

45. Brandon English (Ph.D. 2010)
Thesis entitled: *A Divergent Synthesis of Secologanin Derived Natural Products*
Current position: Assistant Professor at Red Rocks Community College, Lakewood, Colorado.

46. Tatyana Sabodash (M.S. 2011)
Thesis entitled: *Studies Toward the Total Synthesis of Lydiamycin A*
Current position: Trainee European patent attorney, Lederer and Keller, Germany

47. Timmy McAfoos (Ph.D. 2011)
Thesis entitled: *Studies on the Biosynthesis of the Stephacidins and Notoamides. Total Synthesis of Notoamide S and Notoamide T and Progress Toward the Synthesis of Chrysogenamide A.*
Current position: Research Associate at the M.D. Anderson, Cancer Research Center, Texas

48. Jennifer Finefield (Ph.D. 2011)
Thesis entitled: *Studies on the Biosynthesis of Prenylated Indole Secondary Metabolites from Aspergillus versicolor and Aspergillus sp. and A Novel Approach to Tumor Specific Drug Delivery: Use of a Naphthyridine Drug Linker with a DNA Hairpin.*
Current position: Technology Transfer Manager, Indiana University, Bloomington, Indiana

49. Ryan Rafferty (Ph.D. 2011)
Thesis entitled: *Total Synthesis of Hapalindoles J and U, Formal Synthesis of Hapalindole O, Synthesis of of the Proposed Biosynthetic Precursor to Hapalindole K and Work Towards the Ambiguine Family of Alkaloids.*
Current position: Assistant Professor, Kansas State University, Manhattan, Kansas.

50. Paul Schuber (Ph.D. 2011)
Thesis entitled: *Studies on the Total Synthesis of MPC1001*
Current position: Research Associate at the M.D. Anderson, Cancer Research Center, Texas

51. Guojun Pan (Ph.D. 2011)
Thesis Entitled: *Total Syntheses of (+)-Fawcettimine, (+)-Fawcettidine, (+)-Lycoflexine and (+)-Lycoposerramine B.*
Current Position: post-doctoral research associate with Prof. Liming Zhang, University of California, Santa Barbara

52. Timothy R. Welch (Ph.D. 2012)
Thesis entitled: *Epidithiodioxopiperazines: Synthetic Studies of (+)-Chetomin and (-)-Sporidesmin A.*
Current Position: Consulting Scientist, Wilson, Sonsini, Goodrich & Rosati, San Francisco, California

53. Aaron Pearson (Ph.D. 2013)
Research Project: *Asymmetric Total Synthesis of Zetekitoxin and Saxitoxin*
Current Position: post-doctoral research associate with Prof. Peter G. Schultz, Scripps Research Institute, La Jolla, California

54. Marie Trujillo (M.S. 2013)
Research Project: *Synthesis & Biosynthesis of the Notoamides*
Current Position: QC Analyst at Leprino Foods, Greeley, Colorado

55. Alberto Jimenez (Ph.D. 2013)
Thesis Entitled: *Synthetic Studies on (-)-Lemonomycin: Construction of the Tetracyclic Core.*
Current Position: Industrial post-doctoral Research Associate at Emory Institute for Drug Development, Emory University.

56. Michelle Sanchez (Ph.D. 2014)
Thesis Entitled: *The Synthesis of the Pentacyclic Carbon Framework of the PF1270 Family of Natural Products.*
Current Position: Post-doctoral research associate at the University of Pennsylvania with Prof. Jeff Winkler, Philadelphia, Pennsylvania.

Former Postdoctoral Fellows of Robert M. Williams
Department of Chemistry, Colorado State University

1. Dr. Jen-Sen Dung (January 1982-October 1984)
Research Project: *Total Synthesis of Bicyclomycin and Bicyclomycin Analogs*
Current position: Research Scientist at Johnson Matthey Pharmaceuticals, West Deptford, N.J.

2. Dr. Kohtaro Tomizawa (December 1984 - December 1986)
Research Project: *Mechanism of Action of Bicyclomycin*
Current position: Associate Professor at Suzuka College of Tech. Shiroko-cho, Suzuka, Nie 510-02, Japan

3. Dr. Mark Kirms (January 1985 - April 1987)
Research Project: *Development of Synthetic Methodology for the Synthesis of Carbocycles*
Employed at Astronautics Laboratories, AL/LSX, Building 8451, Edwards, AFB, CA 93523
Current position: Associate Professor at Southern Oregon State College

4. Dr. Dongguan Zhai (April 1985 - November 1986)
Research Project: *Practical Synthesis of the Williams Amino Acid Templates and the Asymmetric Synthesis of α-Amino Acids*
Current position: Research Professor, at the Chinese Academy of Sciences, Chengdu Institute of Organic Chemistry, Chengdu, PRC

5. Dr. Tomasz Glinka (April 1985 - February 1987 and April 1989 - November 1990)
Research Project: *Asymmetric total synthesis of Brevianamide B*
Research Scientist at the Polish Academy of Sciences, Warsaw, Poland 1987-1989
Current position: Independent consultant

6. Dr. Eduard J. Brunner (April 1987 - November 1987)
Research Project: *Total Synthesis of Verruculotoxin*
Current position: Production Chemist at Novartis Widhagweg, Kaiseraugst, Switzerland

7. Dr. Byung H. Lee (September 1985 -January 1988)
Research Project: *Synthesis of the First [1,3]-Bridged β-Lactam*
Current position: Research Scientist in Drug Discovery at Pfizer, Kalamazoo, Michigan

8. Dr. Weixu Zhai (December 1985 -December 1987)
Research Project: *Methods Development for the Asymmetric Synthesis of α-Amino Acids and Peptide Isosteres.*
Associate Professor at the Lanzhou Institute of Chemical Physics, Academia Sinica, Chinese Academy of Sciences, Lanzhou, Gansu, PRC
Current position: Research Scientist at Bristol-Myers Squibb PRI, Wallingford, Connecticut.

9. Dr. Ewa Kwast (April 1987-October 1988)
Research Project: *Asymmetric total synthesis of Brevianamide B*
Current position: Translator for the Polish Government

10. Dr. Andrejz Kwast (April 1987-October 1988)
Research Project: *Development of Synthetic Methodology to Synthesize Bicyclomycin Analogs*
Current position: Research Scientist at the Polish Academy of Sciences, Institute of Organic Chemistry

11. Dr. Daimo Chen (April 1987-April 1989 and September 24, 1999 - December 8, 2000)
Research Project: *Practical Synthesis of the Williams Lactone and Synthesis of TAN-1057 Analogs*
Current position: Research Scientist at the Chinese Academy of Sciences, Chengdu Institute of Organic Chemistry)

12. Dr. Maria Wudlikow (April 1989 - November 1990)
Research Project: *Asymmetric Total Synthesis of Brevianamide B*
Current position: Research Scientist in Drug Discovery at Essential Therapeutics Co., Mountainview, CA

13. Dr. Mark Sabol (October 1987-August 1989)
Research Project: *Total Synthesis of Carbabicyclomycin*
Current position: retired

14. Dr. David J. Aldous (February 1988-October 1989)
Research Project: *Asymmetric Synthesis of α-Amino Acids via [1,3]-Dipolar Cycloadditions; Synthesis of Ethynylglycine*
Current position: Director of Drug Discovery at Sanofi-Aventis Pharma, Bridgewater, N.J.

Former Postdoctoral Fellows of Robert M. Williams (continued)

15. Suzanne C. Aldous (July 1989-October 1989)
Research Project: *Asymmetric Synthesis of α-Amino Acids via [1,3]-Dipolar Cycloadditions; Synthesis of Ethynylglycine*
Current position: Research Scientist in Drug Discovery at Sanofi-Aventis Pharmaceutical Co., Bridgewater, N.J.

16. Dr. Gyoosoon Park (October 1988-February 1, 1990)
Research Project: *Studies on the Total Synthesis of Quinocarcin and Quinocarcin Analogs*
Current position: Professor of Chemistry at Kookmin University, Seoul, Korea)

17. Dr. Hee-do Kim (November 1989-September 1990)
Research Project: *Total Synthesis of Carbabicyclomycin*
Current position: Assistant Professor at Soak Myoung Woman's University, School of Pharmacy, Seoul, Korea

***18. Dr. Nobuyoshi Yasuda** (July 1988 - August 1989)
Research Project: *Approach to the Total Synthesis of FR900482*
Current position: Senior Investigator, Process Research & Development at Merck & Co. Rahway, NJ

19. Dr. Norbert Richter (February 1991-August, 1991)
Research Project: *Development of Synthetic Methodology for the Asymmetric Synthesis of Amino Acids*
Current position: Research Scientist at Boehringer Mannheim, Germany

***20. Dr. Mary Dosch-Doubleday** (July 1991-December 3, 1992)
Research Project: *Display of Phage Combinatorial Libraries*
Merck Postdoctoral Fellow (1991-1993)
Current position: Research Scientist at Protarga Co., Exton, Pennsylvania

***21. Dr. Yusuke Amino** (July 1991-August, 1993)
Research Project: *Asymmetric Total Synthesis of Actinoidic Acid*
Current position: Research Scientist in Drug Discovery at Ajinomoto Co., Japan

22. Dr. Pierre-Jean Colson (March 1, 1994 - August 31, 1994)
Research Project: *Asymmetric Synthesis of Hydroxymethylene Peptide Isosteres and a Total Synthesis of Statine*
Current position: Research Scientist in Drug Discovery at G.D. Searle Co., Skokie, Illinois

***23. Dr. Matt A. Peterson** (December 1, 1992 - December 1, 1994)
Research Project: *Display of Phage Combinatorial Libraries*
**NIH Postdoctoral Fellow, Colorado State University, 1992-1994*
Current position: Associate Professor of Chemistry, Brigham Young University, Provo, Utah

***24. Dr.Monica Baloga** (June 1, 1994-May 31, 1995)
Merck Postdoctoral Fellow, Colorado State University 1994-1995
Research Project: *Display of Phage Combinatorial Libraries*
Current position: Assistant Professor at Florida Institute of Technology, Melbourne, Florida

25. Dr. Jiwen Liu (October 29, 1996 - October 15, 1997)
Research Project: *Asymmetric Synthesis of 2,7-Diaminosuberic Acid*
Current position: Research Scientist in Drug Discovery at Amgen, Inc., SanFrancisco, California)

26. Dr. Claude Quesnelle (September 23, 1996 -July 17, 1998)
Research Project: *Total Synthesis of Lightly Oxygenated Natural Taxoids*
Current position: Research Scientist at Bristol-Myers Squibb Pharmaceutical Co., New Jersey

27. Dr. Florenci V. Gonzalez Adelantado (July 8, 1998 - October 30, 1998, Visiting Professor from Spain)
Research Project: *Total Synthesis of Asperparaline A*
Current position: Assistant Professor at the University of Jaume, Campus de Borriol, Spain

28. Dr. David Hennings (December 1, 1997 - November 30, 1999)
Research Project: *Asymmetric Total Synthesis of Mureidomycin*
Current position: Research Scientist at Array Biopharma Co., Boulder, Colorado

29. Dr. Jetze Tepe (January 9, 1998 - July 12, 2000)
Research Project: *Synthesis of Photoactivated Progenitors of Dehydromonocrotaline*
Current position: Associate Professor at Michigan State University

30. Dr. Alfredo Vazquez (March 1, 1999 - August 15, 2000)
Total Synthesis of Lightly Oxygenated, Natural Taxoids, Asymmetric & Total Synthesis of Bioxalomycin α_2.
Current position: Assistant Professor at the University of Mexico

Former Postdoctoral Fellows of Robert M. Williams (continued)

***31. Dr. Hidekazu Tsujishima** (May 10, 1999 - March 31, 2000)
JSPS Fellowship at Colorado State University
Research Project: *Asymmetric Total Synthesis of Paraherquamide A*
Current position: Research Scientist at Tanabe Pharmaceutical Co., Japan

***32. Dr. Masahiko Kinugawa** (October 4, 1999 - September 30, 2000)
Research Project: *Asymmetric Total Synthesis* of *Bioxalomycin α2*
Current position: Research Scientist at Kyowa Hakko Kogyo Pharmaceutical Co., Japan

33. Professor Juan F. Sanz-Cervera (June 24, 1996 - August 31, 1996; July 9, 1997 - September 12, 1997; July 8, 1998 - September 11, 1998; June 10, 1999 - September 25, 1999; June 1, 2000 - September 25, 2000; February 1, 2001 - September 15, 2001 Associate Professsor from the University of Valencia)
Research Project: *Asymmetric Total Synthesis of (+)-Paraherquamide B, Biomimetic Total Synthesis of Brevianamide B, Biomimetic Total Synthesis of VM55599, Biosynthesis of Paraherquamide A, Brevianamide B, Austamide and VM55599.*
Current position: Professor at the University of Valencia, Spain.

34. Dr. Yutaka Aoyagi (February 18, 1997 - August 12, 1998)
Research Project: *Asymmetric Synthesis of Amino Acids and Peptide Isosteres*
Current position: Professor, College of Pharmacy, Kinjo Gakuin University, 2-1723 Omori, Moriyama-ku, Nagoya

35. Dr. Rajendra P. Jain (July 3, 2000 – June 28, 2002)
Research Project: *Asymmetric Synthesis of Amino Acids and Peptide Isosteres*
Current position: Associate Director, Medivation, Inc. India.

***36. Dr. Kosuke Namba** (April 26, 2001-May 1, 2003)
Research Project: *Asymmetric Total Synthesis of Palau'amine*
**JSPS Postdoctoral Fellow*
Current position: Professor at Tokushima University, Tokushima, Japan.

37. Dr. Sammy Metobo (Ph.D. from Rutgers University with Prof. Leslie S. Jimenez)
Research Project: *Asymmetric Total Synthesis of Bioxalomycin α2*
Current position: Research Scientist at Gilead Sciences, California

38. Dr. Rhona J. Cox (Ph.D. from Oxford University, UK, with Prof. Sir Jack E. Baldwin)
Research Project: *Biomimetic Total Synthesis of Paraherquamide A*
Current position: Research Scientist at Astra-Zeneca Pharmaceutical Co., Loughborough, UK

39. Dr. Mick Grady (Ph.D. from The University of Bristol with Prof. Kevin I. Booker-Milburn)
Research Project: *Asymmetric Total Synthesis of TMC-95A-D Analogs*
Current position: Research Scientist at Rhodia ChiRex, UK

***40. Dr. Makoto Mori** (Ph.D. from Gifu University)
Research Project: *Asymmetric, Stereocontrolled Total Synthesis of Quinine*
Current position: Research Scientist at Sankyo Pharmaceutical Co., Japan

41. Dr. Kim Dastlik (Ph.D. from Murdoch University, Australia)
Research Project*: Asymmetric Synthesis of Amino Acids*
Current position: unknown

***42. Dr. Tomoyuki Onishi** (PhD from Tokyo Institute of Technology)
Research Project: *Asymmetric Synthesis of Spirotryprostatin A and the Asymmetric Synthesis of Peptide Isosteres*
Current position: Research Scientist at Ajinomoto Co., Japan

43. Dr. Guiru Zhang (Ph.D. from Vanderbilt University with Prof. Ned Porter)
Research Project: *Asymmetric Total Synthesis of Mitomycin C*
Current position: Research Scientist at Procter & Gamble Co., Cincinnati, Ohio

44. Dr. Uta Sundermeier (Ph.D. from the Institut für organische Katalyseforschung an der Universität Rostock, Germany with Prof. Matthias Beller)
Research Project*: Asymmetric Synthesis of styloguanidine*
Current position: Research Scientist at Henkel KGAA, Duesseldorf, Germany

Former Postdoctoral Fellows of Robert M. Williams (continued)

45. Dr. Alan Stewart (Ph.D. from Imperial College with Prof. Donald Craig)
Research Project*: Asymmetric Synthesis of Stephacidins A,B, avrainvillamide and paraherquamide F*
Current position: Postdoctoral fellow in Finland

***46. Dr. Kateri Ahrendt** (PhD, University of California, Berkeley with Profs. Jon Ellman and Robert Bergman).
Research Project: *Asymmetric Synthesis of Nakadomarin.*
**NIH post-doctoral fellow*
Current Position: Associate Professor at Regis College, Denver, Colorado

47. Dr. Luke Adams (Ph.D. from the University of Bristol, England with Prof. Russell J. Cox)
Research Project: *Asymmetric Total Synthesis of Asperparaline A*
Current Position: Research Scientist at Cellaura Co., Nottingham, UK

48. Dr. Pascal Ducept (Ph.D. from Imperial College, London with Prof. Donald Craig)
Research Project: *Synthesis of Biological Probes Based on FR900482*
Current Position: unknown

***49. Dr. Tohru Horiguchi** (Ph.D. from Tohoku University, Japan with Prof. Kyouzou Suyama).
Research Project: *Synthesis of Taxol Biosynthetic Intermediates.*
**JSPS Postdoctoral Fellow*
Current Position: Research Associate at Nagase & Co., LTD, Japan

***50. Dr. Jonathan Lane** (Ph.D. from the University of Colorado, Boulder, Colorado with Prof. Randall Halcomb).
Research Projects: *Asymmetric Total Synthesis of Jorumycin, Renieramycin G, Saframycin A and Communesin.*
**NIH post-doctoral fellow*
Current Position: Research Scientist at Array BioPharma, Boulder, Colorado

***51. Dr. Yasuo Noguchi** (Ph.D. from Tokyo University with Prof. Susumu Kobayashi).
Research Project: *Total Synthesis of FK228.*
**Sponsored by Sankyo Co., Japan*
Current Position: Research Scientist at Sankyo Co., Japan

52. Dr. Hidenori Namiki (Ph.D. from Hoshi University with Prof. Toshio Honda).
Research Project: *Asymmetric Synthesis of Biosynthetic Intermediates for FR900482 and Mitomycin C*
Current Position: Research Scientist at Sankyo Co., Japan

***53. Dr. Konrad Sommer** (Ph.D. from the University of Göttingen, Germany with Prof. Lutz Tietze).
Research Project*: Asymmetric Synthesis of Paraherquamide F and Paraherquamide Biosynthetic Intermediates.*
Current Position: Research scientist at Carbogen Amcis, Switzerland.

***54. Dr. Gerald D. Artman III.** (Ph.D. from Pennsylvania State University with Prof. Steven M. Weinreb)
Research Project: Asymmetric Total Synthesis of Stephacidin A, Stephacidin B and Notoamide B.
**NIH post-doctoral fellow*
Current Position: Research Scientist at Kalexsyn, Inc., Kalamazoo, Michigan.

55. Dr. Esther González Cantalapiedra (Ph.D. from the Universidad Autónoma de Madrid with Prof. Antonio Echavarren)
Research Project: *Asymmetric Total Synthesis of Nakadomarin A*
Current Position: Research Scientist at the Medicinal Chemistry Department at the Spanish National Cancer Research Centre (Centro Nacional de Investigaciones Oncologicas-CNIO) in Madrid.

56. Dr. Guillaume Vincent (Ph.D. from Laboratoire de Synthese et Methodologie Organiques Universite Claude Bernard Lyon with Prof. Marco Ciufolini)
Research Project: *Asymmetric Total Synthesis of Cribrostatin-4*
Current position: *"Chargé de Recherche"* CNRS at the Université Paris-Sud XI at Orsay

***57. Dr. Deidre M. Johns** (Ph.D. from the University of Colorado, Boulder, Colorado with Prof. Tarek Sammakia).
Research Project: *Asymmetric Total Synthesis of Quinine and Antimycin A3.*
**American Cancer Society post-doctoral fellow*
Current Position: Research Assistant Professor at the Oregon State University

58. Dr. Dan Fishlock (Ph.D. from the University of Waterloo with Prof. Eric Fillion)
Research Project: *Asymmetric Total Synthesis of Ecteinascidin 743*
Current Position: Research Scientist at Hoffman-La Roche, Inc., Basel, Switzerland.

Former Postdoctoral Fellows of Robert M. Williams (continued)

59. Dr. Stephen Chamberland (Ph.D. from the University of California, Irvine with Prof. Keith Woerpel)
Research Project: *Synthesis of isotopically labeled mitomycin C and FR900482 biosynthetic intermediates*
Current Position: Assistant Professor of Chemistry, Central Washington University

60. Dr. Thomas J. Greshock (Ph.D. from Pennsylvania State University with Prof. Raymond L. Funk)
Research Project: *Total Synthesis of FK228;Total Synthesis of Stephacidins A&B; Total Synthesis of Palau'amine*
Current Position: Research Scientist at Merck & Co., West Point, Pennsylvania

61. Dr. Aaron Smith (Ph.D. from the University of North Carolina at Chapel Hill with Prof. Michael Crimmins)
Research Project: *Asymmetric Total Synthesis of Quinine and Oligonucleotide Silencing of Gene-Shuffling in Cancer Cells*
Current Position: Research Scientist at Pfizer, Inc., Groton, Connecticut

***62. Albert A. Bowers** (Ph.D. from the University of Illinois, Chicago, with Prof. David Crich)
Research Project: *Total Synthesis of Largazole and Related HDAC Inhibitors*
Current Position: Assistant Professor at University of North Carolina, Chapel Hill

63. Dr. Hui Li (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: *Asymmetric Total Synthesis of Palau'amine and Taxol Biosynthetic Intermediates*
Synthesis of Inducers of Fetal Hemoglobin (HemaQuest Pharmaceuticals)
Current Position: Research Scientist at Anichem

64. Dr. Carolyn Selenski (Ph.D. from the University of California, Santa Barbara with Prof. Thomas R.R. Pettus)
Research Project: *Asymmetric Total Synthesis of Bioxalomycin and Lemonomycin*
Current Position: Research Scientist at Glaxo SmithKline, Research Triangle Park, North Carolina

65. Dr. Kenny Miller (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: *Asymmetric synthesis of Versicolamide B and related prenylated indole alkaloids; synthesis of largazole analogs and other HDAC inhibitors.*
Current Position: Assistant Professor at Fort Lewis College, Durango, Colorado.

66. Dr. Takeshi Yamada (Ph.D. from Osaka City University, Japan with Prof. Yasufumi Ohfune)
Research Project: *Asymmetric Total Synthesis of Nakadomarin A and Et-743*
Current Position: Assistant Professor at the Kitasato Institute, Japan with Prof. Sunazuka

67. Dr. Cameron Burnett (Ph.D. from Colorado State University, with Prof. Robert M. Williams)
Research Project: *Synthesis of Adjuvants* (Infectious Diseases Research Institute)
Current Position: Instructor for the U.S. Naval Academy

***68. Dr. Makoto Inai** (Ph.D. from Shizouka University, Japan with Prof. Toshiyuki Kan)
Research Project: *Asymmetric Total Synthesis of Palau'amine and Et-743*
Current Position: Assistant Professor, Tokushima Bunri University, Tokushima, Japan
**Uehara Foundation Post-doctoral Fellow*

69. Dr. Ludwig Kaspar (Ph.D. at Ludwig-Maximilians-University, Munich with Prof. Dr. Lutz Ackermann)
Research Project: *Synthesis of Adjuvants*
Current Position: Research Scientist at HWR Chemie, Germany

70. Dr. Michael A. Christiansen (Ph.D. from Brigham Young University with Prof. Merit Andrus)
Research Project: Asymmetric Total *Synthesis of Et-743 and the Synthesis of Et-743 Biosynthetic Intermediates.*
Current Position: Assistant Professor at Utah State University

71. Dr. Sumit Dey (Ph.D. from Indian Institute of Chemical Biology, West Bengal, India, with Prof. P.Jaisankar)
Research Project: *Synthesis of Inducers of Fetal Hemoglobin* (HemaQuest Pharmaceuticals); *Synthesis of HDAC Inhibitors; Synthesis of Adjuvants.*
Current Position: currently unemployed

72. Dr. James E. Sunderhaus (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: Total Synthesis of Notoamides and Stephacidins.
Current Position: Generic Pharmaceutical

73. Dr. Jennifer M. Finefield (Ph.D. from Colorado State University, with Prof. Robert M. Williams)
Research project: Synthesis of new HDAC inhibitors.
Current Position: Indiana University, Technology Transfer Officer

Former Postdoctoral Fellows of Robert M. Williams (continued)

74. Dr. Santhosh Reddy Jangari (Ph.D. from the Indian Institute of Chemical Technology, with Prof. B. Venkateswara Rao)
Research Project: *Synthesis of Adjuvants, pkC-δ Inhibitors.*
Current position: unemployed

75. Dr. Masashi Yokoya (Ph.D. from Meiji Pharmaceutical University, Japan with Prof. Naoki Saito)
Research project: *Synthesis of Et743 and Aclindomycin*
Current position: Assistant Professor at Meiji Pharmaceutical University, Japan

76. Dr. Dane Clausen (Ph.D. from the University of Pittsburgh, with Prof. Paul E. Floreancig)
Research Project: Synthesis of Largazole Analogs
Current position: Research Scientist at Merck & Co., Rahway, New Jersey

77. Dr. Amber Somoza (Ph.D. from the University of Southern California, with Prof. Clay C.C. Wang)
Research project: *Synthesis and Biosynthesis of Prenylated Indole Alkaloids*
Current Position: Research Scientist at Gilead Pharmaceuticals, San Dimas, California

78. Dr. Jiyu Wang (Ph.D. from Chengdu Institute of Organic Chemistry)
Research Project: Synthesis of Largazole Analogs & PKC-δ Inhibitors
Current Position: Professor at the Chengdu Institute of Organic Chemistry, Chengdu, China

***79. Dr. Kazutada Ikeuchi** (Ph.D. from Shizuoka University with Prof. Toshiyuki Kan)
Research Project: *Total Synthesis of Citrinalin*
Current Position: Assistant Professor of Chemistry, Kwansei Gaikun University, Japan

**Post-docs with their own fellowship support or corporate sponsor.*

Current Research Group of Robert M. Williams
Department of Chemistry, Colorado State University

Current Graduate Students

1. Vy Le (Ph.D. expected 2015)
Research Project: *Synthesis of Et-743*

2. Nathan Bair (Ph.D. expected 2015)
Research Project: *Synthesis of Okaramine M*

3. Christine Dunne (Ph.D. expected 2019)
Research Project: (not yet assigned)

4. Jonathan Thielman (Ph.D. expected 2019)
Research Project: (not yet assigned)

5. Kimberly Klas (Ph.D. expected 2019)
Research Project: (not yet assigned)

Current Post-doctoral Associates

1. Dr. Le Zhao (Ph.D. from Tohoku University with Prof. Masahiro Hirama)
Research Project: Synthesis of Largazole Analogs

Former Co-workers of Robert M. Williams in Academia:

Robert W. Armstrong	University of California at Los Angeles, U.S.A. (adjunct)
Scott R. Rajski	University of Wisconsin, Madison, U.S.A.
Jetze Tepe	Michigan State University, U.S.A.
Matt A. Peterson	Brigham Young University, U.S.A.
Lynn Maruyama-Kirms	Southern Oregon State University, U.S.A.
Christopher Sean Esslinger	University of Montana, U.S.A. (deceased)
Jennifer Travers	Oregon State University, U.S.A.
Ryan E. Looper	University of Utah
Monica Baloga	Florida Institute of Technology, U.S.A.
Kohtaro Tomizawa	Suzuka College of Technology, Japan
Gyoosoon Park	Kookmin University, Korea
Dongguan Zhai	Chengdu Institute of Organic Chemistry, China
Daimo Chen	Chengdu Institute of Organic Chemistry, China
Hee-do Kim	Soak Myoung Woman's University, Korea
Florenci V. Gonzalez Adelantado	University of Jaume, Spain
Alfredo Vazquez	University of Mexico, Mexico
Juan F. Sanz-Cervera	University of Valencia, Spain
Yutaka Aoyagi	Kinjo Gaikuin University, Japan
Stephen Chamberland	Central Washington University
Kenny Miller	Fort Lewis College, Colorado
Brandon English	Red Rocks Community College
Tenaya Newkirk	Colorado State University
Takeshi Yamada	Kitasato Institute, Japan
Makoto Inai	University of Shizuoka, Japan
Kosuke Namba	Tokushima University, Japan
Michael Christiansen	Utah State University
Deidre M. Johns	Oregon State University
Ryan E. Looper	University of Utah
Daniel Gubler	Brigham Young University
Kazutada Ikeuchi	Kwansei Gaikun University, Japan
Masashi Yokoya	Meiji Pharmaceutical University, Japan

Former Co-workers of Robert M. Williams in Industry:

Robert W. Armstrong	Eli Lilly Pharmaceutical Co. (retired as Vice President)
Andrew O. Stewart	Abbott Laboratories (retired)
Peter J. Sinclair	Merck & Co. (retired)
Paul P. Ehrlich	Bayer, AG
James A. Hendrix	Aventis Pharma
Glenn J. Fegley	Onconova Therapeutics, Inc.
Timothy D. Cushing	Amgen (retired)
Gregory F. Miknis	Array Biopharma; Colorado State University C2D2 Program
Mark E. Flanagan	Pfizer, Inc.
Steven M. Rubenstein	Albany Molecular Co.
Chester C. Yuan	Amgen
David M. Bender	Eli Lilly Pharmaceutical Co.
Brad Herberich	Amgen, Inc.
Jeffrey Cao	Merck & Co.
Jack D. Scott	Merck & Co.
Emily M. Stocking	R.W. Johnson Pharmaceutical Research Institute
Christi Kosogof	Abbott Laboratories
Paul R. Sebahar	Myriad Pharmaceuticals
Steven Lenger	Array BioPharma
Duane E. DeMong	Merck & Co.
Jen-sen Dung	Johnson Matthey
Tomasz Glinka	Rempex Pharmaceuticals
Eduard J. Brunner	Novartis
Byung H. Lee	Pfizer, Inc.
Maria Wudlikow	Essential Therapeutics

Mark Sabol	Dow Chemical Co.
David J. Aldous	Aventis Pharma
Suzanne C. Aldous	Aventis Pharma
Nobuyoshi Yasuda	Merck & Co.
Norbert Richter	Boehringer Mannheim AG
Mary Dosch-Doubelday	Bristol-Myers Squibb Co.
Yusuke Amino	Ajinomoto Co.
Pierre-Jean Colson	Pfizer, Inc.
Jiwen Liu	Amgen, Inc.
Claude Quesnelle	Bristol-Myers Squibb Pharmaceutical Co.
David D. Hennings	Array Biopharma
Hidekazu Tsujishima	Tanabe Pharmaceutical Co.
Masahiko Kinugawa	Kyowwa-Kirin Co.
Rhona J. Cox	Astra-Zeneca Pharmaceutical Co.
Tomoyuki Onishi	Ajinomoto Co.
Guiru Zhang	Procter & Gamble Co.
Uta Sundermeier	Henkel KGAA (Duesseldorf, Germany)
Jonathan Lane	Array BioPharma
Hidenori Namiki	Daiichi Sankyo Co.
Yasuo Noguchi	Daiichi Sankyo Co.
Meriah W.N. Valente	Bristol-Myers Squibb Co.
Brian K. Albrecht	Constellation Pharmaceuticals
Alan R. Grubbs	Ardea Biosciences
Gerald D. Artman III	Kalexsyn, Inc.
Dan Fishlock	Hoffman-La Roche, Inc.
Deidre M. Johns	Eli Lilly
Aaron Smith	Pfizer, Inc.
Thomas J. Greshock	Merck & Co.
Carolyn Selenski	Glaxo SmithKline
Andrea Geiser	Merck & Co.
Ann E. Troutman	Merck & Co.
Ludwig Kaspar	HWR Chemie
Dane Clausen	Merck & Co.

Collaborations and Biological Support. We have ongoing formal collaborations with the following laboratories around the world that are relevant to numerous ongoing research efforts.

Collaborator	Institution	Role on Project/Expertise
Prof. James E. Bradner	Dana-Farber Cancer Research Inst.	Biology & biochemistry of HDAC inhibitors. NIH sub-contractor.
Prof. Kimberly Stegmaier	Dana-Farber Cancer Research Inst.	Biology & biochemistry of HDAC inhibitors. NIH sub-contractor.
Prof. Olaf Wiest	University of Notre Dame	Design & conformational properties, protein binding of HDAC inhibitors. NIH sub-contractor.
Prof. David Sherman	University of Michigan	Biosynthesis of secondary metabolites from microorganisms and plants; Meta-omics, biosynthetic gene identification, cloning and expression. NIH sub-contractor.
Prof. Sachiko Tsukamoto	Kumamoto University, Japan	Biosynthesis of stephacidins, notoamides
Prof. Douglas V. Faller	Boston University Medical Center	Inhibitors of Protein Kinase C-δ
Prof. Susan M. Perrine	Boston University Medical Center	Development of drugs for hemoglobinopathies
Dr. William V. Williams	Incyte & Sapientia Therapeutics	Inhibitors of Protein Kinase C-δ
Prof. Chris Walsh	Harvard Medical School	Biosynthesis of the kutznerides
Prof. Stuart L. Schreiber	Harvard University The Broad Institute of Harvard & MIT	Cytotoxicity & mode of action of HDAC inhibitors
Prof. Douglas Thamm	Colorado State University Animal Cancer Center	Cytotoxicity of HDAC inhibitors, anti-cancer agents
Prof. Dan Gustafson	Colorado State University Animal Cancer Center	Pharmacokinetics of HDAC inhibitors
Prof. Hiroaki Suga	University of Tokyo	Preparation of HDAC inhibitor libraries
Dr. James R. Berenson, M.D.	Institute for Myeloma and Bone Cancer Research, Los Angeles	HDAC inhibitors for treating multiple myeloma
Prof. George Stamatoyannopoulos	Markey Molecular Medicine Center University of Washington	HDAC inhibitors as erythropoiesis agents.
Dr. Jens C. Frisvad	Technical University of Denmark	Fungi that produce prenylated indole alkaloids.
Prof. Ian Orme	Colorado State University	Synthesis of drugs that inhibit the growth of drug-resistant *Mycobacteria tuberculosis*
Prof. Rod Croteau	Washington State University	Taxol biosynthesis: genetics, cloning, *in vivo* incorporation, metabolite identification, biotransformation, enzymology.
Prof. Juan F. Sanz-Cervera	University of Valencia (Spain)	Visiting scholar: structure determination, synthetic co-worker biosynthesis.
Prof. Hideo Hayashi	Osaka Prefecture University (Japan)	Asperparaline A biosynthesis in *Aspergillus japonicus* strains.
Professor Karolin Luger	Colorado State University	Interaction of FR900482 with nucleosome core particles.
Professor Raymond Reeves	Washington State University	High Mobility Group A1 (HMG A1) oncoprotein interactions with FR900482 and related antitumor agents.
Professor Yutaka Aoyagi	Kinjo Gakuin University	Asymmetric synthesis of amino acids.
Dr. Tomoyuki Onishi	Ajinomoto Co. (Japan)	Asymmetric synthesis of important chiral compounds.
Prof. James B. Gloer	University of Iowa	Biosynthesis of sclerotiamide, versicolamide
Dr. Hiroyuki Osada	RIKEN Institute (Japan)	Cytotoxicity and mammalian cell cycle inhibition assays; microtubule disruption.

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
U.S. Patent 8,497,393

DECLARATION OF ROBERT R. RUFFOLO, Jr., Ph.D. IN SUPPORT OF PATENT OWNER RESPONSE TO PETITION

TABLE OF CONTENTS

I have been retained by the law firm of Wilson Sonsini Goodrich & Rosati ("WSGR") as an expert consultant to United Therapeutics Corporation ("UTC") in connection with the above-identified matter to provide expert testimony concerning U.S. Patent No. 8,497,393 ("the '393 patent", Ex. 1001) by Batra *et al.*, entitled "Process to prepare treprostinil, the active ingredient in Remodulin®," issued on July 30, 2013. At the request of Counsel for UTC, I hereby submit this expert declaration.

I. Qualifications and Background

A. Education and Experience

1. I am the retired (as of 2008) President of Research and Development for Wyeth Pharmaceuticals (now Pfizer Inc.) and Corporate Senior Vice President of Wyeth (now Pfizer Inc.). I am currently Managing Director of Ruffolo Consulting, LLC, a consulting company serving the pharmaceutical and biotechnology industries.

2. I have studied, researched, taught (in medical and pharmacy schools), worked and managed all aspects of the pharmaceutical drug discovery and development fields for over 35 years. I received my Bachelor of Science (B.S.) degree in Pharmacy (*summa cum laude,* and *With Distinction*) in 1973 from The Ohio State University, and was licensed to practice Pharmacy in 1973. I received my Doctor of Philosophy (Ph.D.) degree in Pharmacology in the fields of autonomic and cardiovascular pharmacology in 1976 also from The Ohio State University. My doctoral research included the areas of drug-receptor interactions, autonomic pharmacology, cardiovascular pharmacology, adrenergic drugs, stereochemistry and the study of the stereochemical aspects of adrenergic drugs and their receptors. During the period of my undergraduate and graduate education, I authored or co-authored a number of peer-reviewed research articles describing that work.

3. Upon earning my Ph.D. degree, I remained at The Ohio State University as a Postdoctoral Fellow for six months, and extended my research on drug-receptor interactions and drug-receptor theory. From 1977-1978, I worked as a Staff Fellow and Postdoctoral Fellow [Pharmacology Research Associate Training (PRAT) Fellow] at the National Heart Lung and Blood Institute of the National Institutes of Health (NIH) in the laboratory of Dr. Marshall Nierenberg (Nobel Laureate for breaking the genetic code), where my research focused on neurobiology, and in particular on synapse formation in brain, spinal cord and skeletal muscle.

4. In 1978, I began my independent career in the pharmaceutical industry at Eli Lilly & Company as Senior Pharmacologist in the Department of Cell Biology. I subsequently became Senior Pharmacologist in the Department of Cardiovascular Pharmacology in 1981, and was promoted to Research Scientist in 1982. I then became Chairman of the Cardiovascular Research Committee in 1983, where I continued my research in cardiovascular pharmacology, adrenergic drugs, drug-receptor theory, stereochemistry and the stereochemical basis of drug action. My work also expanded into the area of structure-activity relationships and drug design. Shortly after joining Eli Lilly & Company, I was also assigned to supervise a medicinal chemistry laboratory that was dedicated to my work in stereochemistry and structure-activity relationships, and which I personally directed. While working at Eli Lilly & Company, I was credited with discovering the complex mechanism of action of the newly marketed drug for the treatment of acute congestive heart failure, dobutamine (Dobutrex®), which involved the complex interplay of the different pharmacological activities of both enantiomers of the drug, each acting on multiple adrenergic receptors and their subtypes..

5. In 1984, I joined SmithKline Beckman Pharmaceuticals (now GlaxoSmithKline PLC) as Director of Cardiovascular Pharmacology, where I continued my work in cardiovascular

pharmacology, adrenergic drugs, drug-receptor theory, stereochemistry, the stereochemical basis of drug action, structure-activity relationships and drug design. As Director of the Department of Cardiovascular Pharmacology, I supervised a staff of approximately 40 researchers and scientists in the field of cardiovascular drug discovery and development. Throughout my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities that changed through mergers and acquisitions), I also maintained my own laboratory and conducted studies on the pharmacology of cardiovascular drugs, drug-receptor interactions, adrenergic pharmacology, stereochemistry, the steric aspects of drug action, and structure-activity relationships related to new drug discovery.

6. I remained at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) for approximately 17 years, over which time I rose to the position of Senior Vice President and Director of Biological Sciences Worldwide, where I was responsible for a staff of approximately 500 scientists. During my last year at the company, I became the Senior Vice President and Director of all Discovery Research for the Corporation Worldwide, which included all of the areas of Biological Sciences, Chemical Sciences, Medicinal Chemistry, Physical Chemistry, Process Chemistry, Molecular and Cellular Biology, and Genetics, with responsibility for a staff of approximately 1,700 scientists and an annual budget of approximately $1.2 billion.

7. It was during my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) that I was personally responsible for the discovery and subsequent development of Coreg® (carvedilol) for the treatment of chronic congestive heart failure, for which I was awarded the *Discoverers Award* in 2008 by the Pharmaceutical Research and Manufacturers Association (PhRMA), which is the major trade association for the

pharmaceutical industry and is comprised by Industry CEOs and Senior Executives, as well as a group of my peers (i.e., Presidents of R&D). Coreg® revolutionized the treatment of chronic congestive heart failure by markedly reducing death, hospitalization and morbidity from this devastating disease. Coreg® is now the "standard of care" for the treatment of congestive heart failure. The FDA approved Coreg® in 1997, after more than 10 years of research and development work that I researched and personally led, and the drug is currently prescribed globally to treat congestive heart failure. The drug has saved tens of millions of lives throughout the world.

8. Also during my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) beginning in 1984, I personally led and managed the discovery of ropinirole (Requip®) for the treatment of Parkinson's disease. Ropinirole is a highly selective dopamine DA2 receptor agonist. Ropinirole was approved by the FDA in 1997 for the treatment of the signs and symptoms of Parkinson's disease, both as monotherapy and as adjunctive treatment in combination with Levodopa.

9. Also during my tenure at SmithKline Beecham Pharmaceuticals (and its subsequent corporate identities), I personally initiated and led the Angiotensin II Receptor Antagonist Program, and I was personally involved in the discovery and development of the marketed angiotensin II receptor antagonist, eprosartan mesylate (Teveten®), which was approved by the FDA in 2001 for the treatment of hypertension.

10. As a result of my research at The Ohio State University, Eli Lilly & Company and SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities), I gained considerable experience in all aspects of drug discovery and development. In addition, throughout this entire period, I maintained my own personal laboratories and conducted my own

independent research in cardiovascular pharmacology, drug-receptor theory, autonomic pharmacology, stereochemistry and the stereochemical requirements of drug action and structure-activity relationships. It was during this period that my laboratory was the first to discover that three subtypes existed for both alpha-1 and alpha-2 adrenoceptors, which was subsequently proven to be correct when the human genome was sequenced a decade later, confirming indeed that three subtypes existed for each of these two adrenoceptor subtypes. My personal laboratory also collaborated with many internationally recognized scientists and their laboratories throughout the world. In addition, I have been invited to lecture at international symposia and at leading research institutions and hospitals around the world on most areas of my research.

11. In 2000, I assumed the positions of Executive Vice President of Research and Development at Wyeth Pharmaceuticals as well as Corporate Vice President, and I was appointed to the Corporate Management Committee and the Board of Directors (as a non-voting member), both of which were chaired by the CEO. Eighteen months later, I was promoted to the positions of President of Research and Development, as well as Corporate Senior Vice President, and I was also appointed as Chair of the Science Subcommittee of the Board of Directors. I was responsible for a staff of approximately 7,000 employees globally, with an annual budget in excess of $3 billion. During this period, I was credited with changing the paradigm for drug discovery and development at Wyeth by markedly improving R&D productivity. This work has been highlighted in *BusinessWeek* magazine, and was the subject of a "Case Study" conducted by the Harvard Business School, which was published in the *Harvard Business Review* in 2007. The Harvard Business School "Case Study" has been covered extensively in business school textbooks, and is a commonly taught case study in many leading business schools throughout the

world, including the Harvard Business School, Wharton Business School, Columbia Business School, Duke University Business School and the London School of Economics. The re-engineering of Research and Development at Wyeth under my direction was also the subject of many articles appearing in major newspapers and trade journals globally. In my role as a scientist and senior pharmaceutical executive, I oversaw and managed each and every aspect of the pharmaceutical drug discovery and development processes. My areas of responsibility included Pharmacological Sciences, Biological Sciences, Biochemical Sciences, Medicinal Chemistry, Physical Chemistry, Molecular Modeling, Spectral Sciences, Pharmaceutics and Pharmaceutical Sciences, Drug Safety and Toxicology, Drug Metabolism, Clinical R&D (which included all clinical trials from Phase 1 through Phase 3), Regulatory Affairs [for FDA (U.S.), EMA (Europe), PMDA (Japan) and every regulatory agency in the world], Medical Affairs, Global Safety Surveillance and Epidemiology, Process Chemistry at the pilot plant and kilo plant levels, as well as the transfer of chemical processes to manufacturing scale, and Post-Marketing Research and Surveillance for all Wyeth drugs throughout their lifetimes on the market.

12. Following my retirement from Wyeth in 2008, I served for one year as a consultant to Wyeth Pharmaceuticals and Pfizer, Inc. Since then, I have been a consultant to most of the major large and mid-sized pharmaceutical companies and many biotechnology companies, as well as other industries outside of biomedical research, as Managing Director of Ruffolo Consulting, LLC. My consulting responsibilities include the areas of R&D Leadership, Leadership Development, Management of Scientific Innovators, Managing Innovation and Managing Organizational Change.

13. During my career as an executive in the pharmaceutical industry, both at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) and Wyeth

Pharmaceuticals, I managed and oversaw the discovery and development of over two-dozen innovative new drugs that were approved by the FDA and other regulatory agencies around the world.

14. During my career, I have authored or co-authored nearly 500 full-length scientific publications, over 200 abstracts, and I have edited 17 books. I was founder and editor-in-chief of three international scientific journals, and have served on the editorial boards of 29 international scientific journals devoted to the fields of pharmacology, biochemistry, pharmaceutical sciences, medicinal chemistry, physical chemistry, analytical chemistry, stereochemistry and stereoselectivity of drugs. I have lectured extensively in scientific and industrial forums worldwide. I have also been invited to speak extensively on the topics of Pharmaceutical Research and Development Management, Research and Development Productivity, Organizational Change, Federal Regulation of Drug Approval and the Principles of Executive Leadership at national and international scientific and management meetings and symposia, and since my retirement, also as a consultant to most of the mid-sized and large pharmaceutical companies and many biotechnology companies..

15. I am a member of several professional organizations including the American Society for Pharmacology and Experimental Therapeutics (ASPET), the British Pharmacological Society, the International Union of Pharmacology (IUPHAR), where I was also Chairman of the Committee on Drug Receptor Nomenclature which was responsible for the naming of all drug receptors and ion channels worldwide, and the professional organization comprised of the international Presidents of Research & Development from large Pharmaceutical Companies (a group called "Hever"). I have served as an elected officer of many of these organizations.

16. I have received a number of prestigious awards for accomplishments throughout my career, including two *Lifetime Achievement Awards* (one from the Scrip Awards and the other from The Ohio State University; one of only three ever to be awarded), two *Honorary Doctorates* (one from the University of Catania, Italy, and the other from West Virginia University), *Chief Scientific Officer of the Year* (for being the best leader of R&D in the pharmaceutical and biotechnology industries), the *John Jacob Able Award*, the *Lorenzini Gold Medal for Biomedical Research*, and the *Prix Galien Special Commendation for Excellence and Innovation in Research* to name but a few. I was also the winner of *"The Great Oxford Debate"* at the world-renowned Oxford Union of Oxford University, UK. Recently, the American Society for Pharmacology and Experimental Therapeutics (ASPET) has established an annual award in my name to honor the contributions that I have made to drug discovery and development; the Award is entitled the "*Robert R. Ruffolo Career Achievement in Pharmacology Medal,*" which is awarded annually to the most prestigious scientists in the world at the height of their careers. The American Society for Information Science & Technology has designated me as a *Highly Cited Scientist* for being among the top 100 most cited Pharmacologists in the world for over two decades.

17. My *curriculum vitae* is submitted herewith as Ex. 2023.

II. Legal Standards Provided By Counsel

18. I have been informed by Counsel that because each claim defines a separate invention, the validity of each claim in a patent is addressed independently of the validity of the other claims in that patent.

19. I have also been informed by Counsel that the claims of the '393 patent are "product-by-process" claims. I have also been informed by Counsel that the "product" of

product-by-process claims include structural and functional differences that are present even if they are not explicitly claimed.

20. I understand from Counsel that, in addition to considering the prior art, certain objective indicia may also provide evidence that a claimed invention is not obvious. I am informed by Counsel that these objective indicia, which are also referred to as secondary considerations, may include factors such as commercial success, unexpected results, the resolution of long-felt, but previously unmet needs, skepticism by others prior to achieving the invention, failure of others to achieve the invention, praise from others for the invention, and copying by others.

21. I have been informed by Counsel that a patent is to be interpreted from the perspective of a hypothetical person referred to as the person of ordinary skill in the art ("POSA") to which the patent pertains. I have also been informed by Counsel that a determination of the level of ordinary skill is based on, among other things, the type of problems encountered in the art, prior art solutions to those problems, rapidity with which innovations are made, sophistication of the art, and the educational level of active workers in the field. I have been informed that in any particular case, every factor may not be present, and one or more factors may predominate. I understand the POSA is presumed to know all prior art that is reasonably relevant to the subject matter of the claimed invention.

22. I understand from Counsel that the validity of a patent claim must be assessed from the perspective of a POSA at the time of the invention.

23. I have reviewed Dr. Williams' Declaration (Ex. 2020) and his definition of a POSA with respect to the patent-in-suit and I agree with his opinion that a POSA would have had, at the time of the claimed invention, a doctorate degree in chemistry, pharmaceutics,

pharmaceutical sciences, medicine, or a related discipline. Alternatively, the POSA may have had a lesser degree in one of those fields, with correspondingly more experience. To the extent necessary, a POSA may have collaborated with others of skill in the art, such that the individual and/or team collectively would have had experience in synthesizing and analyzing complex organic compounds.

24. I understand that SteadyMed's expert, Dr. Winkler, in his declaration has opined that a POSA would have "a master's degree or a Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively, a person of ordinary skill would include an individual with a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." Ex. 1009 at ¶14.

25. My opinions in this declaration are expressed from the view of a POSA at the time of the priority date of the '393 patent. These opinions apply equally whether Dr. Williams' definition of a POSA or Dr. Winkler's is applied.

III. The '393 Patent

26. This case relates to a process to prepare an improved treprostinil product, the active ingredient in Remodulin®, as described in the '393 patent. As described in the '393 patent, treprostinil is prepared as an improved drug substance and active pharmaceutical ingredient (API) in a more pure form. The new preparation of treprostinil described in the '393 patent also has lower levels of impurities.

IV. Summary of Opinions

27. This report contains a statement of my present opinions and includes the bases and reasons therefore, and the data and other information that I have considered in forming these opinions. In this report, I offer herein my opinions on the importance of drug purity and

impurities, and on the improvements made in these properties as a result of the new preparation of treprostinil as described in this patent.

28. In forming my opinions, I have reviewed several documents, such as the documents cited by SteadyMed and UTC in this case, the '393 patent and its file history, as well as references that I have found through my own research. I have also based my opinions on my own extensive general knowledge, comprising nearly 40 years of experience, of the areas of pharmaceutical drug synthesis, production of API, manufacturing, formulation and preparation of final drug product.

29. If called to testify, I will, as needed, explain the principles and terminology used in this report, as well as in the materials referenced herein. I may use demonstrative aids and exhibits to illustrate these principles and the opinions expressed. I have not yet prepared any such demonstrative aids.

30. I may also testify or provide an opinion in rebuttal to testimony or opinions offered by other witnesses in response to the opinions stated herein. I reserve the right to supplement or otherwise amend my opinions.

31. It is my opinion that the invention of the '393 patent satisfied a long-felt unmet need by providing a commercial scale synthesis of treprostinil that results in a treprostinil product with higher overall purity and lower levels of individual impurities. As with all drug substances such as treprostinil, the FDA seeks to list, quantitate, and minimize impurities, and maximize the overall purity, of such drug substances as much as possible for the benefit of patients. The claimed invention of the '393 patent invention meets this need.

V. Background

A. The Importance of Purity in Pharmaceutical Preparations

32. The purity of a pharmaceutical drug substance, both active pharmaceutical ingredient (API) and final or finished drug product, is of the utmost importance to regulatory agencies, and especially the FDA. Accordingly, the first sentence of the Code of Federal Regulations (C.F.R.) Title 21, Part 610, Subpart B, Section 610.13 is *"Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application."* 21 C.F.R. § 610.13(b) (2015). Although the FDA provides no absolute level of purity required for any given drug, based on my experience of approximately 40 years in the pharmaceutical industry interacting with the FDA on regulatory issues, it is commonly assumed that, with rare exception, licensed drugs will have purities in excess of 99%, and often significantly higher. ICH Impurities in New Drug Substances Q3A(R2) (2006) ("Q3A(R2)", Ex. 2038) at 12; ICH M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 2015 ("ICH M7", Ex. 2039) at 24-25. There is so much concern with the purity of drug substance and drug product that the highest level of purity possible should be achieved, even if that means changing the synthetic method as has been done in the '393 patent. Olsen, Bernard A., *What's New with Impurities in Pharmaceuticals?*, Southern California Pharmaceutical Discussion Group, January 15, 2015 (Ex. 2040) at 14. Drug purity is of such importance to regulatory agencies that the purity level of a drug substance and API ***must*** appear in the drug product specification, which is the quality control document of the drug's Certificate of Analysis for each batch of drug substance to be released for subsequent formulation into the final drug product. 21 C.F.R. § 600.3 (kk). If a batch of drug substance falls short of its lowest purity limit listed in the

specification, that batch of the drug substance must be rejected, even if the deviation in purity is as low as 0.1%. For example, if the actual purity of an API is 99.4% and the lowest limit of purity in the Drug Specification of the Certificate of Analysis is 99.5%, the entire batch of API must be rejected. As the FDA clearly states, *"Each component* [of API] *shall be tested for conformity with all appropriate written specifications for purity, strength and quality." Id.* at § 211.84(d)(2).

33. The FDA defines purity as *"relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to product." Id.* at § 600.3 (r). Any batches of drug substance that fail to meet the levels of purity indicated in the product specification must not only be rejected, but rejected batches must also be *"identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable." Id.* at § 211.110(a). The position of the FDA on the significance of drug purity is absolutely clear, and would be understood by a POSA.

34. The function of the FDA is to approve new drugs based on their safety and efficacy, as well as the balance between the benefits and risks of new drugs to patients. Biotech, Janet Woodcock, The Political Economy of FDA Drug Review: Processing, Politics, And Lessons For Policy, FDA) (Ex. 2041) at 1-2. The FDA's focus on purity relates specifically to their many analyses that are related to the overall assessment of drug safety, and relative risk, of the new product to be marketed, and is done in the interest of patient safety. Ex. 2039 at 5-9, and 20.

35. Guidelines and requirements for the levels of purity of new drug substances and new drug products have been increasing over the past few decades. The FDA's requirements for increases in drug purity are based on their prior experiences (both positive and negative) in

approving drugs with varying degrees of purity. Based on my experience, the FDA understands that the levels of purity that they require are dependent upon improvements in technologies available to purify drug substances, as well as improvements in the levels of detection of various drug components, including impurities, that are available to pharmaceutical companies to produce, and equally important, manufacture, highly pure compounds. Ex. 2038 at 6-9. The trends for improvements in these technologies have unmistakably improved over the decades, and accordingly, so have the FDA's requirements for drug purity. Accordingly, in my experience, the drug purity requirements of the FDA represent a constantly moving (and improving) target.

36. Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of safety to patients. Ex. 2038 at 13-15. As indicated above, impurities are extraneous substances that are present in the API and final dosage form which add no value to the new drug product or to the patient. 21 C.F.R. § 600.3(5)(r). Because impurities add no value or benefit to the new drug product, they are, at best, irrelevant, and at worst, sources of potential adverse toxicities to patients. Impurities, therefore, can only add to the risk assessments, which are often unknown, made by regulatory agencies in the evaluation of new drug products. Ex. 2040 at 3-4 and 5-8.

37. Impurities may be introduced into the API or final dosage form during any of the many steps involved in the synthesis, formulation and manufacturing of the drug product. ICH Q3D Elemental Impurities ("ICH Q3D", Ex. 2043) at 5; Ex. 2038 at 6-7. It has long been the desire of regulatory agencies, and especially the FDA, to require pharmaceutical companies to produce the highest levels of drug purity that are possible and practicable.

38. Regulatory agencies have observed toxicities, or adverse events, resulting from drugs in clinical development as well as approved drugs that were not related to the new drug product itself, but rather to the impurities present in these new drugs (see examples below at ¶58). Because impurities add nothing to the benefit of a new drug, by extension, it is the view of regulatory agencies that impurities represent only potential risk to patients. Ex. 2039 at 12-16. Accordingly, regulatory agencies encourage (and may mandate) pharmaceutical manufacturers to increase levels of purity of their new drug substance. Even for products already approved by the FDA and on the market, it has been my experience that the FDA often encourages manufactures to continue to develop new synthetic and/or manufacturing processes to improve purity, and decrease levels of impurities, even further. This desirable goal is one of the objects of the invention of the '393 patent with respect to the new preparation of treprostinil with a higher level of purity.

39. The opening sentences of ICH M7 begin as follows: "*The synthesis of drug substances involves the use of reactive chemicals, reagents, solvents, catalysts, and other processing aids. As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated drug products.*" *Id.* at 5. These sentences alone address the importance that regulatory agencies, including the FDA, attach to drug impurities, and the significant risks that may be associated with them. These sentences are also among the fundamentals taught in all introductory courses in Pharmacology and Toxicology (as an adjunct faculty member at Baylor University Medical School, West Virginia University Medical School, and The Ohio State University Department of Pharmacology, I teach this very concept to medical and pharmacy students in their second year pharmacology courses).

40. All drug substances contain impurities, and these impurities can range from harmless to extremely toxic, and often regulatory agencies do not know the risks of all (and sometimes any) of the impurities present in new drug substances. Accordingly, regulatory agencies focus intensely on the numbers and levels of impurities that exist in a drug substance, such as API and the finished drug product. Ex. 2043 at 13-16. With the information on impurities provided by pharmaceutical companies, and analyzed by the regulatory agencies, risk assessments are made or estimated by regulators with respect to the relative hazard that impurities, including trace impurities, may have on patients. Ex. 2039 at 6-10. This task becomes more complex and uncertain when one considers that there will also likely exist impurities that are present but not detectable (based on limitations in levels of detection; see penicillin example below at ¶62), yet may still present risks to individual patients, and the population of patients at large, who take any given medication.

41. In their assessment, the FDA and other regulatory agencies attempt to determine whether the impurities that are known to be present in the new drug substance have the capacity to induce injury or adverse effects in patients, as well as the types of injuries or adverse effects that may occur at the anticipated exposure level to the patient. *Id.* In so doing, regulatory agencies rely on the concept of the "threshold" for producing an adverse health effect. With respect to threshold, the concept of Threshold of Toxicological Concern (TTC) is considered (as discussed below), which is most often associated with impurities that are known or suspected to be mutagenic compounds that have the capacity to damage DNA and therefore the possibility of causing cancer, or impurities known to be highly toxic to humans or animals. *Id.* at 7-8 and 12-13. Regulators will also consider the type of adverse health effect that each impurity may produce, such as direct cell, tissue or organ damage, carcinogenicity, teratogenicity, reproductive

injury, chemical injury, and other types of injuries that may occur from exposure to an impurity. These considerations by regulatory agencies will also be viewed within the overall context of the benefit/risk ratio of the drug substance itself, as well as the severity of the disease to be treated, the availability of other potentially safer therapeutic alternatives or options, the types of patients being treated (i.e., male, female, children, elderly) and the duration of treatment (acute vs. chronic). *See, e.g., id.* at 12-16.

42. Among the many considerations that the FDA will explore with respect to impurities in pharmaceutical preparations is the dose-response relationship, or more appropriately, the dose-toxicity relationship, of the impurity. Accordingly, regulatory agencies also rely heavily on the Permitted Daily Exposure (PDE) of the impurity in the individual patient and the population of patients taking the drug (i.e., the maximum allowable level of the impurity that the patient or patient population can be exposed to), the length or duration of exposure to the impurity (acute vs. chronic administration of the drug), and the overall risk assessment made for each impurity. *Id.* at 13-16; *see also*, Ex. 2038 at 14-15; Ex. 2043 at 5-15. This is to say that regulatory agencies are realistic that patients will undoubtedly be exposed to potentially toxic impurities, and as such, regulatory agencies work diligently to determine how many patients may be injured from the anticipated level of exposure to the impurity in the new drug product. *Id.* These assessments are not viewed in isolation, but rather within the context of the other considerations, such as the benefits and risks of the new drug substance, the severity of the disease to be treated and the duration of therapy, as well as the availability of other potentially safer therapeutic alternatives. *Id.* Regulatory agencies will (reluctantly) accept potential or possible risks from impurities when the new drug product is directed to a serious disease with high unmet medical need, and for which there are few or no other therapeutic options. Ex. 2038

at 14-15. But even in these situations, regulatory agencies still press for the highest levels of purity that are possible and practicable. Again, this desire of regulatory agencies for the highest levels of purities in pharmaceuticals is one of the subjects of the inventions in the '393 patent.

43. Based on my personal experience in the pharmaceutical industry, depending upon the nature and number of impurities, and their levels in the drug substance, the FDA may request that individual impurities be synthesized and tested ***directly*** in a variety of animal safety test systems. Alternately, it is possible to evaluate ***indirectly*** the drug substance itself containing the impurities at very high doses in animal safety test systems, such that the absolute level of the impurities are considered to be sufficiently high to provide a level of exposure to the impurity that would be considered to be appropriately high to detect, with a high level of confidence, potentially injurious events in the safety test systems. For practical considerations, the latter indirect course is the most commonly taken path. When one or the other of these approaches is taken to assess potential safety risks in animal safety studies, either directly or indirectly, and the results of those safety studies indicate that little or no risk is likely, the potential safety risks of one or more of the impurities in the new drug substance are considered to be "***qualified***", and generally considered by regulatory agencies to present little or no risk to patients. Ex. 2038 at 9-14. One significant limitation of the testing of impurities in the drug substance to qualify the impurities, as well as the safety testing done for the drug substance itself, is that these safety test systems rely primarily or exclusively on animal safety models and test systems, and these test systems do not always translate with fidelity to the human patient (*see* below at ¶55). Differences in safety assessment of drugs (and impurities) between animals and humans can result because of differences in biological and biochemical processes between animals and humans, differences in metabolism and elimination, or differences in the duration of exposure

(animal testing is relatively limited in duration for a new drug substance or impurity, compared to exposure in humans which may extend for decades). As a result, there is no way for regulatory agencies to be absolutely certain of the safety risks associated with impurities in new drug substances. To accommodate for this, the analyses used by regulatory agencies to assess the risks of impurities are highly conservative, yet they still cannot guarantee that patient safety will not be compromised by an impurity. Ex. 2039 at 13-16. As a result, regulatory agencies require that impurities be limited to the lowest levels that are possible and practicable. Again, lowering the levels of impurities in treprostinil is one of the major benefits of the '393 patent, and results in higher levels of purity for the treprostinil product.

44. Because impurities in a pharmaceutical preparation are so critically important, regulatory agencies have issued specific guidelines that are consistent with those guidelines outlined in the ICH Impurities in New Drug Substances Q3A(R2) monograph. Ex. 2038 at 11-12. Inasmuch as most drugs are administered in dosages of less than 2 grams per day, only the guidelines for these drugs will be discussed herein. And indeed, treprostinil and the '393 patent fall into this category.

45. Because of the critical significance attached by regulatory agencies to impurities in pharmaceutical preparations, the levels and types of impurities must be included in routine batch specifications for all new drug substances. Ex. 2038 at 7-10. When levels of individual or total impurities exceed those levels listed in the specifications of the batch records required for the release of the drug substance, the batch must be rejected. 21 C.F.R. §§ 211.1 and 211.22(c). For example, if an individual impurity, or the total of all impurities found in a given batch of drug substance, exceeds the limits set in the specifications, even by amounts as low as 0.1%, the entire batch must be rejected. Accordingly, even very small differences in the levels of purity, or

the levels of impurities, can mean the difference between acceptance or rejection of an entire batch of drug substance.

46. Regulatory agencies categorize the treatment of impurities based on the levels that exist in the drug substance, and they categorize them into three different ways: a) Reporting Threshold, b) Identification Threshold and c) Qualification Threshold. Ex. 2038 at 10-12. These threshold levels are extremely low (i.e., conservative) because of the importance assigned by regulators to impurities, and the potential for toxicities caused by them. The specific requirements, as set forth by the ICH, are described below:

47. Reporting Threshold: when a given impurity is present at a level that is less than or equal to 0.05%, the impurity must be ***reported*** in the specification of the drug substance for manufacturing. *Id.*

48. Identification Threshold: when the amount of a given impurity exists at a level that is equal to or greater than 0.1%, or when the total dose of impurity administered per day is equal to or greater than 1.0 mg per day (whichever is lower), the impurity must be ***identified*** chemically in the specification of the drug substance for manufacturing. *Id.*

49. Qualification Threshold: when a given impurity exists at a level that is equal to or greater than 0.15%, or when the total dose administered per day is equal to or greater than 1.0 mg per day (whichever is lower), the impurity must be ***qualified***, which means that the biological safety must be established in standard safety and toxicology studies as described above. *Id.; see also* 13-15. This may either be done indirectly by studying in animal safety models very high doses of the drug substance containing sufficiently high levels of the impurity to be ***qualified***, or directly by studying the isolated and purified impurity to be ***qualified*** by itself in the safety studies. *Id.* at 9-10. In practice, ***qualification*** of an impurity is most commonly done indirectly

by studying high doses of the drug substance containing the impurity in animal safety test systems.

50. As a result of these thresholds, by definition, the limit of detection for impurities (and therefore total related substances) *must be* at least as low as 0.05%.

51. Often the FDA also requires a measure of the Total Related Substances, which is a measurement of all of the identified impurities added together. *See, e.g.*, Ex. 2006 at 6. This number is essentially a measurement of the purity of the sample itself.

52. For each impurity measurement and for the total related substances measurement, the Drug Specification in the Certificate of Analysis also includes a limit for those impurities, reported as "Not More Than", or "NMT", a certain percentage. *Id.* This number is not a measurement of the error, but is an indication of what level in the API or final product the FDA has permitted for that impurity.

53. An assay is a measure of the purity of a sample of the drug substance (or drug product) often performed in comparison to a "reference standard". *See, e.g.*, Ex. 2006 at 6; *see also* ICH Guidance For Industry: Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients (2001) ("Q7A", Ex. 2044) at 34-35; *see also* Reviewer Guidance: Validation of Chromatographic Methods (1994) ("Reviewer Guidance", Ex. 2035) at 8-11. The "reference standard" is a high quality and relatively highly purified sample against which all future samples of the drug are compared and measured. Ex. 2035 at 8. It is important to note that these reference standards do not represent absolute purity (which is impossible), and they also do not represent the maximum level of purity that can be obtained for any given drug. Reference standards are simply deemed to be high quality standards that all future samples of the drug will be measured against. A reference standard should always be included in assays required for

determination of the acceptability or unacceptability of manufacturing batches of API or final product in accord with the Drug Specification of the Certificate of Analysis. Ex. 2035 at 9-10. Accordingly, it is possible, and quite common, for a pharmaceutical company to develop an even more highly purified version of a drug than the reference standard for that drug, and as a result, purity levels for the drug will be in excess of 100% (relative to the reference standard) because it is more pure than the reference standard. This is a highly desirable outcome inasmuch as it indicates that the drug is even more pure than the "accepted" highly purified reference standard. Importantly, purities of a drug in excess of 100% (relative to the reference standard) do ***not*** represent an error in the assay, but simply indicate that the preparation of the drug is ***more pure*** than the reference standard. It cannot be overstated that a reported purity of a drug of over 100% (relative to the reference standard) does not indicate an error in the measurement, but simply that the sample being analyzed has a higher purity than the reference standard. As is the case for most assays, reported assay values may span a range of acceptable limits that can include and go beyond 100%, indicating that certain samples may be even more pure than the reference standard they are compared to.

54. It is also important to note that because some impurities are extremely toxic at very low levels of exposure, Thresholds of Toxicological Concern can, and often are, lowered, beyond the guidelines described above, in the specifications for the synthesis and manufacturing of a drug substance in order to be conservative. Ex. 2039 at 12-15. Conservative specifications, which provide a higher level of certainty that the drug product is safe from the standpoint of impurities, can make it especially difficult for API production plants and manufacturing plants that produce final drug product to meet the specifications established for a new drug substance. This creates the situation where exceedingly small differences in the allowable levels of purity

and impurities make it difficult to meet synthesis and manufacturing specifications. It has been my experience that this is particularly important for drugs of extremely high potency, such as the prostaglandins, prostanoids and prostaglandin-like drugs in general, and treprostinil specifically, which may contain trace amounts of potent structural analogs as impurities. As such, establishing specifications for purity and levels of impurities for extremely potent drugs, such as treprostinil, and being able to meet those specifications for synthesis of API and manufacturing of final drug product, is especially difficult for such highly potent drugs. Accordingly, the potential risk from impurities of even very potent drugs is not minimized because of the lower amount of API used in the final drug product.

55. In addition to the discussion above of the regulatory approach to dealing with the critical issue of impurities in drug substances, there are a number of other issues involved in coping with impurities that regulatory agencies must consider. Among the most important of these other considerations is the fear is that toxic effects of impurities, and especially *qualified* impurities, in drug substances may not be detectable in animal safety and toxicology studies, but yet may still occur in humans, and especially for those drugs that are used on a more chronic basis where they may be administered to patients for decades (especially in view of the relatively short-term nature of animal safety and toxicology testing). Diethylstilbestrol is one such example. This drug was determined to be safe in animal studies, but was subsequently found to produce higher incidences of vaginal and cervical cancer in second-generation female offspring who were exposed to the drug *in utero* while their mothers were being treated. Schrager et al., Diethylstilbestrol Exposure, 2004 (Ex. 2045) at 2-3. These types of results represent false negatives, or Type I Error, in that the animal safety studies failed to predict human toxicities. Carpenter, The Political Economy of FDA Drug Review: Processing, Politics, and Lessons for

Policy (2013) (Ex. 2042) at 4. And the converse may be true as well, as in the case of saccharin, which was found to be carcinogenic in animals, but not in humans. NTP Report on Carcinogens Background Document for Saccharin, March 1999 (Ex. 2046) at 102. This situation represents a false positive, or a Type II error (Ex. 2042 at 4) which will often result in the termination of development of an otherwise safe and potentially highly beneficial and medically necessary new drug. This latter situation is most unfortunate when a medically necessary product never reaches the human patient.

56. For these reasons, and others, the types and levels of impurities in drug substances are of the utmost importance to the FDA, and as a result, the FDA insists on the highest level of purity that is possible in a drug substance, and alternatively, the lowest levels of impurities possible. Although the FDA and ICH make recommendations with respect to the levels of impurities that are present, for example a threshold of 0.1%, or a total dose of 1.0 mg per day, whichever is lower (Ex. 2038 at 12), it is important to note that these are simply guidelines, and previously unknown impurities, or known impurities of high risk, can be held to even lower thresholds at the discretion of the regulatory agencies, or potentially not permissible at all down to the level of detection. Ex. 2039 at 12-16. Based on the present FDA and ICH guidelines, a potentially toxic impurity that is not demonstrated to be a risk in *animals*, could be present in a drug substance at a level resulting in exposures of up to 1.0 mg per day that could, in fact, be toxic to *humans*, and yet may not need to be *identified* or *qualified* (i.e., they were shown or assumed to be safe based on animal studies). Given the number of compounds known to be highly toxic at doses of 1.0 mg or less per day, even these relatively stringent criteria for reporting impurities in specifications for the synthesis and manufacture of drug substance could still expose humans to significant risk, and regulators are acutely aware of this. Accordingly,

any procedure or method that provides for a higher level of drug purity and/or lower levels of impurities is of critical importance to regulatory agencies and to patients in general. Again, one of the major inventions in the '393 patent is a new synthesis of treprostinil that results in a higher level of purity.

57. The regulatory agencies have set specific limits on levels of impurities in drug substances as described above, and they use detailed and complex analyses to assess risk to patients from impurities in drug substances. Accordingly, regulatory agencies insist that drug substances must have the highest levels of purity as possible, and contain the lowest levels of impurities as possible. As a result, new synthetic processes for an existing drug that increase purity and reduce impurities, both in number and level, are considered to be critically important to regulators, patients and public health (Ex. 2040 at 14). Even small increases in purity and decreases in impurities can have significant impacts on patient safety, as well as for acceptance or rejection of batches of manufactured drug substance. My personal experience has been that when considering the safety and toxicology profile of impurities, it is often more efficient to reduce the levels of impurities in the drug substance by altering or changing the synthetic method (*id.*), as opposed to simply adding additional purification steps, to achieve the desired reduction or elimination of impurities, as UTC has done and demonstrated for treprostinil in the '393 patent.

B. Examples of Toxic Contaminants in Pharmaceutical Preparations

58. Toxic impurities in drug substances are not a hypothetical or theoretical discussion. Indeed, highly toxic impurities in drug substances, API and final formulated product are, indeed, a reality with sometimes tragic outcomes. Specific examples of marketed drugs with highly toxic impurities are presented in this section as well as several examples of the negative

impact that toxic impurities in pharmaceutical preparations have had on patients. These examples highlight the critical importance of producing drug product of the highest possible quality and purity and with the lowest possible level of impurities, with both objectives strongly supported and often required by regulatory agencies globally, as reflected in the current ICH Guidelines. Ex. 2039 at 12-16.

59. The existence of highly toxic impurities in commonly used pharmaceutical preparations is not new and is not uncommon. And despite the strict limitations placed on toxic impurities by regulatory agencies, following the recommendations of the ICH discussed above, even newly approved pharmaceuticals may contain highly toxic impurities. It is important to note that even with the precautions taken by regulatory agencies to restrict or limit the levels of toxic impurities present in pharmaceutical substances, and limitations in the maximum allowable level of exposure to these impurities, that have been discussed previously, there is no guarantee that these toxic impurities are harmless even at the low levels that are permitted to exist in drug products. And this is especially true when these assessments are made in experimental animal safety/toxicology studies, which as discussed above, do not necessarily translate to safety in humans. In fact, regulatory agencies acknowledge that even these low levels of impurities allowed by their guidelines can still result in adverse toxicities in patients, and they have even calculated the risks of adverse toxicities, including cancer, that may be associated with these low levels. Ex. 2039 at 13-16; *see also* Ex. 2043 at 15-16.

60. As a result, it is still considered absolutely essential for pharmaceutical manufacturers to produce pharmaceutical products with the highest levels of purity, and the lowest levels of impurities, and I have observed over the course of my career that regulatory agencies have at times recommended changing synthetic and manufacturing procedures to

develop improved procedures to enhance purity and decrease the number and levels of impurities, as has been done in the '393 patent for treprostinil. *Id.*

61. Despite these significant limitations, which are well-known to regulatory agencies, there do exist examples of trace impurities in pharmaceutical substances that have produced significant, and often tragic, toxic adverse effects in patients. Some representative examples of these toxic or potentially toxic effects of impurities in drug substances and drug products are presented below:

62. Penicillins: Penicillins represent a class of drugs that are among the best-known and most extensively studied examples of trace impurities that can cause serious and potentially life-threatening adverse events. Penicillins refer to a group of important antibiotics belonging to the class of beta-lactam antibiotics. FDA Guidance for Industry, Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination, (2013) ("Penicillin Guidance", Ex. 2047) at 4-9. Despite the very high level of safety and efficacy associated with the penicillins (they may be administered at doses of millions of international units, even to newborn babies), they are, as a class, known to produce serious and life-threatening allergic reactions, which, at their most serious levels, can result in anaphylaxis and death. *Id.* Penicillins have been known to be trace impurities in other non-penicillin products that are manufactured by companies that also manufacture penicillins. Penicillin impurities at minute levels have been introduced from the environment (e.g., air in the manufacturing facility) into both the API and finished products manufactured in the same facilities as the penicillins. *Id.* This resulted in immeasurable amounts of penicillin impurities to occur in other unrelated products which, when consumed by patients, have resulted in allergic reactions ranging from minor itching (e.g., rashes) to death (e.g., from anaphylaxis). *Id.* Because penicillins are so highly sensitizing to the human

immune system, and the level of penicillin impurity can be exceedingly low and not quantifiable, the FDA (and now other regulatory agencies) has determined that a zero level of penicillin impurity in a drug substance must be guaranteed. Accordingly, the FDA has mandated that the production of penicillin API and finished product must be performed in entirely different buildings, or buildings that are completely physically isolated from all other buildings, that manufacture other API and finished products for all other drugs. *Id.*

63. Cephalosporins: Cephalosporins are another class of beta-lactam antibiotics that, although chemically different from the penicillins, are associated with the same problem of sensitization and allergic reaction when they exist as trace (and often immeasurable) impurities in the API and finished products of other pharmaceutical drug materials. *Id.* Accordingly, the entire discussion above applies to the cephalosporins as well, including the need for completely isolated synthetic and manufacturing facilities for cephalosporins that are physically (or practically) isolated from all other facilities involved in the production of API and the production of final drug product for all other drugs. *Id.*

64. Human Insulin: Human insulin is an important protein that regulates glucose levels in the blood. In patients with diabetes, there is a low level of insulin secreted from the pancreas, and as a result, blood glucose levels rise producing the signs and symptoms of diabetes. Heinemann and Richter, Clinical Pharmacology of Human Insulin, 1993 (Ex. 2048) at 3. For decades, patients with diabetes were treated with insulin isolated from the pancreases of pigs inasmuch as no human source of insulin was available. With the advent of recombinant DNA technology, it became possible to produce human insulin in the bacteria *E. coli* using large bioreactors. Ex. 2048 at 2. The *E. coli* containing human insulin is then recovered, and the human insulin is isolated free from the *E. coli* and purified for human use to treat diabetes (this

represents the first drug to be produced by recombinant DNA technology). This was, and is today, considered to be a major advance in human health. Initially, in the isolation of human insulin from *E. coli*, the bacteria in which it was produced, the drug product contained trace impurities from *E. coli*, despite the fact that the human insulin was considered to be highly pure. As a member of the Department of Cell Biology at Eli Lilly and Company that developed recombinant human insulin, I am personally aware that when human insulin was initially developed to treat diabetes, allergic reactions were experienced in some diabetic patients, and these reactions were ultimately attributed to the trace impurities of antigens (foreign proteins capable of producing an allergic reaction) derived from the *E. coli* that was used to produce the human insulin. Ex. 2048 at 2. Subsequently, the company needed to manufacture an even more highly purified formulation of human insulin to ensure that the bacterial contaminants were minimized or eliminated. This phenomenon is not limited to human insulin, but is a fairly common occurrence among human proteins produced as therapeutic drugs through recombinant DNA technologies where the human protein is produced in non-human, and therefore potentially antigenic, cells (many human therapeutic proteins are produced in *E. coli* or Chinese Hamster Ovary Cells, or CHO cells, and trace quantities of foreign *E. coli* or CHO proteins can exist in the final product and elicit allergic responses).

65. These representative examples described above, and many others, highlight the importance of drug purity in pharmaceutical preparations, and more importantly, the risks that impurities existing in pharmaceutical API or finished drug product can have on patients. It is for these reasons that regulatory agencies encourage the highest levels of purity possible, and the lowest levels of impurities possible, in pharmaceutical product synthesis and production of drug

product. Ex. 2038 at 5-9. These highly valued goals of regulatory agencies with respect to purity and impurities are embodied in the new preparation of treprostinil described in the '393 patent.

VI. The Invention of the '393 Patent Met a Long-Felt Unmet Need

66. I have reviewed the letter submitted by UTC to the FDA on January 2, 2009 including the revised Drug Substance Specification (Ex. 2006), as well as Dr. Williams' Declaration. It is my opinion that the reduction of individual impurities and the change to the specification of the drug substance of treprostinil indicates that the invention of the '393 patent met a long-felt unmet need by providing a higher purity drug substance. If there is any doubt as to the extreme concern that the FDA places on purity, one need only read the letter submitted by UTC to the FDA on January 2, 2009 where UTC responds to the concerns raised by the FDA with respect to the request to change the Drug Specification for treprostinil in the Certificate of Analysis. The FDA raised only three specific concerns with respect to the Supplemental New Drug Application for treprostinil, and two of these concerns were related to impurities. Ex. 2006 at 1-7. In FDA Comment 1, the FDA has expressed concern about impurities in the supplies of [redacted] that are sourced through outside vendors, and which could potentially carry through to the final product in the synthesis of treprostinil. *Id.* at 1-3. And in FDA Comment 3, the FDA raises a concern about the "residual [redacted] present in treprostinil", which is a potential impurity. *Id.* at 6-7.

67. I have also reviewed Table 1 from Dr. Williams' Declaration where I observe that the level of Total Related Substances (i.e., total impurities) has been reduced from a level of 0.9545% using the Moriarty Process to [redacted] using the '393 patent process, which I calculate to represent a reduction of approximately [redacted] in total impurities. In addition, a reduction was observed in 7 out of 8 impurities using the '393 patent process (compared to the Moriarty

Process), and three impurities were essentially eliminated. Ex. 2020 at ¶¶94-96. These results would most definitely be considered to be significant and clinically meaningful to the FDA, and is consistent with the fact that the FDA allowed the Drug Specification in the Certificate of Analysis to be changed accordingly.

68. Specifically, as UTC explained to the FDA, the '393 patent process was not previously used in their prior process, but the use of the diethanolamine salt intermediate (UT-15C) "affords an additional purification step and an improvement in the process to synthesize treprostinil API" which showed the reduction of all but one of the impurities. Ex. 2006, at 2-3. I understand that the prior synthesis used by UTC was the same as Moriartycited by SteadyMed. *See*, Ex. 1004, *see also*, Ex. 1001, Ex. 6. I have also reviewed the analysis of Dr. Williams and it is my opinion that the removal of [redacted] impurities ([redacted]) was a long-felt unmet need given the FDA's desire to minimize impurities. I note specifically that the FDA concern identified in FDA Comment 1 of the UTC letter to the FDA dated January 2, 2009 demonstrates FDA's worries over the levels of [redacted], which was one of the impurities that was significantly reduced through the '393 patent process. Ex. 2006 at 1-3. As a result, I do not believe that it can be reasonably argued that the improvement in purity, and the reduction in impurities, resulting from the '393 patent process can represent anything other than a long-felt unmet need.

69. Additionally, UTC noted that there was a [redacted] variability in the assay, which meant that the specification was allowable for [redacted] above and below the average purity for the prior synthesis. Ex. 2006 at 3. This variability does not indicate an error in the measurement, but rather a limit on the acceptable specification, so that subsequent batches can be reliably and consistently approved for use by the current manufacturer, and subsequently by generic

manufactures, as a practical and realistic matter, which is also of relevance to the FDA. I have repeatedly observed during the course of my career that the FDA balances their strong desire for the highest levels of purity against the practical need for a company to be able to manufacture the drug product reliably. There are numerous examples of failures to supply patients with much needed medications because Drug Specifications could not be met consistently, resulting in shortages in drug supply. FDA: Drug Shortages, FDA.gov (Ex. 2049) at 1-5. This has been the focus of much attention in recent years, so much so that the FDA has developed an App so that patients can monitor supplies, and observe shortages in drug supply when they occur *Id.* A specification that is too limited or restrictive could result in multiple failing batches, and ultimately the inability to provide a medically necessary drug to patients on a consistent and reliable basis. As a result, there is a [redacted] range in the specification, and this does not represent an error in the assay, but in contrast represents a level of purity that assures the FDA that batches of treprostinil can be consistently manufactured and supplied to patients that need the medication on a reliable basis, while at the same time maintaining levels of purity that are consistent with patient safety.

70. As a result of the change in the synthesis to use the '393 patent process and product, the assay value was changed from a range of [redacted] to [redacted], because the increased purity of the drug substance prepared by the '393 patent process could now be centered at an increased mean of [redacted], and no longer at the [redacted] level as it was for the Moriarty process. Ex. 2006 at 3-4 and 6. This is typical of how assays are reported. *See, e.g.*, Guidance for Industry: Changes to an Approved NDA or ANDA, (2004) (Ex. 2050) at 20. The change in the upper limit for the assay from [redacted] to [redacted] did ***not*** indicate an increase in error from [redacted] to [redacted], but simply indicated that the average purity of the samples ***increased***, from a

midpoint of [redacted] which as indicated above, is always the objective of the FDA. Simply put, the original range of purity ([redacted]) of [redacted] (i.e., +/- [redacted], the variability in the assay) from [redacted] was shifted upward to a range of [redacted] ([redacted]), which unmistakably can ***only*** represent an upward shift in overall purity of the product, which is clearly a primary objective of the FDA and a benefit to patients. Likewise, the increase in the lower limit of purity of [redacted] using the old Moriarty process to [redacted] with the '393 patent process unquestionably represents an ***increase*** of the minimum level of purity required for release of any API or final product batch of the drug. This cannot be argued to represent anything other than an ***absolute increase*** in the required level of purity in the Drug Specification for the treprostinil product. It is also not relevant whether a previous process (*i.e.*, Moriarty) produces batches that are above this new level of [redacted]. What is relevant is that any process that results in a lower routine level of purity, as is the case for the Moriarty process, will have a higher probability of failing to meet the new minimum release specification of [redacted] than a process that routinely produces a higher level of purity, such as the '393 patent process, simply because its lower level of purity is that much closer to the lower limit of [redacted] required for release. Additionally, as shown by the 175 batch records, the average purity of the treprostinil product prepared by the process of the '393 patent is [redacted] while the average purity of the Moriarty product is 99.05%. Ex. 2020 at ¶99 and Appendices A-B. Thus, the average purity of the treprostinil product prepared by the process of the '393 patent has a [redacted] higher average purity than the Moriarty product.

71. As I discussed above, the assay values are a relative measurement that provide a comparison to a reference standard. Therefore, this change in the assay value indicates that the new batches made by the '393 patent process resulted in several batches with purities above that

of the highly purified reference sample. No matter how an assay is performed, an increase in the minimum level of purity from [redacted] in the Drug Specification can ***only*** represent an increased level of required purity of the API and final drug product required for release of any given batch. This clearly represents an improvement in quality and purity, and a reduction in the level of impurities, which is a major goal of the FDA.

72. This change in the assay value of the Drug Specification for treprostinil represents a significant change for the FDA. Specifically, any change in the synthesis or manufacture of the drug substance that may affect its impurity profile and/or the physical, chemical, or biological properties of a drug is considered a major change. Ex. 2050 at 17. Because the FDA allowed the drug specification for purity to be changed to reflect the higher level of purity, from a lower limit of [redacted], around means of [redacted], respectfully, resulting from the '393 patent process, it is clear that the FDA considered this to represent a major/significant change.

73. I have also reviewed the declaration and deposition transcript of Dr. Jeffery D. Winkler. In his opinion, a difference of 0.4% in a purity measurement "would be attributable to experimental error, and thus the claimed degree of purity under the claimed processes of the '393 patent presents no distinction from the prior art." Ex. 1009 at ¶¶ 68-69. I understand that he also acknowledged that he did not know how a purity specification for an FDA-approved product could change from [redacted], and stated that he viewed purity levels above 100% as errors: "I think the thing that I am able to conclude from the data that is on page 6 of this, of this letter [Ex. 2006] is that the error in the HPLC assay could be as high as [redacted] percent in the first column and by my analysis could be as high as [redacted] percent in the second column." Ex. 2051 at 86:15-87:9.

74. I disagree with Dr. Winkler. As I explain above, assay measurements are comparisons to reference standards and therefore can easily be greater than 100%. This is

different than the purity measurement reporting Total Related Substances reported by Dr. Walsh and separate from the Total Related Substances in the drug specification. Dr. Winkler admits he does not know the difference. *Id.* at 89:2-6.

75. It is therefore my opinion that the invention of the '393 patent met a long-felt unmet need that led to an increase in the purity of the treprostinil drug substance by reducing and/or removing several individual impurities and improving the overall purity as analyzed by Dr. Williams. It is because of this major change that the FDA permitted the change in the Drug Specification assay measurement submitted by UTC to be included in the revised specifications.

IPR2016-00006
patent 8,497,393

I declare under penalty of perjury that the foregoing is true and correct.

Date: July 6, 2016

Robert R. Ruffolo, Jr., Ph.D.

CURRICULUM VITAE

Robert R. Ruffolo, Jr., Ph.D., D.Sc. (*honoris causa*), D.Eng. (*honoris causa*), F.C.P.P.

Residence (Preferred Mailing Address)

725 Pughtown Road
Spring City, Pennsylvania 19475

Telephone: 610-469-9308
Fax: 610-469-8711

Office

101 Lindenwood Drive, Suite 225
Malvern, PA 19355

Telephone: 484-467-4982
Fax: 484-875-3101
E-Mail Address: rruffolo@comcast.net

Personal

Born:	April 14, 1950, Yonkers, New York
Marital Status:	Married, Stephany Ruffolo
Children:	Michael Robert Ruffolo, Born October 4, 1983
	Brian John Ruffolo, Born February 7, 1989
	Jennifer Suzanne Ruffolo, Born June 18, 1991

Education

1968-1973	The Ohio State University	B.S., *summa cum laude* and with Distinction in Pharmacy.
1973-1976	The Ohio State University	Ph.D. in Pharmacology

Professional Experience

2008- Present	President, Ruffolo Consulting, LLC (Pharmaceutical Consulting)
2008-2009	Consultant, Wyeth Pharmaceuticals
2002-2008	President, Research and Development, Wyeth (Responsibility for approximately 9,000 staff, >$3 Billion Budget)
2002-2008	Senior Vice President, Wyeth (Corporation)
2007-2008	Director, Wyeth Pharmaceuticals Inc. (Domestic Entity)
2007-2008	Director, Genetics Institute Europe, Inc.
2000-2002	Executive Vice President, Research and Development, Wyeth Research (Responsibility for approximately 4500 staff, $1.6 Billion Budget)
1998-2000	Senior Vice President and Director, Research Worldwide, SmithKline Beecham Pharmaceuticals (acting, while Head of Research recovered

from automobile accident) (Responsible for 1700 staff, $180 Million Budget) [Received a Special Commendation for performance]

Professional Experience (Continued)

1998-2000	Senior Vice President and Director, Biological Sciences, Worldwide, SmithKline Beecham Pharmaceuticals (Responsibility for approximately 500 staff, $105 Million Budget)
1995-1998	Vice President and Director, Pharmacological Sciences, Worldwide, and Medicinal Chemistry, Europe, SmithKline Beecham Pharmaceuticals (Responsibility: >300 staff)
1992-1995	Vice President and Director, Pharmacological Sciences, U.S., U.K., Europe and Australia, SmithKline Beecham Pharmaceuticals (Responsibility: >300 staff)
1990-1992	Vice President and Director, Pharmacological Sciences, U.S., SmithKline Beecham Pharmaceuticals (Responsibility: 120 staff)
1989-1990	Vice President, Pharmacological Sciences, Smith Kline & French Laboratories (Responsibility: 120 staff)
1987-1989	Group Director, Department of Pharmacology and Department of Molecular Pharmacology, Smith Kline & French Laboratories (Responsibility: 140 staff)
1985-1987	Director, Cardiovascular and Renal Pharmacology, Smith Kline & French Laboratories (Responsibility: 41 staff)
1984-1985	Director, Cardiovascular Pharmacology, Smith Kline & French Laboratories (Responsibility: 24 staff)
1983-1984	Chairman, Cardiovascular Research Committee, Lilly Research Laboratories
1982-1984	Research Scientist, Lilly Research Laboratories, Department of Cardiovascular Pharmacology
1981-1982	Senior Pharmacologist, Lilly Research Laboratories, Department of Cardiovascular Pharmacology
1978-1981	Senior Pharmacologist, Lilly Research Laboratories, Department of Cell Biology
1977-1978	Staff Fellow, Postdoctoral Research Associate, National Heart, Lung and Blood Institute, The National Institutes of Health, Laboratory of Biochemical Genetics (Chief, Dr. Marshall Nirenberg, Nobel Laureate)
1976-1977	Postdoctoral Research Associate, The Ohio State University
1973-1976	Graduate Fellow, The Ohio State University (Thesis Advisor, Dr. Popat N. Patil)

Honors, Awards and Recognitions

2013	Resolution: Scroll of Appreciation, West Virginia University Foundation
2013	Chauncey D. Leake Award, The Ohio State University, Columbus, Ohio
2012	Robert R. Ruffolo Career Achievement in Pharmacology Award (Medal) established by the American Society for Pharmacology and Experimental Therapeutics (ASPET)
2011	Elected, Fellow of The College of Physicians of Philadelphia (FCPP)
2009	Winner, Great Oxford Debate, Oxford, England
2009	Lifetime Achievement Award, The Ohio State University, Columbus, Ohio
2009	Target Leadership Lectureship, College of Pharmacy, University of Florida
2008	Scrip Lifetime Achievement Award, Scrip Annual Awards, London, UK
2008	"Best Pharmaceutical Company, R. Ruffolo, Scrip Awards, London, UK
2008	Legislative Commemoration, the State of South Dakota. Awarded for Discovery and Development of Coreg
2008	Tribute, Office of the Governor, State of Delaware. Awarded for Discovery and Development of Coreg
2008	Citation, Commonwealth of Pennsylvania, House of Representatives. Awarded for Discovery and Development of Coreg
2008	Resolution of Congratulations, Senate of the State of Pennsylvania. Awarded for Discovery and Development of Coreg
2008	Maurice Seevers Lectureship, Department of Pharmacology, Univ. Michigan
2008	William E. Hassan Distinguished Rho Chi Memorial Lecture, Massachusetts College of Pharmacy and Health Sciences
2008	Discoverer's Award. Awarded for Coreg® (Carvedilol/Kredex) by PhRMA
2008	David Perlman Memorial Lectureship. American Chemical Society
2008	"Top Ten Pipeline: Strongest Women's Health Pipeline". Awarded to Robert R. Ruffolo by R&D Directions at the Drug Development Summit
2008	RADEX (R&D Executive Committee) Award for re-building Wyeth's R&D
2008	Visionary Leadership Award, Wyeth Pharmaceuticals
2008	Pharmaceutical R&D Achievement Award, Accenture

Honors, Awards and Recognitions (Continued)

2008	Sino-American Pharmaceutical Professionals Special Service Award
2007-2013	Professor of Physiology and Pharmacology. West Virginia University
2007	Honorary Doctorate of Science (D.Sc.). West Virginia University
2007	Honorary Doctorate in Engineering (D.Eng.). University of Catania, Italy
2007	Best Drug Development Pipeline. Awarded to R. Ruffolo for Wyeth Pipeline
2007	John S. O'Brien Memorial Lectureship. University of Pennsylvania
2007	138th Commencement Speaker, West Virginia University School of Medicine. West Virginia University, Morgantown, WV
2007	Profiled in The Star Ledger of New Jersey. Feature article entitled "Researcher Makes His Mark", April 25, 2007.
2007	Profiled in Harvard Business Review. Wyeth Pharmaceuticals: Spurring Scientific Creativity with Metrics, February 2, 2007.
2007	"Top Ten Pipeline: Strongest CNS Pipeline". Awarded to Robert R. Ruffolo by R&D Directions at the Drug Development Summit.
2006	Profiled in BusinessWeek Magazine. Feature article on re-engineering R&D, February 6, 2006.
2006	Centennial Award for Drug Discovery. Awarded for the discovery and development of Coreg® (Carvedilol) by Temple University.
2006	Listed as one of the "Most Notable Graduates" of The Ohio State University
2006	Research's Shining Star. Designated by Med Ad News for Leading and Re-engineering Wyeth's R&D
2006	Key To The City, Catania, Italy (awarded by Mayor)
2006	Designated as "Notable Alumni" by The Ohio State University.
2006	SAPA Special Award for Outstanding Contributions. Awarded by Sino-American Pharmaceutical Professionals Association (SAPA).
2006	Management Team of the Year. Awarded to R. Ruffolo by Scrip for his leadership of the Wyeth Research and Development Executive Committee
2006	Renowned Pharmaceutical Scientist. Named to list of top 200 Renowned Pharmaceutical Scientists by Pharmer.

Honors, Awards and Recognitions (Continued)

2006	"Top Ten Pipelines: The Strongest Primary Cure Pipeline". Awarded to Robert R. Ruffolo by R&D Directions and Drug Discovery Summit.
2005	Profiled in Philadelphia Inquirer. Feature article entitled "Wyeth's Front Man", June 5, 2005.
2005	Designated as One of the 100 Most Inspiring People in the Life-Sciences Industry. Awarded by PharmaVOICE
2005	George B. Koelle Award for Scientific Excellence. Awarded by the Mid-Atlantic Pharmacology Society.
2005	Executive of The Year Finalist. Scrip Awards.
2005	"Top Ten Pipelines: The Pipeline to Watch". Awarded to Robert R. Ruffolo by R&D Directions and Drug Discovery Summit.
2005	SAPA-GP Excellence Award. Awarded by Sino-American Pharmaceutical Professionals Association
2004	Chief Scientific Officer of the Year. Awarded at the Third Annual Pharmaceutical Achievement Awards for exemplary innovation, scientific competence, leadership and organizational creativity in managing R&D.
2004	Corporate Recognition Award for Innovation by American Chemical Society
2002-2008	Principal Corporate Officer, Senior Vice President, Wyeth (Corporation)
2002	Corporate Officer, Senior Vice President, Wyeth (Corporation)
2002	Designated ISI Highly Cited Researcher, in recognition of being in the top 100 cited Pharmacologists worldwide over the past 20 years (1981-2001)
2002-2008	Member, Pharmaceutical Research and Manufacturing Association (PhRMA) Foundation Board
2003-2008	Member, BIO Board of Directors
2002-2008	Member, Robert F. Furchgott Endowed Chair Committee
2001	Corporate Officer, American Home Products Corporation
2001-Present	Member, Pharmaceutical Research and Manufacturers Association (PhRMA), Science and Regulatory Executive Committee
2000	SmithKline Beecham "Battlefield Award" for exceptional performance in SmithKline Beecham in litigation on Phentermine. Cash Award.
1999-2000	Principle Actor; National television and print media campaign for the Pharmaceutical Research and Manufacturers of America. Selected for the discovery and development of Coreg (carvedilol) for congestive heart failure

Honors, Awards and Recognitions (Continued)

1999	Lorenzini Gold Medal for Biomedical Research, Lorenzini Medical Science Foundation
1998	Member (elected), Mark Nickerson Lecture Award Endowment Committee
1998	Chairman, International Union of Pharmacology (IUPHAR) Committee on Receptor Classification and Nomenclature
1998	John V. Croker Lecturer, The American Society for Pharmacology and Experimental Therapeutics
1998	Special Commendation awarded by SmithKline Beecham's Corporate Management Team for supervising Worldwide Discovery while head of Discovery recuperated from a serious automobile accident. Cash award.
1997	Visiting Lecturer of the Hungarian Academy of Sciences (elected by HAS), Budapest, Hungary
1997	Albert Szentgyörgy Medal, Szentgyörgy Medical School, Szeged, Hungary
1997	U.S. Pharmaceutical President's Award in Recognition of Outstanding Contribution for Research on Carvedilol (Coreg), U.S. Pharmaceuticals Division, SmithKline Beecham Pharmaceuticals
1997	R&D President's Gold Impact Award in Recognition of Outstanding Contribution to Carvedilol (Coreg) Approval for Congestive Heart Failure, Research and Development, SmithKline Beecham Pharmaceuticals.
1994-1997	Secretary/Treasurer, American Society for Pharmacology and Experimental Therapeutics (ASPET; Elected Position)
1996	Prix Galien Research Award Citation and Commendation for Innovation and Excellence in Research; Nonpeptide Endothelin Receptor Antagonists UK
1996	Prix Galien Innovation Research Award. United Kingdom
1995	Maloney-Booker Lecturer in Pharmacology. College of Medicine, Howard University, Washington, D.C.
1995	Distinguished Service Award, Publications Committee, Federation of American Societies for Experimental Biology
1995	Simply the Best Award, presented by CEO of SmithKline Beecham
1994	Distinguished Visiting Professor, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas
1991	Diploma - El Postgrado de Farmacologia, Universidad Central de Venezuela, Facultad de Farmacia, Caracas, Venezuela

Honors, Awards and Recognitions (Continued)

1989	Distinguished Alumni Award, The Ohio State University
1989	Certificate of Appreciation, Food and Drug Administration, Committee for Advanced Scientific Education
1988	John Jacob Abel Award in Pharmacology, American Society for Pharmacology and Experimental Therapeutics
1988	R&D President's Award, SmithKline Beckman Pharmaceuticals, for Exceptional Performance with CEO in presentation of R&D Pipeline to Analysts and Investors. Cash Award.
1984	Travel Award granted by the American Society for Pharmacology and Experimental Therapeutics to attend the Ninth IUPHAR International Congress for Pharmacology, London, England.
1982	Queen Beatrix Medal, Koningin Der Nederlanden, The Netherlands.
1981	Travel Award granted by the American Society for Pharmacology and Experimental Therapeutics to attend the Eighth IUPHAR International Congress for Pharmacology, Tokyo, Japan
1977-1978	PRAT Fellow: Fellow of the National Institutes of General Medical Sciences. Pharmacology Research Associate Training Fellowship
1976	Phi Kappa Phi Honor Society, The Ohio State University
1973-1976	Fellowship from the American Foundation for Pharmaceutical Education
1973	B.S. *Summa cum laude*, The Ohio State University
1973	Rho Pi Phi Scholastic Award, The Ohio State University
1973	George B. Kauffman Memorial Award for Scholarship, The Ohio State University
1972	Rho Chi Pharmaceutical Honor Society, The Ohio State University
1972	Rho Chi Scholarship Recognition Award, The Ohio State University
1972	Miami Valley Pharmaceutical Association Scholastic Award, The Ohio State University

Major Personal Role in the Discovery and Development of the Following Marketed Products

- Dobutrex (Dobutamine) for Congestive Heart Failure
- Requip (Ropinerole) for Parkinson's Disease
- Teveten (Eprosartan) for Hypertension
- Coreg/Kredex (Carvedilol) for Congestive Heart Failure and Acute Myocardial Infarction

The Following Products Were Approved During Tenure as President of Research & Development, Wyeth Pharmaceuticals

- Effexor for Depression and multiple indications
- Enbrel for Rheumatoid Arthritis and other Indications
- Pristiq (Desvenlefaxine) for Depression
- Relistor (Methylnaltrexone) for Opiate-Induced Consitpation
- InFuse (rhBMP-2) for Bone Healing
- Torisel (Temsirilomus) for Renal Cancer
- Lybrel (Levo Ethynylestradiol) for Continuous Contraception
- Xyntha (rhFactor VIII) for Hemophilia
- Tygacil (Tygacycline) Broad Spectrum Injectable Antibiotic
- Mylotarg (Gemtuzumab Ozogamicin) for Acute Myelogenous Leukemia
- Premarin (Conjugated Estrogens), Low Dose
- PremPro (Conjugated Estrogens/Progestin), Low Dose
- DuaVee (Conjugated Estrogens/Bazedoxifene) for Menopausal Symptoms
- Prevnar and Prevnar 13 vaccines for Pneumococcal Disease (children and adult)

Philanthropic Activities

- Established the *Ruffolo Charitable Fund* to support many charities, humanitarian projects and educational programs throughout the World

- With the American Society for Pharmacology and Experimental Therapeutics (ASPET), established the *Robert R. Ruffolo Career Achievement in Pharmacology Award (Medal) in Pharmacology*

- Underwrote the renovation of the *Robert & Stephany Ruffolo Lecture Hall* in the College of Pharmacy at The Ohio State University

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at The Ohio State University College of Pharmacy

- Established the *Popat N. Patil Endowed Scholarship* at The Ohio State University College of Pharmacy in honor of his former Professor and Advisor

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at The Ohio State University Fisher School of Business

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at West Virginia University School of Pharmacy

- Established the *Robert & Stephany Ruffolo Endowed Research Fellowship* at West Virginia University School of Pharmacy

- Funded over 20 Non-Endowed *Robert & Stephany Ruffolo Scholarships* at West Virginia University College of Pharmacy

Philanthropic Activities (Continued)

- Established the *Robert & Stephany Ruffolo Endowed Research Fellowship* at the University of Florida College of Medicine
- Major Donor to the *Mali Health Organization Project*
- Named *"American Patriot"* by Bill O'Reilly live "on air" on the Fox News Network and on the Bill O'Reilly website for his "*Generous Donation to the Fisher House Foundation*" which is dedicated to help fallen and disabled military and their families.

Ruffolo Consulting, LLC, Clients (Past and Present)

- Wyeth Pharmaceuticals
- Pfizer Pharmaceuticals
- Takeda Pharmaceuticals
- Merck, Sharp & Dohme
- EMD Serono Pharmaceuticals (Merck KGaA)
- Johnson & Johnson
- Novartis
- GlaxoSmithKline
- Teva Pharmaceuticals
- Shire Pharmaceuticals
- The Carlyle Group
- PPD (Pharmaceutical Product Development)
- Accenture
- McKinsey
- UCB
- Alcon
- Highfields Capital Management
- Tessella
- Trevena Pharmaceuticals
- Keddem Biotech
- Gillead
- HemoShear Technologies
- GLG Institute
- Gardner Roberts, LLP
- Goodwin-Proctor, LLP

Board Memberships and Consultancies (Past and Present)

- Trevena Pharmaceuticals (Board of Directors and Scientific Advisory Board)
- West Virginia University Foundation (Board of Directors)
- Gene Network Sciences (Scientific Advisory Board)
- HemoShear Pharmaceuticals (Scientific Advisory Board)
- Ore Pharmaceuticals (Scientific Advisory Board)
- West Virginia University School of Pharmacy Dean's Board of Directors
- University of Michigan Health Sciences Center
- The Ohio State University School of Business
- Mali Health Organization Project Board
- EMD Serono (International Advisory Board)
- DNDi (Drugs for Neglected Diseases Initiative) (Scientific Advisory Board)
- Sapience Pharmaceuticals (Board of Directors)
- Sigilon Pharmaceuticals (Board of Directors)
- Aridis Pharmaceuticals (Board of Directors)

Expert Witness in Lawsuits

- SmithKline Beecham Litigation on Diet Drug-induced Primary Pulmonary Hypertension and Valvular Heart Disease (1998-2000)
- Wyeth Pharmaceuticals Hormone Therapy Litigation following publication of the Women's Health Initiative (2007)
- Patent Litigation, Gardner Roberts LLP (2009-2010)
- Patent Litigation; Goodwin-Procter LLP (2010-2013)

Academic Appointments

2007-2014	Adjunct Professor of Physiology and Pharmacology, School of Medicine, West Virginia University
2004-2008	Corporate Advisory Board for the University of Michigan Medical School
2004-2008	Corporate Advisory Board, University of Pennsylvania School of Nursing
1989-Present	Adjunct Professor, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio
2004-2008	University of Pennsylvania School of Nursing Board of Overseers
2004-2009	Ohio State University College of Pharmacy Dean's Corporate Council
2000-2008	West Virginia University School of Pharmacy Dean's Advisory Board
1990-2001	Adjunct Professor, Department of Pharmacology, School of Medicine, Baylor University, Houston, Texas
1982-1989	Adjunct Professor, Department of Pharmacology, School of Medicine, McGill University, Montreal, Canada
1993-1996	Board of Visitors, School of Pharmacy, University of Wisconsin, Madison, Wisconsin

Professional Affiliations

Coalition Against Major Diseases, The Brookings Institute (Chaired by Dr. Mark McClellan, Former Commissioner of the FDA and CMS)

American Society for Pharmacology and Experimental Therapeutics (ASPET)

British Pharmacological Society (BPS)

International Union of Pharmacology (IUPHAR)

Federation of American Societies of Experimental Biology (FASEB)

Experimental Biology (EB)

Society of Critical Care Medicine (SCCM)

Mid-Atlantic Pharmacology Society (MAPS)

Professional Affiliations (Continued)

Screen Actors Guild (SAG); Principal Performer in Television Advertisement for Pharmaceutical Research and Manufacturers of America

SAPA Board of Advisors

Center for Biomedical Innovation; MIT

Drugs for Neglected Diseases (DNDi)

University of Florida College of Pharmacy Dean's National Advisory Board

Editorial Responsibilities

Editor-in-Chief, Current Opinions in Pharmacology (2000-2008)

Founding Editor, Current Opinions in Pharmacology (2000)

Editor-in-Chief, Pharmacology Reviews and Communications (1996-2000)

Founding Editor, Pharmacology Reviews and Communications (1996)

Editor, British Journal of Pharmacology (1998-2000).

Editor-in-Chief, Pharmacology Communications (1991-1996).

Founding Editor, Pharmacology Communications (1991)

Editor, Critical Reviews in Pharmacology (1993-1995)

Editor, The Journal of Pharmacology and Experimental Therapeutics; Autonomic Pharmacology (1985-1992).

Editor, Cardiovascular Drugs and Therapy; Adrenergic Modulation (1994-Present)

Editor, Cardiovascular and Renal Drugs, Current Opinion in Investigational Drugs (1992-present).

Co-Editor, Neuropharmacology, Textbook of Basic and Clinical Pharmacology (1990-1994)

Guest Editor, Autonomic Pharmacology, The Journal of Pharmacology and Experimental Therapeutics, 1982-1985.

Editorial Advisory Boards

British Journal of Pharmacology (1998 - 2000)
The Journal of Pharmacology and Experimental Therapeutics (1981 - 1986)
Journal of Cardiovascular Pharmacology (1991 - present)
Cardiovascular Drugs and Therapy (1993 - present)
Annual Reviews of Pharmacology (1991 - 1995)
Journal of Autonomic Pharmacology (1984 - present)
Fundamental and Clinical Pharmacology (1986 - 1992)
Journal of Medicinal Chemistry (1989 - 1995)
Trends in Pharmacological Sciences (1990 - present)
The Spilker Report (2004 - present)
Medicinal Research Reviews (1994 - 2003)
FASEB Journal Publications Committee (1989 - 1995)
Receptor (1989 - 1996)
Drug Development Research (1995 - Present)
Year Book of Pharmacology (1988 - 1992)
Journal of Chirality (1988 - 1996)
Drug News & Perspective (1989 - present)
Pharmacology Communications (1991 - 1996)
CRC Critical Reviews in Pharmacology (1992 - 1996)
Investigational Drugs Database (1993 - present)
Pharmaceutical News (1994 - present)
Research Biochemicals International (1993 - 1997)
Current Protocols in Pharmacology (1995 - 2001)
Receptors and Signal Transduction (1995 - 1996)
Pharmacology Reviews and Communications (1996 - Present)
Current Opinions in Pharmacology (2000 - Present)
Phacilitate: R&D Leaders Forum (2001 - Present)
Expert Review on Drug Metabolism and Toxicology (2004 – Present)
Expert Opinion on Drug Discovery (2005 – Present)

Reviewer for Research Grants

National Institutes of Health, National Institute on Drug Abuse, Study Section: Drug Abuse Biomedical Research Review Committee, 1984, Special Review Consultant

National Institutes of Health, Bio-organic and Natural Products Chemistry Study Section, 1984, Special Review Consultant

National Science Foundation, Molecular and Cellular Neurobiology Section 1984

Medical Research Council of Canada, Montreal

Tobacco and Health Institute, Lexington, Kentucky

National Institutes of Health, Study Section, Cardiovascular Pharmacology; Special Review Consultant, 1986

National Institutes of Health Study Section, Autonomic Pharmacology; Special Review Consultant, 1986

National Institutes of Health Study Section, Receptor Pharmacology; Special Review Consultant, 1986

National Institutes of Health, Study Section; Special Review Consultant, 1987

Member, Site Visit Committee, National Heart, Lung and Blood Institute, Stanford University, 1988

Member, Site Visit Committee, National Heart, Lung and Blood Institute, University of Chicago, 1988

American (ASPET) and International (IUPHAR) Pharmacology Society Roles

Council Member, Drug Discovery, Drug Development and Regulatory Affairs, Division of the American Society for Pharmacology and Experimental Therapeutics (1999-2008)

Officer, IUPHAR 2002 World Congress, 1996-2002.

Chairman, IUPHAR Committee for Receptor Nomenclature and Drug Classification (1998 - 2002)

ASPET Sollmann Award Selection Committee (1997)

Chairman, IUPHAR Finance Committee, International Union for Pharmacology (1995 - 2002)

IUPHAR Subcommittee for Receptor Nomenclature and Drug Classification: Endothelin Receptors (1995 - 2002), International Union for Pharmacology (IUPHAR)

ASPET Sollmann Award Selection Committee (1995)

IUPHAR Committee for Receptor Nomenclature and Drug Classification (1994-present), International Union for Pharmacology

Member, ASPET Council (1994-1997)

Member, ASPET Finance Committee (1994-1997)

Chairman, ASPET Finance Committee (1995-1996)

Member, ASPET Investment Subcommittee (1995-1996)

Secretary/Treasurer, American Society for Pharmacology and Experimental Therapeutics (1994-1997) (Elected Position)

Committee on Industrial - Academic Relations, American Society for Pharmacology and Experimental Therapeutics (1993 - 1994)

Nomination Committee, American Society For Pharmacology and Experimental Therapeutics (1992 - 1993) (Elected Position).

Subcommittee on Pharmacology in Industry, (1990-1992), American Society for Pharmacology and Experimental Therapeutics (ASPET)

IUPHAR Subcommittee for Receptor Nomenclature and Drug Classification: Adrenoceptors (1990-present), International Union for Pharmacology (IUPHAR).

Publications Committee, the FASEB Journal (1989-present), Federation of American Societies for Experimental Biology (FASEB)

American (ASPET) and International (IUPHAR) Pharmacology Society Roles (continued)

Selection Committee (1985), Pharmacology Research Associate Training (PRAT) Fellowships National Institute of General Medical Sciences, National Institutes of Health

Program Committee (1984-1989), American Society for Pharmacology and Experimental Therapeutics

Invited Lectures

University of Kansas, Department of Pharmacology, Lawrence, Kansas. Synapse turnover: A mechanism for acquiring synaptic specificity, 1978.

Mayo Clinic, Department of Pharmacology, Rochester, Minnesota. Alpha-Adrenergic Activity of Clonidine-like Imidazolines, 1979.

University of Connecticut, Department of Neurobiology, Storrs, Connecticut. Synapse Turnover: A Mechanism for Acquiring Synaptic Specificity, 1979.

Northeastern University, Section of Pharmacology, College of Pharmacy, Boston, Massachusetts. Alpha-Adrenergic Effects of Imidazolines and β-Phenethylamines, 1981.

University of Kentucky, Department of Pharmacology, College of Medicine, Lexington, Kentucky. Peripheral and Central Effects of Adrenergic Agonists, 1981.

Merck Institute for Therapeutic Research, Department of Cardiovascular Pharmacology, West Point, Pennsylvania. Evaluation of Clonidine-like Imidazolines from the Standpoint of Receptor Theory and Antihypertensive Activity, January 18, 1982.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Quantitative Analysis of Drug-Receptor Interactions in Classical Pharmacological Studies, March 2, 1982.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. An Evaluation of the Antihypertensive Activity of Clonidine-Like Imidazolines From the Standpoint of Classical Receptor Theory, March 3, 1982.

Dow Chemical Company, Department of Cardiovascular Pharmacology, Zionsville, Indiana. Central Alpha-Adrenergic Mechanisms in the Regulation of Blood Pressure. Evaluation From the Standpoint of Receptor Theory, May 3, 1982.

Laboratoires D'Etudes et de Recherches SYNTHELABO, Department of Cardiovascular Pharmacology, Paris, France. Stereochemical Requirements of Alpha-2 Adrenergic Receptors, May 24, 1982.

University of Amsterdam, Department of Pharmacodynamics, Amsterdam, The 1982. Differences Between the Interactions of Imidazolines and Phenethylamines with Alpha-Adrenergic Receptors, October 25, 1982.

Emory University, Department of Pharmacology, Atlanta, Georgia. Peripheral and Central Effects of α-Adrenergic Agonists, November 5, 1982.

Emory University, Department of Pharmacology, Atlanta, Georgia. The Current Status of α-Adrenergic Receptors. November 5, 1982.

Invited Lectures (Continued)

Smith Kline & French Laboratories, Department of Pharmacology, Philadelphia, Pennsylvania. Stereochemical requirements of α_1 and α_2-adrenoceptors. July, 1982.

State University of New York at Buffalo, Department of Biochemical Pharmacology, Buffalo, New York. Role of Central and Peripheral α-Adrenergic Receptors in the Regulation of Blood Pressure. December 1, 1982.

The Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. The Role of Central and Peripheral α-Adrenergic Receptors in the Regulation of Blood Pressure. February 9, 1983.

Tulane University, Department of Pharmacology, School of Medicine, New Orleans, Louisiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. May 24, 1983.

University of Mainz, Institute of Pharmacology, School of Medicine, Mainz, West Germany. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. June 21, 1983.

University of Wurzburg, Department of Pharmacology, School of Medicine, Wurzburg, West Germany. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. June 22, 1983.

Doctor's Hospital, Section on Critical Care Medicine, Grand Rounds, Columbus, Ohio. Central and Peripheral Regulation of the Cardiovascular System. September 7, 1983.

Mayo Clinic, Department of Physiology and Biophysics, Rochester, Minnesota. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. December 19, 1983.

Sloan-Kettering Memorial Hospital, Department of Critical Care Medicine, Grand Rounds, New York. Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. December 16, 1983.

Cook County Hospital, Department of Critical Care Medicine, Grand Rounds, Chicago. Central and Peripheral Regulation of the Cardiovascular System. May 10, 1984.

University of Illinois, Department of Neural and Behavioral Biology, Urbana, Illinois. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November 22, 1983.

Indiana University, Department of Pharmacology, Indianapolis, Indiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. December 2, 1983.

Invited Lectures (Continued)

Presbyterian University Hospital, Anesthesia Research Conference, Pittsburgh, Pennsylvania. New Insights into the Inotropic Activity of Dobutamine: Future Directions. February 9, 1984.

Mayo Clinic, Department of Physiology and Biophysics, Rochester, Minnesota. The Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors in the Vasculature: Caution about the Calcium Dependence of α_2-Adrenoceptors. December 19, 1983.

Presbyterian University Hospital, Department of Critical Care Medicine, Pittsburgh, Pennsylvania. Central and Peripheral Regulation of the Cardiovascular System. February 8, 1984.

Presbyterian University Hospital, Anesthesia Grand Rounds, Pittsburgh, Pennsylvania. Sympathomimetic Amines in the Treatment of Heart Failure and Shock. February 9, 1984.

University of Brussels, Society of Emergency and Critical Care Medicine. New Insights Into the Inotropic Action of Dobutamine. March 30, 1984.

Laboratoires D'Etudes et de Rechereches SYNTHELABO, Department of Cardiovascular Pharmacology, Paris, France. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature of the Pithed Rat: Possible Relationship to Calcium Utilization. March 26, 1984.

Ohio State University, Department of Clinical Pharmacology, College of Medicine, Columbus, Ohio. Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. March 1, 1984.

Ohio State University, Department of Pharmacology, College of Pharmacy, Columbus, Ohio. Possible Relationship Between Spare α_1- and α_2-Adrenoceptors and the Differential Utilization of Calcium for Vasoconstriction In Vivo. March 1, 1984.

Glaxo Group Research Ltd., Department of Cardiovascular Pharmacology, Ware, England. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature of the Pithed Rat: Possible Relationship to Calcium Utilization. July 26, 1984.

Wishard Hospital, 11th International Cardiovascular Opinion Leaders Meeting, Indianapolis, Indiana. New Insights into the Mechanism of the Inotropic Activity of Dobutamine. May 11, 1984.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Peripheral Adrenoceptors and Dopamine Receptors in the Cardiovascular System. October 2, 1984.

Veterans Administration Hospital, Research Services, Wood (Milwaukee), Wisconsin. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. October 3, 1984.

Invited Lectures (Continued)

Medical College of Pennsylvania, Department of Pharmacology, Philadelphia, Pennsylvania. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November 17, 1984.

Temple University School of Medicine, Department of Pharmacology, Philadelphia, Pennsylvania. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. January 16, 1985.

Medical College of Pennsylvania, Department of Physiology, Philadelphia, Pennsylvania. Graduate Course in The Biology of the Arterial Wall. Vascular Adrenergic Receptors. May 1, 1985.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Advances in α-Adrenoceptor Research. September 17, 1985.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Quantitative Analysis of Drug-Receptor Interactions. September 18, 1985.

Wayne State University, Department of Pharmacology, School of Medicine, Detroit, Michigan. Mechanism for the Positive Inotropic Effect of Dobutamine. November 1, 1985.

University of Utah, Department of Biochemical Pharmacology and Toxicology, Salt Lake City, Utah. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November, 1985.

Louisiana State University, Department of Pharmacology, School of Medicine, New Orleans, Louisiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. April 29, 1986.

West Virginia University Medical Center, Department of Pharmacology and Toxicology, Morgantown, West Virginia. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. September 29, 1986.

Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, Michigan. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature: Possible Relationship to Calcium Utilization. May 14, 1986.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Developments in α-Adrenoceptor Research. September 22, 1986.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature: Relationship to Calcium Utilization. September 23, 1986.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Biology, Paris, France. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. September 15, 1986.

Invited Lectures (Continued)

Allergan, Department of Pharmacology, Irvine, California. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. October 9, 1986.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Pharmacologic Basis of Drug-Receptor Interactions. February 23, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: Distribution and Function of α- and β-Adrenoceptors in the Cardiovascular System. February 24, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. February 25, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. February 26, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: Future Trends in Pharmacological Sciences. February 27, 1987.

Medical College of Pennsylvania, Department of Physiology Graduate Course entitled "Biology of the Arterial Wall". Lecture title: "Vascular Adrenergic Receptors". Philadelphia, Pennsylvania. May 6, 1987.

Analysts' and Investors' Meeting, SmithKline Beckman Corporation, Wyndham Franklin Plaza Hotel. The Future of Smith Kline & French Pharmaceutical R&D. Philadelphia, Pennsylvania. December 16, 1986.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Developments in α-adrenoceptor Research. September 21, 1987.

University of Vermont, Department of Pharmacology, College of Medicine, Burlington, Vermont. The Role of Central and Peripheral α-Adrenoceptors in the Control of Cardiovascular Function. October 8, 1987.

Blood Vessel Club, University of Vermont, Burlington, Vermont. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. October 8, 1987.

American Analysts and Investors' Colloquium, King of Prussia, Pennsylvania. Future Therapeutic Approaches to Congestive Heart Failure. December 2, 1987.

European Analysts and Investors' Colloquium, Welwyn Spring Garden, England. Future Therapeutic Approaches to Congestive Heart Failure. December 4, 1987.

Invited Lectures (Continued)

Food and Drug Administration, Department of Health, Education and Welfare, Bethesda, Maryland. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. February 4, 1988.

Dahlhouse University, Department of Pharmacology, Halifax, Nova Scotia. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. April 12, 1988.

Wayne State University, Department of Pharmacology, School of Medicine, Detroit, Michigan. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. April 1, 1988.

Ohio State University, Department of Veterinary Physiology and Pharmacology, School of Agriculture, Columbus, Ohio. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. May 17, 1988.

Baylor University, School of Medicine, Houston, Texas. Congestive Heart Failure, March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. The Pharmacology of Inotropic Agents. March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. The Pharmacology of Vasodilators, March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. Angina and Antianginal Drugs. March 29, 1988.

Baylor University, School of Medicine, Houston, Texas. Coronary Thrombosis and Thrombolytic Agents. March 29, 1988.

Eli Lilly & Company, Research and Development, Indianapolis, Indiana. 1988 John Jacob Abel Award. Lecture entitled: "The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure". June 22, 1988.

Food and Drug Administration, Department of Health, Education and Welfare, Bethesda, Maryland. Future Therapeutic Uses of α-Adrenoceptor Agonists and Antagonists. January 11, 1989.

Medical College of Pennsylvania, Department of Pharmacology, Philadelphia, PA. The Role of Guanine Nucleotide Regulatory Proteins in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction. May 8, 1989.

Baylor University, School of Medicine, Houston, Texas. Series of six lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs". April 3-6, 1989.

Invited Lectures (Continued)

National Institutes of Health, National Institute of Drug Abuse, Bethesda, Maryland. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins and Calcium Utilization in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". December 6, 1988.

Northeastern University School of Pharmacy, Departments of Pharmacology and Medicinal Chemistry, Boston, Massachusetts. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". December 13, 1988.

Harvard University, The Brigham and Women's Hospital, Department of Medicine, Boston, Massachusetts. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". December 13, 1988.

University of Houston, School of Pharmacy, Department of Pharmacology, Houston, Texas. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". April 4, 1989.

Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". May 12, 1989.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Lecture entitled: "The Physiology and Pharmacology of α_1- and α_2-Adrenoceptors". September 26, 1989.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". September 27, 1989.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Pharmacology, Paris, France. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins and α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". June 28, 1989.

Uniformed Services, Department of Pharmacology, School of Medicine, Bethesda, Maryland. Lecture entitled: "Signal Transduction Processes Involved α-Adrenoceptor-Mediated Vasoconstriction". December 12, 1989.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Pharmacology, Paris, France. Lecture entitled: " Renal α_2-Adrenoceptors". October 16, 1989.

Department of the Navy, Naval Hospital, Naval Medical Research Institute, Bethesda, Maryland. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". January 8, 1990.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure, Inotropic Agents, Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 2-6, 1990.

Invited Lectures (Continued)

Department of Biology, Les Laboratoires Beecham, Rennes, France. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". September 24, 1990.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Biology, Paris, France. Lecture entitled: "Evidence that a Single α_1-Adrenoceptor is Coupled to Two Signal Transduction Processes in the Vasculature". October 15, 1990.

Department of Pharmacology, Philadelphia College of Pharmacy and Sciences, Philadelphia, Pennsylvania. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". December 20, 1990.

Department of Physiology and Biophysics, University of Tennessee, Memphis, Tennessee. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". April 19, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". May 9, 1991.

Department of Pharmacology, University of Vermont, Burlington, Vermont. Lecture entitled: "A Single α_1-Adrenoceptor Subtype is Coupled to Two Signal Transduction Processes in Vascular Smooth Muscle". June 13, 1991.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure, Inotropic Agents, Vasodilators, Angina and Antianginal Drugs, Thrombolytic Agents, Antiarrhythmic Drugs, Diuretics". April 8-11, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Molecular Structure and Function of G-Protein Coupled Receptors". May 6, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Interactions of Receptors with G-Proteins". May 7, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Activated by G-Protein Coupled Receptors". May 8, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Receptor Regulation". May 9, 1991.

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "Cardioprotection by Carvedilol". August 24-25, 1991.

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "The Pharmacology of the Angiotensin II Receptor Antagonists, SK&F 108566 and SB 200220". August 25, 1991.

Invited Lectures (Continued)

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "Basic and Clinical Pharmacology of Carvedilol". August 25, 1991.

Department of Biology, Les Laboratoires Beecham, Rennes, France. Lecture entitled: "Cardioprotection by Carvedilol". September 20, 1991.

Society of Scandinavian Cardiologists, Monte Carlo, Monaco. Lecture entitled: "Cardioprotection by Carvedilol". January 23, 1992.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 27-30, 1992.

Division of Cardiology, Department of Medicine, University of Gothenburg, Gothenburg, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 20, 1992.

Department of Medicine, Malmo University, Malmo, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems; Not Only β-Blockade". May 21, 1992.

Department of Medicine, Karolinska Institute, Stockholm, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 25, 1992.

Department of Cardiology, School of Medicine, Upsala, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 27, 1992.

Department of Medicine, International Cardiological Institute for Therapeutic Research, Oslo, Norway. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol; Not Only β-Blockade". May 26, 1992.

Department of Medicine, University Hospital, Tromso, Norway. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol; Not Only β-Blockade". May 27, 1992.

Toronto Hypertension Society, Toronto, Canada. Lecture entitled: "α-Adrenoceptors in Hypertension". January 26, 1993.

Division of Cardiology, Department of Medicine, University of Toronto, Toronto, Canada. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptors Mediated Vasoconstriction". January 27, 1993.

Department of Medicine, University of Antwerp, Antwerp, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 27, 1992.

Division of Cardiology, Department of Medicine, University Hospital, Ghent, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 28, 1992.

Invited Lectures (Continued)

Department of Cardiology, University Hospital, Leuven, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 29, 1992.

Division of Cardiology, Department of Medicine, University of Brussels, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 29, 1992.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 12-15, 1993.

Department of Pharmacology, College of Medicine, University of Minnesota, Minneapolis, Minnesota. Lecture entitled: "Stereochemical Requirements of α_1- and α_2-Adrenoceptors". June 25, 1993.

Department of Molecular Biology, Human Genome Sciences, Gaithersburg, Maryland. Lecture entitled: "Drug Development Pipeline at SmithKline Beecham Pharmaceuticals". August 9, 1993.

Department of Medicine, University of Hawaii, Honolulu, Hawaii. Lecture entitled: "The Drug Discovery Process in the Pharmaceutical Industry: Myths and Realities". February 2, 1994.

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 7, 1994.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents. Vasodilators; Angina and Antianginal Agents; Thrombolytic Agents; Antiarrhythmic Agents; Diuretics". April 4-7, 1994.

Department of Pharmacology, University of Houston, Houston, Texas. Lecture entitled: "Effect of Point Mutations in Transmembrane Helices II and III on the Stereoselective Interaction of Catecholamines with α-Adrenoceptors". April 6, 1994.

University of Houston, Distinguished Visiting Professor Lecture and Award Ceremony, Houston, Texas. Lecture entitled: "Impact of Health Care Reform on the Pharmaceutical Industry". April 5, 1994.

Department of Pharmacology, University of Kansas, Kansas City, Kansas. Lecture entitled: "The Use of Site-Directed Mutagenesis in α_2-Adrenoceptors to Establish the Molecular Basis of Chirality". November 1, 1994.

Department of Medicine, Baylor University Medical School, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents, Vasodilators; Angina and Antianginal Agents; Thrombolytic Agents; Antiarrhythmic Agents; Diuretics". March 20-24, 1995.

Invited Lectures (Continued)

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 6, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Merck Corporate Conference Center, Woodridge, New Jersey. Lecture entitled: "Principles of Pharmacodynamics I". April 4, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Merck Corporate Conference Center, Woodridge, New Jersey. Lecture entitled: "Principles of Pharmacodynamics I". April 4, 1995.

Department of Pharmacology, Stanford University, College of Medicine, Palo Alto, California. Lecture entitled: "Molecular Basis of Chirality for the Interaction of Catecholamines with the Adrenoceptors". July 5, 1995.

Department of Medicine, Stanford University, College of Medicine, Palo Alto, CA, California. Lecture entitled: "The Pharmacology of Carvedilol". July 6, 1995.

World Bank, Washington, D.C. Lecture entitled: "The Process of Drug Discovery and Development: Risks and Pitfalls". June 8, 1995.

National Hospital, Department of Medicine, Oslo, Norway. Lecture entitled: "The Pharmacology of Carvedilol; Rationale for Use in Congestive Heart Failure". June 19, 1995.

Department of Medicine, Bergen, Norway. Lecture entitled: "The Pharmacology of Carvedilol; Rationale for Use in Congestive Heart Failure". June 20, 1995.

Department of Pharmacology, Institute of Pharmacological Sciences, University of Milan, Milan, Italy. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-adrenoceptors". October 25, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Arlington, Virginia. Lecture entitled: "Principles of Pharmacodynamics I". October 17, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Arlington, Virginia. Lecture entitled: "Principles of Pharmacodynamics II". October 17, 1995.

Department of Molecular and Medical Pharmacology, University of California, Los Angeles, California. Lecture entitled: "Molecular Basis of Chirality for the Interaction of Catecholamines with the Adrenoceptors". November 8, 1995.

Invited Lectures (Continued)

Congestive Heart Failure Advisory Board Meeting on Carvedilol, Philadelphia, Pennsylvania. Lecture entitled: "The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure". October 12, 1995.

Department of Pharmacology, College of Medicine, Howard University, Washington, D.C. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". November 29, 1995.

Department of Pharmacology, University of Vancouver, Vancouver, Canada. Lecture series entitled "Frontiers in Cardiovascular Research". Lecture entitled: "The Pharmacology of Carvedilol". May 17, 1996.

Laboratoires D'Etudes et de Recherches Synthelabo, Division of Biological Research, Paris, France. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". January 26, 1996.

Laboratoires D'Etudes et de Recherches Synthelabo, Division of Biological Research, Paris, France. Lecture entitled: "The Use of Genomics to Discover Novel Drug Targets". January 26, 1996.

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 5, 1996.

Department of Physiology, University of Glasgow, Glasgow, Scotland. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". September 26, 1996.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of Lectures entitled: "Congestive Heart Failure; Inotropic Agents, Vasodilators; Angina and Antianginal Drugs". August 8-9, 1996.

Department of Cardiology, Hospital General Universitari Vall D'Hebron, Barcelona, Spain. Lecture entitled: "The Antioxidant Activity of Carvedilol: Clinical Implications". December 13, 1996.

Italian Ministry of Health, Instituto di Sanita, Rome, Italy. Lecture entitled: "Innovation in Research and Development of Drugs: A Global Approach". May 28, 1997.

Joint Research & Development, Boehringer Mannheim and Daiichi Pharmaceutical Companies, Tokyo, Japan. Lecture entitled: "The Antioxidant Activity of Carvedilol and its Relationship to Congestive Heart Failure". September, 1997.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of Lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; β-Blockers in Heart Failure; Angina and Antianginal Drugs". August 18-19, 1997.

Invited Lectures (Continued)

Grand Rounds; Department of Medicine, Louisiana State University, New Orleans, Louisiana. Lecture entitled: "Rationale for the Use of β-Blockers in Congestive Heart Failure: Experience with Carvedilol". October 31, 1997.

Yamanouchi Tsukuba Research Center, Tsukuba, Japan. Lecture entitled: "The Research and Development Pipeline at SmithKline Beecham". September 17, 1997.

Hungarian Academy of Sciences, Budapest, Hungary. Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Drugs with α-Adrenoceptors". October 14, 1997.

Albert Szentgyörgy Medical School, Department of Pharmacology, Szeged, Hungary. Lecture entitled: "The Pharmacology of Carvedilol: Use in Congestive Heart Failure". October 17, 1997.

Department of Pharmacology, University of West Virginia, Morgantown, Virginia. Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Drugs with α-Adrenoceptors". November 17, 1997.

Department of Medicine, Albert Einstein University, College of Medicine, Bronx, New York. Lecture entitled: "The Discovery and Development of Carvedilol: From the test-tube to the Patient". December 17, 1997.

John V. Croker Lecture, The American Society for Pharmacology and Experimental Therapeutics, San Francisco, California. Lecture entitled: "Pharmacology and the Pharmaceutical Industry: An assessment of the present and a prediction of the future". April 19, 1998.

Program Introduction Meeting, SB The Netherlands, New York, New York. Lecture entitled: "The Effect of Teveten on the Sympathetic Nervous System". April 26, 1998.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of lectures entitled: "Congestive Heart Failure, Angina and Antianginal Drugs". August 13, 1998.

Wharton Business School, Philadelphia, Pennsylvania: Lecture entitled: "Drug Discovery in the Pharmaceutical Industry". April 26, 1999.

Phentermine Legal Defense Team (approx. 120 Attorneys), Wyndham Franklin Plaza Hotel, Philadelphia, Pennsylvania: Lecture entitled: "The Pharmacology of Phentermine and Related Sympathomimetic Amines". January 14, 1999.

Department of Medicine, Tulane University and Louisiana State University, New Orleans, Louisiana. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". June 16, 1999.

Clinical Development Partners Annual Meeting, Phoenix, Arizona. Lecture entitled: "The Impact of Genomics on Drug Discovery". April 30, 1999.

Invited Lectures (Continued)

Meeting of the Key Opinion Leaders in Nephrology, New York, NY. Lecture entitled: "The Renal Protective Effects of Carvedilol (Coreg®) and Rosiglitazone (Avandia®)". April 19, 1999.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of lectures entitled: "Congestive Heart Failure, Angina and Antianginal Drugs". August 23, 1999.

Grand Rounds, Department of Medicine, Tallahassee, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". September 1, 1999.

Grand Rounds, Department of Medicine, Tampa, Florida. Lecture entitled: " New Frontiers in the Pharmacotherapy of Congestive Heart Failure: Recent Experience with β-Blockers". November 11, 1999.

Grand Rounds, Department of Medicine, Panama City, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". November 17, 1999.

Division of Pharmacology, Johns Hopkins University, Baltimore, Maryland: Lecture entitled: "The Discovery and Development of Carvedilol: From the Test-Tube to the Patient". March 1, 2000.

Division of Pharmacology, Johns Hopkins University, Baltimore, Maryland: Lecture entitled: "The Molecular Basis of the Stereoselective Interactions of Catecholamines with the Adrenoceptors". March 1, 2000.

Grand Rounds, Tampa, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". March 7, 2000.

Wharton Business School, Philadelphia, Pennsylvania: Lecture entitled: "Drug Discovery in the Pharmaceutical Industry". May 1, 2000.

Grand Rounds, Department of Medicine, Panama City, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". May 11, 2000.

Grand Rounds, Department of Medicine, Pensacola, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". May 12, 2000.

Grand Rounds, Department of Medicine, Tampa, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". November 2, 2000.

Invited Lectures (Continued)

Grand Rounds, Department of Medicine, Fort Walton Beach, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". October 12, 2000.

Grand Rounds, Department of Medicine, Mount Sinai School of Medicine, New York. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". October 19, 2000.

University of Minnesota, College of Medicine; Department of Pharmacology, Minneapolis, Minnesota. Lecture entitled: "Modern Drug Discovery and Development", 2002.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 4, 2002.

Accenture Discovery Advisory Board, Philadelphia, Pennsylvania. Lecture entitled: "Increasing Productivity in the R&D Process". June 3, 2002.

Heidrick and Struggles Global Healthcare Meeting, Princeton, New Jersey. Lecture entitled: "Filling the R&D Pipeline". April 29, 2002.

University of Minnesota, Department of Pharmacology, College of Medicine, Minneapolis, Minnesota. Lecture entitled: "Drug Discovery in the New Millennium". April 25, 2003.

Division of Pharmacology, School of Medicine, John Hopkins University, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". February 11, 2004.

College of Pharmacy, The Ohio State University, Columbus, OH. Lecture entitled: "The Discovery and Development of Carvedilol: From the Test Tube to the Patient". May 7, 2004.

FDA Grand Rounds, Rockville, Maryland. Lecture entitled: "Issues Affecting R&D Productivity: Obstacles and Solutions". September 2, 2004.

Grand Rounds, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida. Lecture entitled: "The Future of Women's Health: A Pipeline Perspective". November 19, 2004.

Grand Rounds, Department of Cardiology, University of Florida, Gainesville, Florida. Lecture entitled: "The Discovery of Carvedilol (Coreg) for the Treatment of Congestive Heart Failure: From the Test Tube to the Patient". November 19, 2004.

Grand Rounds, Department of Obstetrics and Gynecology, Emory University, Atlanta, Georgia. Lecture entitled: "The Future of Women's Health: A Pipeline Perspective". March 30, 2005.

Invited Lectures (Continued)

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 3, 2005.

Department of Pharmacology, Vanderbilt University, College of Medicine. Lecture entitled: "Opportunities in the Pharmaceutical Industry in the 21st Century". Nashville, Tennessee, July 26, 2005.

Grand Rounds, Health Sciences Grand Rounds, West Virginia University Medical School. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: The Saga of Carvedilol". Morgantown, West Virginia, August 31, 2006.

Grand Rounds, Department of Medicine, West Virginia University School of Medicine. Lecture entitled: "Drug Discovery and Development: Impact on the Cost of Health Care Delivery". Morgantown, West Virginia, September 1, 2006.

Distinguished Lecture Series, Temple University. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol): A New Paradigm for the Treatment of Congestive Heart Failure". Philadelphia, Pennsylvania, November 30, 2006.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 10, 2006.

Health Sciences Center, West Virginia University Medical School. Lecture entitled: "Management of Change to Increase Productivity in a Scientific/Technical Environment". Morgantown, West Virginia, January 16, 2007.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 12, 2007.

Massachusetts College of Pharmacy and Health Sciences. Lecture entitled: "The Trials and Tribulations of a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Boston, Massachusetts, April 17, 2008.

Department of Pharmacology, University of Michigan School of Medicine. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Ann Arbor, Michigan, October 8, 2008.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 4, 2008.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". February 2, 2009.

Invited Lectures (Continued)

University of Delaware, Department of Psychiatry, Newark, Delaware. Lecture entitled: "The Drug Discovery and Development Process". April 1, 2009

Johns Hopkins University, School of Medicine, Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg). April 1, 2009.

Columbia University, School of Business, New York, New York. Lecture entitled: "The Drug Discovery and Development Process". April 3, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "Overview of the Pharmaceutical Industry; The World's Most Unique Industry". May 6, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "The Research & Development Process in the Pharmaceutical Industry; The Source of Most New Medicines". May 6, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "The Interplay Between the Pharmaceutical Industry, Academia, Research Institutes and the Government in the Development of New Drugs". May 7, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "Training Needs for the Pharmaceutical Industry; Expectations of Graduate Education". May 7, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "My Journey from OSU to the Pharmaceutical Industry and Back Again to OSU". May 8, 2009.

The Great Oxford Debate, Oxford University, United Kindgom. The Role of the Pharmaceutical Industry and Academia in Conducting Clinical Trials. September 23, 2009.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". August 18, 2009

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". January 22, 2009

Department of Pharmacology, University of Florida, College of Pharmacy. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Gainesville, Florida, February 19, 2009.

Invited Lectures (Continued)

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 31, 2010

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 30, 2011

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 4, 2012

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2013

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 2, 2014

IMPACT Branding & Design, LLC, Wallingford, Connecticut: Lecture entitled: "The Qualities of Leadership". September 19, 2014.

Invited Lectures (Continued)

The Hartford, Hartford, Connecticut. Lecture Entitled: "Leadership Practices Relevant to the 21st Century Business Environment". June 18, 2010

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2009

XenoBiotic, Plainsboro, New Jersey. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". September 29, 2011.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 29, 2010.

Chauncey D. Leake Award Lecture, Columbus, Ohio. Lecture entitled: "The Drug Discovery and Development Process: A Case Study with Carvedilol for the Treatment of Heart Failure". Spring, 2013

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 26, 2012.

University of Florida, College of Pharmacy, Orlando, Florida. Lecture entitled: "Drug Discovery and Development: Past Present and Future". November 8, 2012.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2013.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2014.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2015.

Servier, Paris, France. Lecture entitled: "Qualities of Leadership Necessary to Change the Direction of a Large R&D Organization", September 7, 2015.

Invited Symposium Speaker

American Society for Pharmacology and Experimental Therapeutics, Graduate Student Convocation, Columbus, Ohio. Lecture Title: Pharmacological and Biochemical Characterization of Adrenoceptors. August, 1977.

International Symposium on Neuroreceptors. Lecture entitled: Alpha-Adrenoceptors. Terre Haute Center for Medical Education, Terre Haute, Indiana, September 25, 1982.

International Symposium on Neuroreceptors. Lecture entitled: The Use of Isolated Intact Tissues to Study Neurotransmitter Receptors. Terre Haute Center for Medical Education, Terre Haute, Indiana, September 25, 1982.

International Symposium, "Stereochemistry and Biological Activity of Drugs". Stereoselectivity in Adrenergic Agonists and Adrenergic Blocking Agents. Noordwijkerhout, Holland, October 21-22, 1982.

American Chemical Society Symposium: "Alpha$_2$-Adrenergic Receptors". Stereochemical Requirements of Alpha$_2$-Adrenergic Receptors. Seattle, Washington, March 21, 1983.

Society of Critical Care Medicine Symposium, Plenary Lecture: Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. New Orleans, Louisiana, May 27, 1983.

Society of Critical Care Medicine Symposium, Pharmacological Vignettes: Dobutamine Enantiomers, Contribution of α- and β-Adrenoceptor Activity to Inotropic Selectivity. New Orleans, Louisiana, May 26, 1983.

American Society for Pharmacology and Experimental Therapeutics, Symposium on "Peripheral Alpha-Adrenergic Receptors". Interactions of Agonists with Peripheral Alpha-Adrenergic Receptors. Philadelphia, Pennsylvania, August 11, 1983.

Symposium on Centroperipheral Resetting Loops, organized by the Department of Medical Pharmacology, Pharmacology Institute of Milan, Subclassification of Adrenoceptors, Florence, Italy, November 13-17, 1983.

Fourth International Symposium of Intensive Care and Emergency Medicine, Plenary Lecture. The Role of Central and Peripheral Alpha- and Beta-Adrenoceptors in the Control of Cardiovascular Function. Brussels, Belgium, March 28-30, 1984.

Workshop on Alpha-Adrenoceptors, Session Title: How Many Types of α-Adrenoceptors are Currently Required. Lecture Title: Agonist Potency Series, Ross Priory on Loch Lomondside, Glasgow, Scotland, July 27-28, 1984.

Satellite Symposium of the 9th International Congress of Pharmacology, Pharmacology of Adrenoceptors: Selective α_1-Adrenoceptor Agonists and Antagonists. Manchester, England, August 6, 1984.

Invited Symposium Speaker (Continued)

American Society for Pharmacology and Experimental Therapeutics, Symposium entitled "α-Adrenoceptor Distribution and Function". Lecture Title: Distribution and Function of Peripheral α-Adrenoceptors. Indianapolis, Indiana, August, 1984.

Symposium on Contemporary Issues in the Management of Chronic Congestive Heart Failure. Lecture Title: Importance of Receptor Regulation in the Pathophysiology and Therapy of Congestive Heart Failure, Baltimore, Maryland, May 3, 1985.

Federation of American Societies for Experimental Biology (FASEB) Symposium Title: "Subtypes of Alpha-Adrenoreceptors in Systemic and Pulmonary Vascular Beds". Lecture Title: Spare Alpha-Adrenoceptors: Excitation-Contraction Coupling. Anaheim, California, April, 1985.

Joint Statistical Meetings of the American Statistical Association Symposium entitled "Statistical Aspects of Drug-Receptor Interactions". Lecture Title: Pharmacological Basis of Drug-Receptor Interaction. Las Vegas, Nevada, August 5-8, 1985.

International Symposium entitled "Brain Epinephrine Neuronal Functions". Lecture Title: Alpha-Adrenoceptor Coupling to Vasoconstriction in the Peripheral Circulation. Baltimore, Maryland, September 29 - October 2, 1985.

Symposium entitled "The Adrenergic Receptors". Lecture Title: Alpha-Adrenoceptor Location and Function in the Cardiovascular System. University of Michigan, Ann Arbor, Michigan, May 15, 1986.

Symposium entitled "The Adrenergic Receptors". Lecture Title: The Role of Central and Peripheral Alpha-Adrenoceptors in the Regulation of Blood Pressure. University of Michigan, Ann Arbor, Michigan, May 15, 1986.

Symposium Honoring The Centennial Celebration for The Ohio State University College of Pharmacy. Lecture Title: Visions of the Future in Pharmacology. Columbus, Ohio, September 13, 1985.

Future of Cardiovascular/Renal Therapeutics Symposium. Lecture Title: The Functional Role of α_2-Adrenoceptors in the Peripheral Arterial and Venous Circulation. Naples, Florida, February 22, 1986.

Smooth Muscle Function Symposium, Satellite Symposium of XXX International Congress of the Physiology. Lecture Title: Pharmacologic Basis of Drug-Receptor Interaction. Banff, Alberta, Canada, July 2, 1986.

International Committee on Medicinal Chemistry Symposium entitled "Recent Advances in Receptor Chemistry". Lecture Title: The Mode of Action and Structure - Activity Relationships Among Imidazoline-like Compounds Acting at the α-Adrenoceptor. Camerino, Italy, September 6-10, 1987.

Invited Symposium Speaker (Continued)

New York Academy of Sciences and the Giovanni Lorenzini Medical Foundation Symposium on "Calcium Antagonists: Pharmacology and Clinical Research". Lecture Title: Causes of Heterogeneity in the Importance of Calcium Entry in Vascular Smooth Muscle. New York, February 11-13, 1987.

Satellite Symposium to the 11th Scientific Meeting of the International Society of Hypertension entitled "Adrenergic Receptor Function and Cardiovascular Reactivity in Human Hypertension". Lecture Title: Arterial α_2-Adrenoceptor Blockade: A New Approach to Antihypertensive Therapy. Essen, Federal Republic of Germany, September 8, 1986.

Vascular Neuroeffector Mechanisms 6th International Symposium, session on "α-Adrenoceptors in the Vasculature". Lecture Title: The relationship between agonist efficacy and receptor reserve to the sensitivity of α-adrenoceptor-mediated vasoconstriction to inhibition by calcium entry blockers. Melbourne, Australia, August 30-September 2, 1987.

Federation of the American Societies for Experimental Biology (FASEB) Symposium entitled "Vasomotor Regulatory Mechanisms: Central and Peripheral Aspects". Lecture title: Adrenergic Receptor Distribution and Function. Washington, DC, April, 1987.

American Society for Pharmacology and Experimental Therapeutics (ASPET) Symposium entitled "Spare α-Adrenoceptors in the Vasculature". Lecture title: The relationship between α_1-adrenoceptor reserve and calcium utilization in the vasculature. Honolulu, Hawaii, August, 1987.

14th Annual Harvard Medical School Postgraduate Course entitled "Intensive Care Medicine-Mastering the New Skills". Lecture title: Clinical Implications of Adrenergic Physiology. Boston, Massachusetts, April 27-29, 1987.

Federation of the American Societies for Experimental Biology (FASEB). Catecholamine Club Annual Dinner. Lecture title: Pharmacologic Differentiation Between Pre- and Postjunctional α_2-Adrenoceptors. Washington, DC, March 31, 1987.

Future of Cardiovascular/Renal Therapeutics Symposium. Lecture title: The Pharmacologic Differentiation Between Pre- and Postjunctional α_2-Adrenoceptors: Relevance to Cardiovascular Disease. Tempe, Arizona, March 14, 1987.

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Renal α_2-Adrenoceptors". Lecture title: Pharmaceutical Aspects of α_2-Adrenoceptors. Las Vegas, Nevada, May, 1988.

Xth International Congress of Pharmacology, Symposium entitled "Drug Metabolism and its Pharmacokinetic and Pharmacodynamic Consequences". Lecture title: Enantioselectivity: Its Biological Basis and Pharmacological Consequences. Sydney, Australia, August 27, 1987.

Invited Symposium Speaker (Continued)

Future Therapeutic Approaches to Congestive Heart Failure. Lecture title: "α-Adrenoceptor Antagonists", London, England, November 7-8, 1987.

Future Therapeutic Approaches to Ischemic Heart Disease. Lecture title: "Acute Myocardial Infarction: The Role of Thromboxane A_2 Receptor Antagonists in Coronary Thrombosis and Thrombolysis", San Juan, Puerto Rico, February 12-14, 1988.

Mid-Atlantic Pharmacology Society Symposium entitled "Molecular Pharmacology: Future Drug Development". Lecture title: "Molecular Pharmacology of α-Adrenoceptors: Future Drug Development", Jefferson University, Philadelphia, Pennsylvania, April 8, 1988.

International Carvedilol Symposium. Lecture entitled: "Preclinical Pharmacology of Carvedilol". Nice, France, June 11, 1988.

Federation of the American Societies for Experimental Biology (FASEB). Overview entitled "Distribution Function, Isolation and Subclassification of α_2-Adrenoceptors. Las Vegas, Nevada, May 2, 1988.

16th Annual Harvard Medical Course in Intensive Care Medicine, Harvard Medical School. Lecture title: "The α-Adrenergic Receptor: New Insights into Lung and Cardiovascular Function", Boston, Massachusetts, April 27, 1989.

American Motility Society Symposium on "Cell Membrane Receptors". Lecture title: "Adrenergic Receptors", Monterey, California, October 3, 1988.

National Institutes of Health Symposium on "Animal Use in Research". Lecture title: "Use of Animal Model Systems in Drug Discovery". Washington, D.C. May 1-2, 1989.

Federation of American Societies for Experimental Biology (FASEB) symposium entitled "Subclassification of α-Adrenoceptors". Lecture entitled: Interaction of Vascular α_1-Adrenoceptors with Multiple Signal Transduction Pathways". Washington, DC, 1990.

13th Annual Conference on Shock, symposium on "Basic Pharmacologic Principles Applied to Shock Research" Lecture entitled: "Characterization of Receptors by Physiologic Assays". Durango, Colorado, June 11, 1990

XIth International Congress of Pharmacology, Satellite Symposium entitled "Pharmacology of Adrenoceptors." Lecture entitled: "Structure Activity Relationships". Manchester, England, June 27, 1990.

10th International Symposium on Intensive Care and Emergency Medicine. Lecture entitled: "The Role of α_1- and α_2-Adrenoceptors in the Regulation of the Cardiovascular System". Brussels, Belgium, March 28, 1990.

Invited Symposium Speaker (Continued)

Roussel Scientific Institute - Table Ronde on "Chirality and Drug Design". Lecture entitled: "Stereospecificity at Receptors". Oxford, England, July 12-13, 1990.

Federation of American Societies for Experimental Biology (FASEB) symposium entitled: "Impact of Federal Agencies on Drug Development and Utilization". Lecture entitled: "Identification of Novel α-Adrenoceptor Agonists and Antagonists for Drug Development". Washington, DC, 1990.

Second Congress on "Strategies and Prospects in Cardiovascular Research", Satellite symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol". Antwerp, Belgium, February 20, 1990.

International Symposium on Presynaptic Receptors and Neuronal Transporters. Lecture entitled: "Possible Heterogeneity Between Prejunctional and Postjunctional $\alpha 2$-Adrenoceptors in the Cardiovascular System". Rouen, France, June 26-29, 1990.

Vascular Neuroeffector Mechanism 7th International Symposium. Lecture entitled: "Interaction of Vascular α_1-Adrenoceptors with Multiple Signal Transduction Pathways". Bonn, West Germany, July 8-11, 1990.

Thrombolytics Symposium. Lecture entitled: "The Future of Cardiovascular Therapeutics". Bermuda, March 30, 1990.

Intensive Care and Emergency Medicine Symposium; Tutorial entitled: Adrenergic Receptors, Brussels, Belgium, March 29, 1990.

Twentieth Annual Meeting of New England Pharmacologists, Wallingford, Connecticut. Lecture entitled: "Signal Transduction Processes Involved α-Adrenoceptor-Mediated Vasoconstriction". February 1, 1991.

International Symposium on "Carvedilol; Refining Antihypertensive Therapy". Lecture entitled "The Pharmacology of Carvedilol", Paris, France, October 19, 1990.

XIth International Congress of Pharmacology, Satellite Symposium entitled "Pharmacology of Adrenoceptors". Lecture entitled: Molecular Structure and Genetics of Adrenoceptors. Manchester, England, June 27, 1990.

International Symposium Entitled "Receptors: Actualization and Clinical Importance". Lecture entitled: "Adrenoceptors". Santiago, Chile, July 8-31, 1991.

International Symposium on "Carvedilol; Refining Antihypertensive Therapy". Lecture entitled "Antihypertensive Drugs and the Coronary Circulation", Paris, France, October 19, 1990.

First International Symposium on "Imidazoline Specific Receptors". Lecture entitled: "Evolution of the concept from $\alpha 2$-adrenoceptors to imidazoline specific receptors". Paris, March, 1992.

Invited Symposium Speaker (Continued)

Twentieth Annual Meeting of New England Pharmacologists, Wallingford, Connecticut. Lecture Panel on "Pharmacology in the Industrial Setting". February 1, 1991.

Symposium in Medicinal Chemistry on "Structure-Activity Relationships". Lecture entitled: "Structure-activity relationships of α-adrenoceptor agonists and antagonists". Minneapolis, Minnesota, June 28, 1991.

German-Austrian Society of Critical Care Medicine. Lecture entitled: "Preclinical Pharmacology of Fenoldopam". Hannover, Germany, October 25, 1991.

Symposium on "Carvedilol: The Promise of the Future". Lecture entitled: "The Pharmacology of Carvedilol", Handbury Manor, England, March 23, 1991.

Swedish Academy of Pharmaceutical Sciences Symposium on "Neuromedicinal Chemistry of G-Protein Coupled Receptors". Lecture entitled: Medicinal Chemistry of Adrenoceptor Agonists", Lund, Sweden, May 20-22, 1992.

Conference on Vascular Smooth Muscle. Lecture entitled: "Signal transduction processes involved in α-adrenoceptor mediated vasoconstriction", Burlington, Vermont, June 13, 1991.

Annual Conference of the Chairmen of Pharmacology in United States Medical Schools. Lecture entitled: "Training Needs of the Pharmaceutical Industry in Pharmacology", Cloisters, Georgia, February 15, 1992.

Annual Medical Sciences Symposium of the University of Calgary. Lecture entitled: "Signal transduction processes involved in α-adrenoceptor mediated vasoconstriction", The University of Calgary, Calgary, Alberta, Canada, March, 1992.

International Cardiological Institute for Therapeutic Research Symposium on "Carvedilol: Wider Therapeutic Potential in Cardiovascular Syndromes", Satellite Symposium of the 13th Congress of the European Society of Cardiology. Lecture entitled: Cardio-protective Potential of Carvedilol, Scheveningen, The Netherlands, August 23, 1991.

International Symposium Entitled "Hypertension and Concomitant Diseases in the Elderly: New Perspectives". Lecture entitled: "Carvedilol - Novel Pharmacology with Far Reaching Clinical Potential". Monte Carlo, Monaco, January 22-25, 1992.

International Symposium Entitled "From α_2-Adrenoceptors to the Imidazoline-Preferring Receptors". Satellite Symposium of the 7th International Catecholamine Symposium. Keynote lecture entitled "From α_2-Adrenoceptors to the Imidazoline-Preferring Receptors: An Historical Overview". Paris, France, June 29-30, 1992.

International Symposium entitled: "Peptide Therapies in Developmental Gastroenterology and Nutrition". Lecture entitled "The G-Protein Family of Peptide Receptors", Columbus, Ohio, October 8-10, 1992.

Invited Symposium Speaker (Continued)

International Symposium entitled: "Anti-atherosclerotic Drugs: Medicinal, Chemical and Biochemical Aspects". Sponsored by the Division of Medical Chemistry, American Chemical Society. Lecture entitled: "Adrenoceptors and Lipid Lipoprotein Metabolism", Cincinnati, Ohio, May 28, 1992.

International Symposium entitled: "Vasodilating β-Blockers: Hemodynamics, Clinical Implications and Promises for the Future". Continuing Medical Education Symposium. Lecture entitled: "Cardioprotective Potential of Carvedilol". Reims, France, April 28, 1992.

American Society for Pharmacology and Experimental Therapeutics (ASPET) Symposium entitled: "Graduate Pharmacology Instruction in the Age of Molecular Biology". Lecture entitled: "Traditional vs. Molecular/Cellular-Based Pharmacology: A View from Industry". Orlando, Florida, August 14-18, 1992.

Twenty Second Annual Meeting of the New England Pharmacologists. Lecture entitled: Training Needs in the Pharmaceutical Industry in Pharmacology. Lake Morey Resort, Sarlee, Vermont, February 2-6, 1993.

International Symposium on Carvedilol. Lecture entitled: "Major Organ Protection by Carvedilol". Brussels, Belgium, October 26-30, 1992.

First International Symposium on Imidazoline Preferring Receptors. Led and Chaired Discussion Group. Lecture entitled: "Future Directions", Paris, France, June 30, 1992.

International Symposium on Polychlorinated Biphenyls (Sponsored by the Environmental Protection Agency). Lecture entitled: "Receptor Theory: Agonists, Partial Agonists and Competitive Antagonists". Chicago, Illinois, August 17, 1992.

International Symposium on Carvedilol. Lecture entitled "Major Organ Protection by Carvedilol". London, England, October 21-22, 1992.

Australian Society of Pharmacology/Western Society of Pharmacology Joint Meeting. Symposium entitled "Novel Nonpeptide Angiotensin II Receptor Antagonists". Lecture entitled: "Cardiovascular Effects of the Nonpeptide Angiotensin II Receptor Antagonists". Lake Tahoe, Nevada, February 1-5, 1993.

International Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: Major Organ Protection". Stockholm, Sweden, March 9, 1993.

Pharmaceutical Manufactures Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics I". Philadelphia, Pennsylvania, February 23, 1993.

Pharmaceutical Manufactures Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics II". Philadelphia, Pennsylvania, February 23, 1993.

Invited Symposium Speaker (Continued)

XIIIth International Symposium on Medicinal Chemistry. Symposium on "Low Urinary Tract Diseases". Lecture entitled: "Recent Progress in the Treatment of Low Urinary Tract Diseases", Paris, France, September 19-23, 1994

International Symposium on "Multiple Action Antihypertensives, Hemodynamics, Clinical Implications and Promises for the Future: An Update. Lecture entitled: "Free radicals in ischemic tissue damage; role for carvedilol. Luxemburg, May 8, 1993.

Satellite Symposium of the XVth Congress of the European Society of Cardiology Symposium entitled: "The Heart Failure Syndrome - From Prevention to Treatment". Lecture entitled: "A Role for the Antiproliferative and Antioxidative Effects of Carvedilol". Nice, France, August 29, 1993.

Symposium entitled "Ischemic Episodes: Strategies in Cardioprotection and the Role of Free Radicals". Lecture entitled: "The Promise of Protection: Carvedilol is a Potent Antioxidant. Oslo, Norway, March 8, 1993.

International Launch Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: Antiproliferative and Antioxidant Activities. Oslo, Norway, August 27, 1993.

37th Annual Meeting of the Western Pharmacology Society. Symposium on "α_1-Adrenoceptors". Lecture entitled: "The Pharmacological Classification of α_1-Adrenoceptors". January 30 - February 4, 1994, Kona, Hawaii.

8th Annual Meeting on Adrenergic Mechanisms. Symposium on "Adrenoceptors and Second Messengers". Lecture entitled: "Signal Transduction Mechanisms Utilized by Vascular α-Adrenoceptors". September 19-22, 1993, Porto, Portugal.

Symposium entitled "The Pharmacology of Adrenoceptors". Satellite symposium to the XII IUPHAR Congress. Lecture entitled "Overview: α_1-Adrenoceptors". July 21, 1994, King of Prussia, Pennsylvania.

2nd International Meeting on Imidazoline Receptors. Satellite symposium to the XII IUPHAR Congress. Lecture entitled: "Relationships Between Imidazoline and α_2-Adrenergic Receptors". July 19, 1994, New York, New York.

Pharmaceutical Manufacturers Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics I". Washington, D.C., September 27, 1993.

Pharmaceutical Manufacturers Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: General Principles: Pharmacodynamics II". Washington, D.C., September 27, 1993.

8th International Symposium on Vascular Neuroeffector Mechanisms. Lecture entitled "Receptor, Receptor Interactions and Vascular Smooth Muscle Function: Overview". August 3, 1994, Kananaskis, Alberta, Canada.

Invited Symposium Speaker (Continued)

The Irvington Institute for Medical Research Symposium on "New York Area Careers in Industry". Lecture entitled "Career Opportunities in the Pharmaceutical Industry". October 15, 1993, New York, New York.

Pharmacology-Medicinal Chemistry Annual Symposium, University of Minnesota. Lecture entitled: "Structure-Activity Relationships and Stereochemical Requirements for α_1- and α_2-Adrenoceptors". June 23, 1993. Minneapolis, Minnesota.

Oxford International Biomedical Centre symposium on "Biomedicine Today and Tomorrow". Lecture entitled: "Drug Discovery Into the Next Millennium: From the Gene to the Human". December 17, 1993, King of Prussia, Pennsylvania.

XIIth World Congress of Cardiology and XVth Congress of the European Society of Cardiology symposium entitled "Beta-Blockers in Heart Failure: Myths and Realities". Lecture entitled: Vasodilating beta-blockers in heart failure: their experimental potential. September 10-14, 1994, Berlin, Germany.

Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: antiproliferative and antioxidant activities". Copenhagen, Denmark, February 22, 1994.

International Symposium on Carvedilol. Lecture entitled: Major organ protection with carvedilol, a potent antioxidant and antiproliferative agent. Budapest, Hungary, April 9, 1994.

International Symposium on Vasodilating β-Blocking Drugs: A New Generation of Antihypertensives. Lecture entitled: Protection of cardiovascular organs by carvedilol. Helsinki, Finland, April 18, 1994.

Maine Society of Hospital Pharmacists. Lecture entitled: Impact of Health Care Reform on the Pharmaceutical Industry". Portland, Maine, October 27, 1994.

Pharmacology of Adrenoceptors. Satellite Symposium to the 12th International Congress of Pharmacology. Lecture entitled: "The Adrenoceptors: Historical perspectives, current status and future directions". King of Prussia, Pennsylvania, July 21, 1994.

Western Pharmacology Society Annual Meeting. Lecture entitled: α_1-Adrenoceptors: Pharmacological Subclassification and Newer Therapeutic Applications." Maui, Hawaii, January 23, 1995.

Society of Neurosciences, Lecture to the Catecholamine Club. Lecture entitled: Molecular Basis of Chirality in the Interaction of Ligands with Adrenoceptors." San Diego, California, November 15, 1995.

Invited Symposium Speaker (Continued)

Joint Meeting of the Inter-American Congress of Cardiology and the International Society for Heart Research: Symposium entitled "Cardiovascular Pharmacotherapy: From Prevention to Treatment." Lecture entitled: A Role for Antiproliferative and Antioxidative Effects of Carvedilol in Coronary Artery Disease." Santiago de Chile, December 4, 1995.

European Congress of Pharmacology. Symposium entitled: "Adrenoceptor Pharmacology". Lecture entitled "New Concepts in Adrenoceptor Pharmacology". June 16-19, 1995, Milan, Italy.

Vascular Biology Symposium, University of Vermont. Lecture entitled "The Discovery and Development of Carvedilol for Congestive Heart Failure". May 19, 1995, Burlington, Vermont.

American College of Cardiology, Carvedilol Investigators Symposium entitled "Carvedilol in Congestive Heart Failure: Results of Phase III Clinical Trials". Lecture entitled "The Pharmacology of Carvedilol: Role of Antiischemic Activity to Congestive Heart Failure". March 19, 1995, New Orleans, Louisiana.

Experimental Biology Symposium entitled: Industrial-Academic Relations". Lecture entitled "Industrial Perspectives". April 12, 1995, Atlanta, Georgia.

Symposium/Panel Discussion on Barriers to Health Care. Lecture entitled: The Role of the Pharmaceutical Industry in Health Care Reform". Albert Einstein College of Medicine, May 17, 1995, Bronx, New York.

Symposium entitled "Alpha-1 Adrenoceptor Subtype Selectivity." Lecture entitled "Current Status of α_1-Adrenoceptor Nomenclature." November 6, 1995, London, England.

Association of American Medical Colleges Symposium on "Reassessing the Biomedical Ph.D." Lecture entitled: Industrial Expectations for the New Generation of Biomedical Ph.D.s." October 8, 1995, Ft. Lauderdale, Florida.

Scientific Therapeutics Information "Consensus Conference on Carvedilol." Lecture entitled: "Overview of the Pharmacology of Carvedilol: Rationale for Use in Cardiovascular Disease." October 11, 1995, Philadelphia, Pennsylvania.

International Symposium on "New Drugs for the Treatment of Congestive Heart Failure." Lecture entitled: "The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure." October 1, 1995, Oslo, Norway.

American Heart Association Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure." November 12, 1995, Anaheim, California.

Association of Black Cardiologists Symposium. Lecture entitled: "Protection of Major Organ Systems by Carvedilol." November 11, 1995, Anaheim, California.

Invited Symposium Speaker (Continued)

Hypertension Investigators Meeting on Carvedilol. Lecture entitled: "The Use of Carvedilol in Hypertension: Prevention of Secondary Organ Damage." November 11, 1995, Anaheim, California.

Congestive Heart Failure Investigators Symposium. Lecture entitled: "Carvedilol Rationale for Use in Congestive Heart Failure." November 11, 1995, Anaheim, California.

International Symposium on "Cell Cycle Regulation". Lecture entitled: "Regulation of the Cell Cycle: Overview". November 5, 1995, King of Prussia, Pennsylvania.

EUROCONFERENCE on "Receptors in Cardiovascular Diseases as Drug Targets". Lecture entitled: "The Role of α- and β-Adrenoceptors in Cardiovascular Diseases: Recent Developments". October 3-4, 1996, Paris, France.

Winter Cardiology Meeting Symposium. Lecture entitled: "The Pharmacology of Carvedilol". February 26, 1996, Sugar Bush, Vermont.

European Society of Cardiology Symposium on "Advances in the Treatment of Congestive Heart Failure: Therapeutic Targets for the New Millennium". Lecture entitled: "The Prevention of Disease Progression: New Approaches". August 25, 1996, Birmingham, United Kingdom.

Symposium on Carvedilol in Congestive Heart Failure. Lecture entitled: Carvedilol: Basic Pharmacology and Ancillary Properties. February 23, 1996, Dallas, Texas.

Symposium on Basic Cardiovascular Research in Scottish Universities. Lecture entitled: Major Unmet Needs in Cardiovascular Diseases. September 27, 1996, Glasgow, Scotland.

American Society of Hypertension Symposium on Hypertension and the Heart: Left Ventricular Hypertrophy and Heart Failure. Lecture entitled: Recent Observations with ß-Blockade: Beneficial Effects in Hypertension and Heart Failure. May 18, 1996, New York, New York.

American College of Cardiology Symposium on Evolution of Heart Failure Management: Traditional Endpoints Versus New Outcomes. Lecture entitled: Pharmacology of ß-Blockade. March 23, 1996, Orlando, Florida.

Symposium on Carvedilol. Lecture entitled: Unique Activities of Carvedilol and their Relevance to Congestive Heart Failure. April 12, 1996, Budapest, Hungary.

2nd International Conference on Lipoprotein and Atherosclerosis: Biological and Clinical Aspects. Lecture entitled: Carvedilol, an α- and ß-Adrenoceptor Antagonist Inhibiting LDL Oxidation. September 14, 1996, Pavia, Italy.

Invited Symposium Speaker (Continued)

28th Annual Joint Symposium of the German and Austrian Societies of Intensive Care Medicine. Symposium entitled "Antioxidants and Limitation of Reperfusion Damage". Lecture entitled: "Carvedilol: A Potent Antioxidant that Limits Reperfusion Damage in the Heart." November 21-23, 1996, Vienna, Austria

Joint Meeting of the Finnish and Hungarian Cardiac Societies. Symposium on "Update in Heart Failure". Lecture entitled: "Cardiovascular Protective Properties of Carvedilol: Role of Antioxidant Activity". May 25, 1996, Stockholm, Sweden.

9th Meeting on Adrenergic Mechanisms. Lecture entitled: "Adrenoceptors." September 23, 1996, Porto, Portugal.

Scottish Biomedical Research Trust Symposium, Cardiovascular Symposium. Keynote Lecture "Major Unmet Needs in Cardiovascular Disease." September 27, 1996, Glasgow, Scotland.

Annual Meeting of the Canadian Cardiovascular Society. Symposium entitled "Evolution of Heart Failure Management: Emerging Role of Beta-Blockers." Lecture entitled: "Pharmacology of Beta-Blockade." October 29, 1996, Montreal, Canada.

American Society of Consultant Pharmacists (ASCP) Annual Meeting. Symposium entitled "Progress in Managing Congestive Heart Failure." Lecture entitled: "New Developments in Heart Failure Management: Role of β-Blockade." November 16, 1996, Nashville, Tennessee.

British Pharmacological Society Meeting. Symposium on "Genetics and the Therapy of Cardiovascular Disease." Lecture entitled: "What Does the Pharmaceutical Industry Expect from Genetic Analysis in Cardiovascular Diseases?" December 13, 1996, Brighton, United Kingdom.

Joint Meeting of the American Society of Pharmacology and Experimental Therapeutics and the British Pharmacological Society. Symposium on "G-Protein Coupled Receptors Regulation: Evolving Concepts and Clinical Implications. Lecture entitled "New Strategies in Drug Development for the Regulation of G-Protein Coupled Receptor Function." March 9, 1997, San Diego, California.

Finnish Society of Cardiology Meeting. Symposium on "The Effect of Medical Treatment on Symptoms and Prognosis of Congestive Heart Failure". Lecture entitled "New Approaches in Preventing the Progression of Heart Failure." October 10, 1996, Helsinki, Finland.

Austrian Cardiac Society Meeting. Symposium on "Congestive Heart Failure". Lecture entitled "The Role of Carvedilol in Preventing the Progression of Congestive Heart Failure." January 16, 1997, Bad Gastein, Austria.

Portuguese Congress of Cardiology Symposium on "Congestive Heart Failure". Lecture entitled "The Pharmacology of Carvedilol." April 21-23, 1997, Lisbon, Portugal.

Invited Symposium Speaker (Continued)

Keynote Speaker, 1997 Pharmacology Day, University of Toronto. Lecture entitled "The History of Adrenergic Pharmacology", May 23, 1997, Toronto, Canada.

Dutch Society of Cardiology Annual Symposium. Lecture entitled: "The Pharmacology of Carvedilol", May 31, 1997, Amsterdam, The Netherlands.

Symposium on Remodeling of Cardiovascular Organs. Lecture entitled: "Overview of Cardiac and Vascular Remodelling". February 17, 1997, King of Prussia, PA.

International Union of Pharmacology (IUPHAR) World Congress Meeting. Symposium on "Receptor Nomenclature: Principles and Applications". Lecture entitled: "The Resolution of the Problem of α-Adrenoceptor Classification". August, 1998, Munich, Germany.

United States Launch Symposium for Coreg (Carvedilol). Lecture entitled: "The Pharmacology of Coreg: Mechanisms for Inhibition of Progression of Congestive Heart Failure". April 28, 1997, Atlanta, GA.

Satellite Symposium to the 45th Annual Meeting of the Japanese College of Cardiology. Symposium entitled: "Cardiovascular Disease and β-Blockers". Lecture entitled: "Multiple Actions of β-Blockers for Cardiovascular Disease". September 25, 1997, Sapporo, Japan.

International Society for Heart Research, World Congress of Cardiology. Symposium entitled: "Adrenergic Receptor Modulation: A Molecular and Pharmacological Adventure in Heart Failure Territory". Lecture entitled: "Adrenergic Receptor Pharmacology". May 27-31, 1998, Rhodes, Greece.

Western Pharmacology Society Annual Meeting. Symposium entitled: "Alpha-1-Adrenoceptors". Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Agonists with α-Adrenoceptors". January 25-30, 1998, Mazatlan, Mexico.

Satellite Symposium to the 5th International Congress on Endothelin. Symposium entitled: "Endothelin in Disease". Lecture entitled: "The Effects of Carvedilol on Endothelin: Inhibition of Endothelin Synthesis". September 12, 1997, Kyoto, Japan.

American Heart Association Annual Symposium. Satellite Symposium on Teveten. Lecture entitled: "The Pharmacology of Teveten". November 8, 1997, Orlando, Florida.

9th International Symposium on Vascular Neuroeffector Mechanisms. Satellite Symposium to the IUPHAR Congress. Symposium entitled "Adrenoceptors". Lecture entitled: "New Perspective in the use of β-blockers in the treatment of congestive heart failure". August 2-5, 1998, Porto, Portugal.

Invited Symposium Speaker (Continued)

International Society of Hypertension. Symposium on "The Importance of Systolic Control: A New Paradigm for Effective Bleed Pressure Management". Lecture entitled: "Pharmacological Mechanisms of Angiotensin II Receptor Antagonists: Implication for the Treatment of Elevated Systolic Blood Pressure". June 7, 1998, Amsterdam, The Netherlands.

XIIIth World Congress of Pharmacology, Satellite Symposium on "α_1-Adrenoceptors as Targets for Therapeutic Agents in Urology". Lecture entitled "Adrenoceptor Pharmacology". July 23, 1998, Paris, France.

World Congress of Neurosciences. Lecture entitled: "Adrenoceptors". July 11-16, 1999, Jerusalem, Israel.

International Symposium on Medicinal Chemistry, European Federation of Medicinal Chemistry. Plenary Lecture "Pharmacotherapy of the Major Cardiovascular Diseases". Bologna, Italy, September 19-23, 2000.

XIV Lorenzini Annual Lecture. 5th International Symposium Multiple Risk Factors in Cardiovascular Disease: Global Assessment and Intervention". Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". Venice, Italy, October 28-31, 1999.

British Pharmacology Society Annual Meeting. Symposium on "Pharmacology for the New Millennium-Directions". Lecture entitled: "Orphan Receptors and the Discovery of Orexins". Cambridge, England, January 5-7, 2000.

Experimental Biology Meeting. ASPET Colloquium on "Functional Genomics and Proteonomics". Lecture entitled: "Functional Genomics: Overview". Boston, Massachusetts, June 4, 2000.

Annual Meeting of the Regional Medical Associates for SmithKline Beecham. Symposium on Coreg. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". Atlanta, GA, November 3, 1999.

10th Meeting on Adrenergic Mechanisms. Session on Adrenoceptors I. Lecture entitled: "Current Status of Adrenoceptors: Concluding Remarks". Porto, Portugal, September 25, 2000.

American Diabetes Association Annual Meeting, Symposium on the Use of Carvedilol (Coreg) in Heart Failure Patients with Diabetes. Lecture entitled: "Carvedilol: Pharmacologic Profile". San Antonio, Texas, June 10, 2000.

Mid-Atlantic Pharmacology Society Annual Meeting. Plenary Lecture entitled: "The Role of β-Blockers in Congestive Heart Failure: Challenging Dogma". Collegeville, Pennsylvania, September 15, 2000.

Invited Symposium Speaker (Continued)

Rapamune Internal Launch Meeting. Session on Shaping the Art of Immunosuppression. Lecture entitled: "Wyeth - A Commitment to R&D". Marbella, Spain, March 4, 2001.

Joint Meeting of the Australasian, British Canadian and the Western Pharmacology Societies. Plenary lecture entitled: "Drug Discovery in the New Millennium". Vancouver, Canada, March 25, 2001.

Heart and Kidney Institute, University of Houston. Lecture entitled: "The Role of β-Blockers in the Management of Congestive Heart Failure ". Houston, Texas, June 14, 2002.

International Congress of Pharmacology. Lecture entitled: "Receptor Classification in the Post Genomic World ". San Francisco, California, July 9, 2002.

Drug Discovery Technology 2003 Symposium. Keynote Lecture entitled: "R&D Productivity in the Pharmaceutical Industry: Issues and Challenges". Boston, Massachusetts, August 12, 2003.

Biopharmaceutical Conference in Europe. Lecture entitled: "Pharmaceutical R&D: Former and Future Perspectives". Monaco, June 20, 2003.

R&D Leader's Forum. Lecture entitled: "R&D Productivity: Can it be Increased?". Coral Gables, Florida, March 1-3, 2004.

Annual Meeting of the Association of American Medical Colleges. Lecture entitled: "Measuring Productivity: Industry Models". Philadelphia, Pennsylvania, October 11, 2002.

Symposium of New Molecules in Cancer Therapeutics. Lecture entitled: "Overview of the Wyeth Oncology Pipeline". Athens, Greece, October 27, 2002.

Symposium on Futures in Biomedical Research: A Look Ahead VII. Lecture entitled: "Building Success in Tomorrow's Pharmaceutical Industry". University of Maryland Baltimore Campus, Baltimore, Maryland, November 12, 2003.

2003 Retail Advisory Board Meeting. Lecture entitled: "R&D Productivity in the Pharmaceutical Industry". Philadelphia, Pennsylvania, August 23, 2003

Research & Development Leaders Forum. Lecture entitled: "How can the Pharmaceutical Industry Counter the Growing Costs of Drug Development Amid Increasingly Stringent Regulatory Requirements?" Coral Gables, Florida, March 1-3, 2004.

Drug Discovery Technology World Congress Summit. Lecture entitled: "Is R&D Productivity Really Falling? Or is this Oft-Quoted "Fact" Based on Outdated Parameters?" Boston, Massachusetts, August 8, 2004.

The World Pharmaceutical Congress. Lecture entitled: "Optimizing R&D Productivity". Philadelphia, Pennsylvania, May 18, 2004.

Invited Symposium Speaker (Continued)

2004 BIO Annual Meeting. Lecture entitled: "Productivity of Drug Discovery". San Francisco, California, June 8, 2004.

Annual Congress of the Florida American College of Obstetrics and Gynecology. Lecture entitled: "The Future of Women's Health". Naples, Florida, July 24, 2004.

11th Annual Human Genome Discovery Symposium. Lecture entitled: "Redefining the Pre-Competitive Opportunity Within the Pharmaceutical Industry". San Francisco, California, March 26, 2004.

Experimental Biology 2004 Annual Meeting. Lecture entitled: "What the Pharmaceutical Industry of the 21st Century is Looking for in a Pharmacologist". Washington, D.C., April 18, 2004.

2004 Retail Advisory Board Meeting. Lecture entitled: "R&D Productivity in the Pharmaceutical Industry: Issues and Challenges". San Diego, California, August 28, 2004.

2004 Windhover AudioConference. Lecture entitled: "Future Direction of R&D at Wyeth". Philadelphia, Pennsylvania, September 17, 2004.

All Florida Obstetrics and Gynecology Symposium. Lecture entitled: "The Future of Women's Health: New Therapies in Development". Orlando, FL, November 20, 2004.

Drug Discovery Technology Europe 2005. Lecture entitled: "Re-Engineering Discovery and Development: Impact on the Pharmaceutical Industry of Tomorrow". London, England, March 14-17, 2005.

Mid-Winter Symposium of the University of South Florida. Lecture entitled: "The Future of Women's Health: New Therapies in Development". February 19, 2005.

Drug Development Summit. Lecture entitled: "Wyeth: The R&D Pipeline to Watch". Phoenix, Arizona, February 7, 2005.

Pharmaceutical Strategy Series: Executive Leadership Summit. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Orlando, Florida, February 10, 2005.

Sino-American Pharmaceutical Professionals Association-Greater Philadelphia Chapter (SAPA-GP). Annual Symposium on Globalization of Pharmaceutical/Biotech R&D. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Blue Bell, Pennsylvania, June 18, 2005.

PhRMA Science and Regulatory Annual Meeting. Town Hall Session. Lecture entitled: "From Basic Research to Marketed Products: What Value Does Each Stake Holder Add". Washington, DC, May 2, 2005.

Invited Symposium Speaker (Continued)

Drug Discovery Summit Genius Symposium. Lecture entitled: "What Role Does Genius Play in Drug Discovery and Development". Phoenix, Arizona, February 8, 2005.

Pennsylvania Biotech Annual Dinner. Lecture entitled: "The Challenges of Drug Discovery and Development". Philadelphia, Pennsylvania, January 26, 2005.

CMR International Workshop on a New Paradigm for Clinical Research. Lecture entitled: "A New Paradigm for Clinical Research – A Path to Improve Drug Development at Wyeth". Washington, D.C., October 4, 2005.

Economist Conference: The 12th Annual Pharmaceuticals Conference. Lecture entitled: "Collaborating for R&D Success". London, England, February 21, 2006.

Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Lecture entitled: "Strategies to Integrate Discovery, Development and Global Marketing". Philadelphia, Pennsylvania, June 18, 2005.

Drug Development Summit. Lecture entitled: "Wyeth Pharmaceutical's Approach to Redesigning R&D to Increase Innovation, Output and to Expedite Discoveries into Therapeutic Products". Phoenix, Arizona, February 14, 2006

Scrip R&D Productivity Summit. Lecture entitled: "If You Can't Measure It, You Can't Manage It; Accurately Measuring and Comparing Productivity Improvements Across Multiple Deliverables". London, England, April 26, 2006.

Drug Discovery Technology and Development World Congress. Lecture entitled: "Growing Costs of Healthcare: Impact on Government, Regulatory Agencies, Industry and Patients". Boston, Massachusetts, August 8, 2005.

R&D Executive Leadership Summit. Lecture entitled: "R&D Productivity Improvements at Wyeth". Boston, Massachusetts, August 9, 2005.

IBC Conference on R&D Productivity. Lecture entitled: "Rethinking the Productivity Challenge". New York, New York, September 27, 2005.

PharmaDiscovery Conference. Lecture entitled: "Maximizing the Tools of Drug Discovery and Development to Improve R&D Productivity". Washington, DC, May 11, 2005.

Drug Discovery & Technology Leaders Summit. Lecture entitled: "Re-Engineering the Drug Discovery and Development Process and Overcoming Technological Challenges in the Drug Discovery Process". Orlando, Florida, February 24, 2005.

Mid-Winter Florida Obstetrics & Gynecology Conference. Lecture entitled: "Developing and Delivering Health Care: The Pharmaceutical Industry Perspective". St. Pete Beach, Florida, February 20, 2005.

2005 BIO Symposium on Intellectual Property. Lecture entitled: "Capturing the Value in Non-Progressed Assets". Philadelphia, Pennsylvania, June 22, 2005.

Invited Symposium Speaker (Continued)

Drug Discovery Technology Europe 2005. Lecture entitled: "Strategies for Re-Engineering Discovery and Development to Address R&D Productivity". London, England, March 14, 2005.

2005 BIO Annual Conference. Lecture entitled: "Maximizing the Tools of Drug Discovery and Development to Improve R&D Productivity". Philadelphia, Pennsylvania, June 21, 2005.

Cambridge Health Institute (CHI) Pharmaceutical Leadership Summit. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Lake Buena Vista, Florida, February 10, 2005.

12th Annual Pharmaceuticals Conference: Building a Strategy for Pharma's New Role. Lecture entitled: "Collaborating for R&D Success". London, England, February 21, 2006.

Annual Meeting of the Directors of Graduate Studies in Pharmacology. Lecture entitled: "Training Needs for the Pharmaceutical Industry". Salt Lake City, Utah, July 25-28, 2007.

2006 Adaptive Designs: Opportunities, Challenges and Scope in Drug Development. Keynote address entitled: "Improving the Efficiency of Drug Development". Bethesda, Maryland, November 13-14, 2006.

Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Keynote lecture entitled: "21st Century Innovation and Globalization of Drug Development: Wyeth's New Clinical Development Paradigm". Philadelphia, Pennsylvania, June 17, 2006.

5th Annual Evolution Summit. Keynote lecture entitled: "21st Century Innovation in the Discovery and Development of New Medicines – Changing the Clinical Development Paradigm". Monte Carlo, Monaco, October 22, 2006.

Ohio State University College of Pharmacy Annual Symposium. Keynote lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Columbus, Ohio, May 16, 2006.

Drug Development Summit. Keynote lecture entitled: "Development of the Wyeth Neurosciences Pipeline: Transformation in Discovery and Clinical Development". Phoenix, Arizona, January 22, 2006.

Enbrel Summit: Progress and Promise: A Decade of Scientific Innovation. Lecture entitled: "Inflammation Research at Wyeth: Bringing Novel Therapies to the Clinic". Munich, Germany, March 15, 2007.

Second Trial Design Innovation Conference. Keynote lecture entitled: "Bringing Statistical Methodology to the Board Room: How Adaptive Designs Influence Portfolio Management Decisions". Washington, D.C., July 16, 2007.

Invited Symposium Speaker (Continued)

40th Mid-Atlantic Graduate Student Symposium. Keynote lecture entitled: "New Drug Development: The Saga of Carvedilol". Morgantown, West Virginia, June 10, 2007.

Annual Meeting of the National Directors of Graduate Studies in Pharmacology. Keynote lecture entitled: "Future Training Needs in Pharmacology". Salt Lake City, Utah, July 25, 2007.

ASPET Centennial Meeting. Symposium entitled "Drug Discovery Paradigms: Past, Present, Future". Lecture entitled: "Drug Discovery of the Future". San Diego, California, April 5-9, 2008.

Asia-Pacific Enbrel Summit: Keynote lecture entitled: "Inflammation Research at Wyeth: Bringing Novel Therapies to the Clinic". Seoul, Korea, July 3, 2007.

John S. O'Brien Memorial Lectureship at the Annual University of Pennsylvania Graduate Students in Pharmacology Symposium. Lecture entitled: "New Drug Development: The Saga of Carvedilol". Philadelphia, Pennsylvania, November 1, 2007.

David Perlman Memorial Lectureship at the American Chemical Society Annual Meeting. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: A Paradigm Shift". Philadelphia, Pennsylvania, August 17, 2008.

WV-INBRE Summer Research Symposium. Keynote lecture entitled: "The Discovery and Development of Carvedilol (Coreg) for the Treatment of Congestive Heart Failure". Morgantown, West Virginia, July 31, 2008.

Induction Ceremony for the Rho Chi Society. Keynote lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: A Paradigm Change". Boston, Massachusetts, April 17, 2008.

Drug Development Summit. Keynote lecture entitled: "Integrated Small Molecule, Biotechnology and Vaccine Technologies in Pharmaceutical Research & Development". Amelia Island, Florida, February 12-15, 2008.

Drug Development Summit. Panel Discussion: "Critical Issues for R&D in 2008". Amelia Island, Florida, February 12-15, 2008.

16th Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Keynote lecture entitled: "Future Trends for Integrated Drug Discovery and Development". Princeton, New Jersey, June 15, 2008.

Institute for Regulatory Science Symposium. Keynote Lecture entitled: "Predictable Outcomes: Why Do Potential Winners Fail". Washington, D.C., September 30-October 1, 2008.

Invited Symposium Speaker (Continued)

University of Florida, College of Pharmacy Annual Research Showcase. Keynote Lecture entitled: "The Trials and Tribulations of a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Gainesville, Florida, February 18, 2009.

University of Florida, College of Pharmacy. Target Leadership Speaker. Lecture entitled: "Leadership Lessons from the School of Life". Gainesville, Florida, February 18, 2009.

FDA/CMS Summit. Plenary Lecture entitled: "Interactions Between the Pharmaceutical Industry and Global Regulatory Agencies". Washington, D.C., December 3, 2009.

West Virginia University, College of Pharmacy, White Coat Induction Ceremony. Lecture Entitled: "The Role the Research Scholarships Played in My Career", Morgantown, West Virginia, December 14, 2009.

Great Oxford Debate, Oxford University. Lecture entitled: "There Exists No Conflict of Interest in the Pharmaceutical Industry Conducting Its Own Clinical Trials". Oxford, England, September 23, 2009.

50th Year Anniversary of the PRAT Program at the National Institute of General Medical Sciences, Lecture entitled: "The Discovery and Development of Carvedilol for the Treatment of Congestive Heart Failure: From the Laboratory Bench to the Patient", Bethesda, MD, November 6, 2015.

Chairman or Organizer of the Following Symposia

American Society for Pharmacology and Experimental Therapeutics, Symposium of Peripheral Alpha-Adrenergic Receptors, Philadelphia, Pennsylvania, August 11, 1983 (Chairman).

Satellite Symposium of the 9th International Congress of Pharmacology entitled Pharmacology of Adrenoceptors, Manchester, England, August 6, 1984 (Organizing Committee).

30th International Congress of Physiology, Satellite Symposium on Smooth Muscle Function. Ligand Binding and Approaches to Receptor Characterization, Banff, Alberta, Canada, July 2, 1986 (Chairman).

Federation of American Societies for Experimental Biology (FASEB), Symposium entitled: The Role of Peripheral Dopamine Receptors in Cardiovascular Function, Anaheim, California, April, 1985 (Organizer).

American Society for Pharmacology and Experimental Therapeutics, Symposium on the Pharmacology of the Prostate, Boston, Massachusetts, August, 1985 (Organizer).

Satellite Symposium to the 11th Scientific Meeting of the International Society of Hypertension. Adrenergic Receptor Function and Cardiovascular Reactivity in Human Hypertension. Session on Postsynaptic α_1- and α_2-Adrenoceptors in Hypertension, Essen, Federal Republic of Germany, September 7-8, 1986 (Chairman).

Symposium Entitled "Cardiac and Renal Failure", Phoenix, Arizona, March 13-15, 1987 (organizer).

Federation of the American Societies for Experimental Biology, Symposium entitled "Vasomotor Regulatory Mechanisms: Central and Peripheral Aspects", Washington, DC, April, 1987 (Chairman).

Vascular Neuroeffector Mechanisms, 6th International Symposium. Symposium entitled "α-Adrenoceptor II", Melbourne, Australia, August 30-September 2, 1987 (Chairman).

Smith Kline & French, Symposium entitled " Future Therapeutic Approaches to Ischemic Heart Disease", San Juan, Puerto Rico, February 12-14, 1988 (Organizer).

UCLA Symposia on Molecular and Cellular Biology, Symposium entitled "Molecular Biology of the Cardiovascular System", Keystone, Colorado, April, 1988 (Organizing Committee).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Renal α_2-Adrenoceptors", Las Vegas, Nevada, May 3, 1988 (Co-Chairman).

Chairman or Organizer of the Following Symposia (Continued)

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Tyrosine Kinase", New Orleans, Louisiana, March 20 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Regulation of Adenylyl Cyclase", New Orleans, Louisiana, March 20 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Calcium, Phosphoinositides, and C Kinase", New Orleans, Louisiana, March 20, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Ion Pumps and Channels", New Orleans, Louisiana, March 22,1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: The Steroid Supra-Family of Receptors", New Orleans, Louisiana, March 22, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Sensory Signal Transduction", New Orleans, Louisiana, March 22, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Guanylate Cyclase", New Orleans, Louisiana, March 23, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled: "Endothelin and Endothelial-Derived Contractile Substance", New Orleans, Louisiana, March, 1989 (Chairman).

Smith Kline & French, Symposium entitled "Future Therapeutic Approaches to Cerebrovascular Disease", Orlando, Florida, March 10-12, 1989 (Organizer).

Satellite Symposium of the 11th International Congress of Pharmacology entitled "Pharmacology of Adrenoceptors". Manchester, England, June 27-29, 1990 (Organizing Committee).

Satellite Symposium of the 11th International Congress of Pharmacology entitled "Presynaptic Receptors and Neuronal Transporters". Rouen, France, June 26-29, 1990 (Organizing Committee).

Chairman or Organizer of the Following Symposia (Continued)

American Society for Pharmacology and Experimental Therapeutics (ASPET).Short Course: Endothelial Biology. Milwaukee, Wisconsin, August, 1990 (Chairman and Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Endothelium-Derived Relaxing Factors", Milwaukee, Wisconsin, August, 1990 (Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Endothelium-Derived Contracting Factors", Milwaukee, Wisconsin, August, 1990 (Chairman and Organizer).

Cardio/Renal Conference entitled "Cell and Molecular Biology of Atherosclerosis", Phoenix, Arizona, February 23-25, 1990 (Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Cellular Interactions of Endothelium", Milwaukee, Wisconsin, August, 1990 (Organizer).

Satellite Symposium of the XIth International Congress of Pharmacology, Session entitled "Molecular Structure and Genetics of Adrenoceptors. Manchester, England, June 27, 1990 (Chairman).

Symposium Entitled" Molecular and Cellular Biology of Atherogenesis: Alteration in Progression/Regression". Phoenix, Arizona, April 5-7, 1991 (Chairman).

International Symposium Entitled "From $\alpha 2$-Adrenoceptors to the Imidazoline-Preferring Receptors". Satellite symposium of the 7th International Catecholamine Symposium. Paris, France, June 29-30, 1992 (Scientific Committee).

Symposium Entitled "Vascular Remodeling: Thrombosis and Smooth Muscle Function". West Palm Beach, Florida, June 26-28, 1992 (Organizer).

International Symposium on Endothelin, Houston, TX, 1993 (Scientific Advisory Board).

XIIIth International Symposium on Medicinal Chemistry, organized by Societe de Chimie Therapeutique on behalf of the European Federation for Medicinal Chemistry. Paris, France, September 19-23, 1994 (International Advisory Board and Chairman).

International Symposium Entitled "Angiotensin II Receptor Antagonists". Organized by the International Academy of Biomedical and Drug Research, Monte Carlo, Monaco, March 20-22, 1993 (Organizing Committee).

8th Meeting on Adrenergic Mechanisms, Symposium on "Adrenoceptors and Second Messengers". Porto, Portugal, September 19-22, 1993 (Chairman).

Chairman or Organizer of the Following Symposia (Continued)

International Symposium entitled "The Pharmacology of Adrenoceptors". Satellite symposium to the 12th International Pharmacology (IUPHAR) Congress. King of Prussia, Pennsylvania, July 21-23, 1994 (Organizer).

Vascular α-Adrenoceptors: From the Gene to the Human. 8th International Symposium on Vascular Neuroeffector Mechanisms, Satellite Symposium to the 12th International Pharmacology Congress, Kananaskis, Alberta, Canada, August 1, 1994 (Chairman).

Fourth International Conference on Endothelin, London, England, April 23-26, 1995 (Scientific Advisory Board).

2nd International Meeting on Imidazoline Receptors. Satellite symposium to the 12th International Pharmacology (IUPHAR) Congress. New York, New York, July 19-20, 1994 (Scientific Advisory Board).

8th International Symposium on Vascular Neuroeffector Mechanisms. Satellite Symposium to the 12th International Pharmacology (IUPHAR) Congress. Kananaskis, Alberta, Canada. August 1-5, 1994 (Co-Organizer).

International Symposium on Cell Cycle Regulation, King of Prussia, Pennsylvania, November 5-7, 1995 (Co-Organizer).

9th International Symposium on Adrenergic Mechanisms, Porto, Portugal, September 23-25, 1996 (Scientific Advisory Board).

6th World Congress of the World Federation of Societies of Biological Psychiatry. Symposium entitled: The Genomic Alliance and Innovative Drug Discovery in the Neurosciences. Nice, France, June 23, 1997 (Co-Chairman).

Fifth International Conference on Endothelin. Kyoto, Japan, September 12-15, 1997 (Scientific Advisory Board and Co-Chairman).

International Society for Heart Research, World Congress of Cardiology Symposium on "Adrenergic Receptor Modulation: A Molecular and Pharmacological Adventure in Heart Failure Territory". Rhodes, Greece, May 27-31, 1998 (Organizer and Chairman).

6th World Congress of Biological Psychiatry. Symposium on "The Genomic Alliance and Innovative Drug Discovery in the Neurosciences". Nice, France, June 23, 1997 (Chairman).

9th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Porto, Portugal, August 2-5, 1998 (Scientific Advisory Committee).

10th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Porto, Portugal, September 24-27, 2000 (Scientific Advisory Committee).

Chairman or Organizer of the Following Symposia (Continued)

Fifth International Conference on Endothelin; Session entitled "Endothelin Receptors and Endothelin Receptor Antagonists". Kyoto, Japan, September 13, 1997 (Chairman).

9th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Session entitled "Adrenoceptors". Porto, Portugal, August 2-5, 1998 (Chairman).

XIIIth World Congress of Pharmacology, Satellite Symposium on "α_1-Adrenoceptors as Targets for Therapeutic Agents in Urology". Paris, France, July 23-24, 1998 (Chairman).

XVI International Symposium on Medicinal Chemistry. Bologna, Italy, September 18-23, 2000 (Scientific Advisory Board).

ET-6 International Symposium, Montreal, Canada, October 10-14, 1999 (Scientific Advisory Board).

Experimental Biology Meeting, ASPET Colloquium on Functional Genomics, Symposium on "Functional Genomics and Proteonomics". Boston, Massachusetts, June 4, 2000 (Chairman).

First International Symposium on PPARs: From Basic Science to Clinical Applications. Florence, Italy, April 4-7, 2001 (International Advisory Board).

11th Meeting on Adrenergic Mechanisms. Porto, Portugal, September 25-27, 2003 (Scientific Advisor).

2nd International Symposium on PPARs: From Basic Science to Clinical Applications. Florence, Italy, March 19-22, 2003 (International Advisory Board).

International Congress of Pharmacology. Symposium on “Receptor Classification in the Post Genomic World”. San Francisco, California, July 9, 2002 (Organizer).

Sixth IUPHAR Satellite Symposium on Adrenoceptors. Rohnert Park, California, July 12-14, 2002 (Organizer).

Second International Symposium on “PPARs: From Basic Science to Clinical Applications”. Florence, Italy, March 19-22, 2003 (International Advisory Board).

World Drug Discovery Congress 2004. Copenhagen, Denmark, January 19-21, 2004 (Scientific Advisory Committee).

rEVOLUTION 2004 Summit for Chief Scientific Officers. Greensboro, Georgia, May 5-7, 2004 (Steering Committee)

Research & Development Leaders Forum. Coral Gables, Florida, May 1-3, 2004 (Scientific Advisory Committee).

Chairman or Organizer of the Following Symposia (Continued)

rEVOLUTION 2005 Summit for Chief Scientific Officers, September 29-30, 2005 (Steering Committee)

4th World Drug Discovery and Development Summit, Copenhagen, Denmark, January 25-26, 2005 (Advisory Board).

IBC Drug Discovery and Technology Conference. R&D Executive Summit. Boston, Massachusetts, August 9, 2005 (Chairman).

PhRMA Science and Regulatory Annual Meeting. Town Hall Session entitled "From Basic Research to Marketed Products". Washington, D.C., May 2, 2005 (Chairman).

Drug Development Summit 2007, Phoenix, Arizona, January 21-24, 2007 (Advisory Board).

ASPET Centennial Meeting. Symposium entitled: "Drug Discovery Paradigms: Past, Present and Future". San Diego, California, April 7, 2008 (Chairman).

Expert Opinion-Evolution Summit 2008. Monte Carlo, Monaco, October 22-24, 2008 (Scientific Advisory Board)..

7th Annual R&D Leaders Forum. San Diego, California, October 28-30, 2008 (Scientific Advisory Board).

rEvolution Summit 2009. Miami, Florida, March 25-27, 2009 (Scientific Advisory Board).

Publications

Books Edited

1. The Alpha-1 Adrenergic Receptors, edited by R.R. Ruffolo, Jr., Humana Press, Clifton, New Jersey, 1987 (543 pages).

2. Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, 1991 (240 pages).

3. Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, edited by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, 1991 (226 pages).

4. Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspective, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1994 (170 pages).

5. Angiotensin II Receptors, Volume 2: Medicinal Chemistry, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1994 (225 pages).

6. Endothelin Receptors: From the Gene to the Human, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1995 (285 pages).

7. Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, London, 1995 (287 pages).

8. G-Protein Coupled Transmembrane Signaling Mechanisms, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, 1995 (204 pages).

9. Inflammation: Mediators and Pathways, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, 1995 (206 pages).

10. Pharmacology of Adrenoceptors, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, London, 1995 (279 pages).

11. Cell Cycle Regulation, edited by R.R. Ruffolo, Jr., B. Metcalf and G. Poste. Harwood Academic Publishers, London, 1997 (174 pages).

12. Carvedilol: A Multiple Action Neurohormonal Antagonists, edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in preparation.

13. Inflammatory Cells and Mediators in CNS Diseases. Edited by R.R. Ruffolo, Jr., G. Feuerstein, J. Hunter, G. Poste and B. Metcalf. Harwood Academic Publishers, London, 1998 (518 pages).

14. The IUPHAR Compendium of Receptor Characterization and Classification. Edited by T. Godfraind, P.M. Vanhoutte, R.R. Ruffolo, Jr. and P. Humphrey. Published by IUPHAR Media, Burlington Press, Cambridge, 1998 (267 pages).

Books Edited (continued)

15. The Alpha-1 Adrenergic Receptors. Edited by J.P. Hieble, A. Leonardi and R.R. Ruffolo, Jr. Humana Press, Totowa, New Jersey, in preparation.

16. Apoptosis in Health and Disease. Edited by R.R. Ruffolo, Jr. and F. Walsh, Harwood Academic Publishers, London, 2000 (249 pages).

17. IUPHAR Compendium of Receptor Characterization and Classification, Volume II. Published by IUPHAR Media, Burlington Press, Cambridge, 398 pages, 2000.

Theses

The Mechanism of Action of Sympathomimetic Amines. Submitted in partial fulfillment for the degree, Bachelor of Science in Pharmacy with Distinction. The Ohio State University, Columbus, Ohio, 1973.

Biochemical and Pharmacological Characterization of the Alpha-Adrenoreceptor. Submitted in partial fulfillment for the degree, Doctor of Philosophy in Pharmacology. The Ohio State University, Columbus, Ohio, 1976.

Publications (Full Papers)

1. Krell, R. D., Ruffolo, R. R., Jr. and Patil, P. N.: Steric aspects of adrenergic drugs. XXI. Drug-induced release of (-)- and (+)- ^{14}C-norepinephrine from the isolated superfused rat vas deferens. Naunyn-Schmiedeberg's Arch. Pharmacol. 274: 394-403, 1972.

2. Witiak, D. T., Sinha, B. K., Ruffolo, R. R., Jr. and Patil, P. N.: cis- and trans-2-Mercaptocyclo-butylamines, their benzylmercapto analogs, and aminomethyl homologs. Influence on bradykinin-induced contraction of the guinea pig ileum. J. Med. Chem. 16: 232-235, 1973.

3. Ruffolo, R. R., Jr. and Patil, P. N.: Catecholamine content of pigmented and nonpigmented tissues of the rabbit. Eur. J. Pharmacol. 25: 255-258, 1974.

4. Bingham, W. G., Ruffolo, R. R., Jr. and Friedman, S. J.: Catecholamine levels in the injured spinal cord of monkeys. J. Neurosurg. 42: 174-178, 1975.

5. Bingham, W. G., Ruffolo, R. R., Jr., Goodman, J. H., Knofel, J. and Friedman, S. J.: Norepinephrine and dopamine levels in normal dog and monkey spinal cord. Life Sci. 16: 1521-1526, 1975.

6. Ruffolo, R. R., Jr., Miller, D. D. and Patil, P. N.: Biochemical correlates for the pharmacological effects of L(+)-isomers and beta-desoxy-sympathomimetic amines. Biochem. Pharmacol. 25: 399-404, 1976.

7. Ruffolo, R. R., Jr., McCreery, R. L. and Patil, P. N.: A kinetic analysis of a catechol-specific binding site in the microsomal fraction from the rabbit aorta. Eur. J. Pharmacol. 38: 221-232, 1976.

8. Ruffolo, R. R., Jr., Fowble, J. W., Miller, D. D. and Patil, P. N.: Binding of ^{3}H-dihydroazapetine to alpha-adrenoreceptor-related proteins from rat vas deferens. Proc. Natl. Acad. Sci. U.S.A. 73: 2730-2734, 1976.

9. Miller, D. D., Hsu, F. L., Ruffolo, R. R., Jr. and Patil, P. N.: Stereochemical studies of adrenergic drugs. Optically active derivatives of imidazolines. J. Med. Chem. 19:1382-1384, 1976.

10. Ruffolo, R. R., Jr., Fowble, J. W., Miller, D. D. and Patil, P. N.: Kinetics of accumulation, efflux and the pharmacological effects of tritiated dihydroazapetine on the rabbit aorta. J. Pharmacol. Exp. Ther. 202:278-286, 1977.

11. Ruffolo, R. R., Jr., Turowski, B. S. and Patil, P. N.: Lack of cross-desensitization between structurally dissimilar alpha-adrenoceptor agonists. J. Pharm. Pharmacol. 29: 378-380, 1977.

12. Ruffolo, R. R., Jr. and Patil, P. N.: Kinetics of blockade of different receptors by chlorpromazine in rabbit stomach strips. Eur. J. Pharmacol. 48: 151-157, 1978.

Publications (Continued)

13. Ruffolo, R. R., Jr., Turowski, B. S. and Patil, P. N.: Further biochemical characterization of ^{3}H-dihydroazapetine binding to alpha-adrenoreceptor-related proteins from the rat vas deferens. J. Pharm. Pharmacol. 30: 498-502, 1978.

14. Ruffolo, R. R., Jr., Eisenbarth, G. S., Thompson, J. M. and Nirenberg, M.: Synapse turnover: A mechanism for acquiring synaptic specificity. Proc. Natl. Acad. Sci. U.S.A. 75: 2281-2285, 1978.

15. Eisenbarth, G. S., Ruffolo, R. R., Walsh, F. W., and Nirenberg, M: Lactose sensitive lectin of chick retina and spinal cord. Biochem. Biophys. Res. Commun. 83: 1246-1252, 1978.

16. Ruffolo, R. R., Jr., Miller, D. D. and Patil, P. N.: Some thoughts on the chemical and pharmacological aspects of adrenoreceptors. In: Recent Advances in the Pharmacology of Adrenoceptors. ed. by E. Szabadi, C. M. Bradshaw and P. Bevan, pp. 45-50, Elsevier/North-Holland Biomedical Press, 1978.

17. Ruffolo, R. R., Jr. and Patil, P. N.: Kinetics of alpha-adrenoreceptor blockade by phentolamine in the normal and denervated rabbit aorta and rat vas deferens. Blood Vessels, 16: 135-143, 1979.

18. Ruffolo, R. R., Jr., Rosing, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. I. Affinity and efficacy for alpha-adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. 209: 429-436, 1979.

19. Ruffolo, R. R., Jr., Dillard, R. D., Rosing, E. L. and Waddell, J.E.: Receptor interactions of imidazolines. II. Affinities and efficacies of hydroxy-substituted tolazoline derivatives in rat aorta. J. Pharmacol. Exp. Ther. 211: 74-79, 1979.

20. Ruffolo, R. R., Jr., Dillard, R. D., Waddell, J. E. and Yaden, E. L.: Receptor interactions of imidazolines. III. Structure-activity relationships governing alpha-adrenergic receptor occupation and receptor activation for mono- and dimethoxy-substituted tolazoline derivatives in rat aorta. J. Pharmacol. Exp. Ther. 211: 733-738, 1979.

21. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. IV. Structural requirements for alpha-adrenergic receptor occupation and receptor activation by clonidine and a series of structural analogs in rat aorta. J. Pharmacol. Exp. Ther. 213: 267-272, 1980.

22. Patil, P. N. and Ruffolo, R. R., Jr.: Evaluation of adrenergic alpha- and beta-receptor activators and adrenergic alpha- and beta-receptor blocking agents. In: The Handbook of Experimental Pharmacology. Vol. 54/1, ed. by L. Szekeres, Springer-Verlag (Berlin), pp. 89-134, 1980.

Publications (Continued)

23. Ruffolo, R. R., Jr., Yaden, E. L., Waddell, J. E. and Dillard, R. D.: Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha-adrenergic receptor subtypes in tissues from the rat. J. Pharmacol. Exp. Ther. 213: 557-561, 1980.

24. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. VI. Significance of carbon bridge separating phenyl and imidazoline rings of tolazoline-like alpha-adrenergic imidazolines. J. Pharmacol. Exp. Ther. 214: 535-540, 1980.

25. Zaborowsky, B. R., McMahan, W. C., Griffin, W. A., Norris, F. H. and Ruffolo, R. R., Jr.: Computerized graphic methods for determining dissociation constants of agonists, partial agonists and competitive antagonists in isolated smooth muscle preparations. J. Pharmacol. Methods 4: 165-178, 1980.

26. Cohen, M. L., Ruffolo, R. R., Jr. and Wiley, K. S.: Antagonist dissociation constants and relative agonist efficacies for compounds interacting with $beta_1$ and $beta_2$ adrenergic receptors in the rat jugular vein. J. Pharmacol. Exp. Ther. 215: 325-331, 1980.

27. Fuller, R. W., Hemrick-Luecke, S., Toomey, R. E., Horng, J.-S., Ruffolo, R. R., Jr. and Molloy, B. B.: Properties of 8,9-dichloro-2,3,4,5-tetrahydro-1H-benzazepine, an inhibitor of norepinephrine N-methyltransferase. Biochem. Pharmacol. 30: 1345-1352, 1981.

28. Ruffolo, R. R., Jr., Waddell, J. E. and Yaden, E. L.: Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of $alpha_2$-adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. 217: 235-240, 1981.

29. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. VII. Peripherally-mediated pressor and centrally-mediated depressor effects of clonidine and a series of mono- and dimethoxy-substituted tolazoline derivatives. J. Pharmacol. Exp. Ther. 218: 154-160, 1981.

30. Ruffolo, R. R., Jr., Spradlin, T. A., Pollock, G. D., Waddell, J. E. and Murphy, P. J.: Alpha- and beta-adrenergic effects of the stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. 219: 447-452, 1981.

31. Hoffman, B. B., Lavin, T. N., Lefkowitz, R. J. and Ruffolo, R. R., Jr.: Alpha-adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. J. Pharmacol. Exp. Ther. 219: 290-295, 1981.

32. Ruffolo, R. R., Jr.: Structure-activity relationships of alpha adrenoceptor agonists. In: Adrenoceptors and Catecholamine Action, Part B, G. Kunos (editor), John Wiley and Sons, Inc., New York, pp. 1-50, 1983.

Publications (Continued)

33. Ruffolo, R. R., Jr., Anderson, K. and Miller, D. D.: Conformational requirements of alpha$_1$- and alpha$_2$-adrenergic receptors. Mol. Pharmacol. 21: 259-261, 1982.

34. Ruffolo, R. R., Jr., Yaden, E. L. and Ward, J. S.: Receptor interactions of imidazolines. VIII. Influence of ionization constant on the diffusion of clonidine and a series of structurally related imidazolidines into and out of the central nervous system. Eur. J. Pharmacol. 81: 367-375, 1982.

35. Ruffolo, R. R., Jr., Waddell, J. E. and Yaden, E. L.: Heterogeneity of postsynaptic alpha-adrenergic receptors in mammalian aortas. J. Pharmacol. Exp. Ther. 221: 309-314, 1982.

36. Ruffolo, R. R., Jr. and Waddell, J. E.: Receptor interactions of imidazolines. IX. Cirazoline is an alpha$_1$-adrenergic agonist and an alpha$_2$-adrenergic antagonist. J. Pharmacol. Exp. Ther. 222: 29-36, 1982.

37. Ruffolo, R. R., Jr. and Waddell, J. E.: Receptor interactions of imidazolines. X. Alpha-adrenergic receptor of rat and rabbit aortae differentiated by relative potencies, affinities and efficacies of imidazoline agonists. Br. J. Pharmacol. 77: 169-176, 1982.

38. Ruffolo, R. R., Jr., Yaden, E. L., Waddell, J. E. and Ward, J. S.: Receptor interactions of imidazolines. XI. Alpha-adrenergic and antihypertensive effects of clonidine and its methylenebridged analog, St 1913. Pharmacology 25: 187-201, 1982.

39. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Stereochemical requirements of alpha$_2$-adrenergic receptors. J. Pharmacol. Exp. Ther. 222: 645-651, 1982.

40. Ruffolo, R. R., Jr.: Stereoselectivity in adrenergic agonists and adrenergic blocking agents. In: Stereochemistry and Biological Activity of Drugs, edited by E. J. Ariens, W. Soudijn, and P. B. M. W. M. Timmermans, Blackwell Scientific Publications, Oxford, pp. 103-125, 1983.

41. Ruffolo, R. R., Jr.: Review: Important concepts of receptor theory. Journal of Autonomic Pharmacology 2: 277-295, 1982.

42. Thompson, J. M., Eisenbarth, G. S., Ruffolo, R. R., Jr. and Nirenburg, M.: Synapse selection based on differences in synapse turnover. Int. J. Devl. Neurosciences 1: 25-30, 1983.

43. Ruffolo, R. R., Jr. and Waddell, J. E.: Stereochemical requirements of alpha-2 adrenergic receptors for α-methyl substituted phenethylamines. Life Sciences 31: 2999-3007, 1982.

44. Ruffolo, R. R., Jr. and Yaden, E. L.: Vascular effects of the stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. 224: 46-50, 1983.

Publications (Continued)

45. Ruffolo, R. R., Jr. and Waddell, J. E.: Aromatic and benzylic hydroxyl substitution of imidazolines and phenethylamines: Differences in activity at alpha-1 and alpha-2 adrenergic receptors. J. Pharmacol. Exp. Ther. 224: 559-566, 1983.

46. Ruffolo, R. R., Jr., Nelson, W. L. and Yaden, E. L.: Blockade of postjunctional vascular $alpha_1$- and $alpha_2$-adrenoceptors in pithed rat by the enantiomers of WB-4101. Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 93-97, 1983.

47. Ruffolo, R. R., Jr., Rice, P. J., Patil, P. N., Hamada, A. and Miller, D. D.: Differences in the applicability of the Easson-Stedman hypothesis to the alpha-1 and alpha-2 adrenergic effects of phenethylamines and imidazolines. Eur. J. Pharmacol. 86: 471-475, 1983.

48. Ruffolo, R. R., Jr., Patil, P. N. and Miller, D. D.: Adrenergic effects of optically active catecholimidazoline derivatives in pithed rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 323: 221-227, 1983.

49. Ruffolo, R. R., Jr.: Drug, neurotransmitter and hormone receptors in the regulation of the cardiovascular system. In: Critical Care: State-of-the-Art, Volume 4, edited by W. Shoemaker and W. L. Thompson, Fullerton, California, pp. (K)1-(K)56, 1983.

50. Ruffolo, R. R., Jr.: The use of isolated, physiologically responding tissues to investigate neurotransmitter receptors. In: Neuroreceptors in Health and Disease, Monographs in Neural Sciences, Volume 10, edited by J. Marwaha and W. Anderson, S. Karger AG, Basel, pp. 53-84, 1984.

51. Ruffolo, R. R., Jr.: Alpha-Adrenoceptors. In: Neuroreceptors in Health and Disease, Monographs in Neural Sciences, Volume 10, edited by J. Marwaha and W. Anderson, S. Karger AG, Basel, pp. 224-252, 1984.

52. Ruffolo, R. R., Jr.: Stereochemical requirements for activation and blockade of $alpha_1$- and $alpha_2$-adrenoceptors. Invited review, Trends in Pharmacological Sciences 5: 160-164, 1984.

53. Dharmsathaphorn, K., Yamashiro, D. J., Lindeborg, D., Mandel, K. G., McRoberts, J. and Ruffolo, R. R., Jr.: Structure-activity relationships of alpha-adrenergic compounds on electrolyte transport in the rabbit ileum and rat colon. Gastroenterology 86: 120-128, 1984.

54. Ruffolo, R. R., Jr., Banning, J. W., Patil, P. N., Hamada, A., and Miller, D. D.: Evaluation of the adrenergic effects of a novel optically active catecholamidine in vitro and in vivo: Differential application of the Easson-Stedman Hypothesis to alpha- and beta-adrenoceptors. J. Pharmacol. Exp. Ther. 226: 469-476, 1983.

Publications (Continued)

55. Ruffolo, R. R., Jr., Timmermans, P. B. M. W. M. and van Zwieten, P. A.: Interaction of clonidine, its methylene-bridged analog, St 1913, and the benzylic hydroxyl-substituted derivative, St 1965, with α_1- and α_2-adrenoceptors. J. Auton. Pharmacol. 3: 185-193, 1983.

56. Timmermans, P. B. M. W. M., Qian, J. Q., Ruffolo, R. R., Jr. and van Zwieten, P. A.: A study of the selectivity and potency of rauwolscine, RX 781094 and RS 21361 as antagonists of alpha-1 and alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 228: 739-748, 1984.

57. Banning, J. W., Rice, P. J., Miller, D. D., Ruffolo, R. R., Jr., Hamada, A. and Patil, P. N.: Differences in the adrenoreceptor activation by stereoisomeric catecholimidazolines and catecholamines. In: Neuronal and Extraneuronal Events in Autonomic Pharmacology, edited by W. W. Fleming, S. Z. Langer, K. H. Graefe and N. W. Weiner, Raven Press, New York, pp. 167-180, 1984.

58. Hynes, M. D., Atlas, D. and Ruffolo, R. R., Jr.: Analgesic activity of HP-aminoclonidine, a novel analog of clonidine: Role of opioid receptors and alpha-adrenoceptors. Pharmacol. Biochem. Behav. 19: 879-882, 1983.

59. Ruffolo, R. R., Jr. and Yaden, E. L.: Selective α_2-adrenoceptor agonist activity of the novel inotropic agent, ASL-7022: Comparison with dobutamine. Eur. J. Pharmacol. 93: 117-120, 1983.

60. Ruffolo, R. R., Jr. and Shaar, C. J.: Relative potency of LY141865 at dopamine DA_2 and histamine H_2 receptors. Eur. J. Pharmacol. 92: 295-296, 1983.

61. Ruffolo, R. R., Jr. and Morgan, E. L.: Interaction of the novel inotropic agent, ASL-7022, with alpha- and beta-adrenoceptors in the cardiovascular system of the pithed rat: comparison with dobutamine and dopamine. J. Pharmacol. Exp. Ther. 229: 364-371, 1984.

62. Ruffolo, R. R., Jr., Yaden, E. L., Timmermans, P. B. M. W. M., van Zwieten, P. A. and Hynes, M. D.: Characterization of the alpha-adrenoceptor and antihypertensive activity of ICI-106270: Comparison with Clonidine. J. Pharmacol. Exp. Ther. 229: 58-66, 1984.

63. Ruffolo, R. R., Jr.: Interactions of agonists with peripheral alpha-adrenoceptors. Proceedings from Minisymposium "Peripheral Alpha-Adrenergic Receptors", sponsored by the American Society for Pharmacology and Experimental Therapeutics. Federation Proc. 43: 2910-2916, 1984.

64. Ruffolo, R. R., Jr., Messick, K. and Horng, J. S.: Interaction of the selective inotropic agents, ASL-7022, dobutamine and dopamine, with alpha- and beta-adrenoceptors in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. 326: 317-326, 1984.

Publications (Continued)

65. Nelson, W. L. Bartels, M. J., Bednarski, P. J., Zhang, S., Messick, K., Horng, J. S. and Ruffolo, R. R., Jr.: The 3,4-catechol derivative of propranolol, a minor dihydroxylated metabolite. J. Med. Chem. 27: 857-861, 1984.

66. Ruffolo, R. R., Jr. and Yaden, E. L.: The existence of spare alpha$_1$-adrenoceptors, but not alpha$_2$-adrenoceptors, for the respective vasopressor effects of cirazoline and B-HT 933 in pithed rat. J. Cardiovasc. Pharmacol. 6: 1011-1019, 1984.

67. Ruffolo, R. R., Jr., Morgan, E. L. and Messick, K.: Possible relationship between receptor reserve and the differential antagonism of α_1- and α_2-adrenoceptor-mediated pressor responses by calcium channel antagonists in pithed rat. J. Pharmacol. Exp. Ther. 230: 587-594, 1984.

68. Ruffolo, R. R., Jr.: The role of central and peripheral alpha- and beta-adrenoceptors in the regulation of cardiovascular function. Anaesthesiology and Intensive Care Medicine, 167, Intensive Care and Emergency Medicine, edited by J. L. Vincent, Springer-Verlag (Berlin), pp. 67-70, 1984.

69. Ruffolo, R. R., Jr. and Messick, K.: Evaluation of the alpha$_1$- and alpha$_2$-adrenoceptor-mediated effects of a series of dimethoxysubstituted tolazoline derivatives in the cardiovascular system of the pithed rat. J. Pharmacol. Exp. Ther. 232: 94-99, 1985.

70. Ruffolo, R. R., Jr., Messick, K. and Horng, J. S.: Interactions of dimethoxy-substituted tolazoline derivatives with alpha$_1$- and alpha$_2$-adrenoreceptors in vitro. J. Auton. Pharmacol. 5: 71-79, 1985.

71. Ruffolo, R. R., Jr.: Selective alpha$_1$-adrenoceptor agonists and antagonists. In: Pharmacology of Adrenoceptors, ed. by E. Szabadi, C. M. Bradshaw, S. Nahorski, Macmillan Press, London, pp. 3-12, 1985.

72. Ruffolo, R. R., Jr.: Introduction: Peripheral Alpha-Adrenoceptors. Federation Proc. 43: 2908-2909, 1984.

73. Ruffolo, R. R., Jr.: On the mechanism of action of dobutamine. Ann Intern. Med. 100: 313-314, 1984.

74. Ruffolo, R. R., Jr., Goldberg, M. R. and Morgan, E. L.: Interactions of epinephrine, norepinephrine, dopamine and their corresponding α-methyl substituted derivatives with alpha and beta adrenoceptors in pithed rat. J. Pharmacol. Exp. Ther. 230: 595-600, 1984.

75. Ruffolo, R. R., Jr.: Relative agonist potency as a means of differentiating α-adrenoceptors and α-adrenergic mechanisms. Clinical Science 68 (Suppl. 10): 9s-14s, 1985.

Publications (Continued)

76. Ruffolo, R. R., Jr.: Stereochemical requirements for activation and blockade of α_1- and α_2-adrenoceptors. In: Receptors Again, edited by J. W. Lamble and A. C. Abbott, Elsevier Science Publications, Amsterdam, pp. 128-137, 1984.

77. Ruffolo, R. R., Jr., Cohen, M. L., Messick, K. and Horng, J. S.: α_2-Adrenoceptor mediated effects of pergolide. Pharmacology 35: 148-154, 1987.

78. Paget, C. J. and Ruffolo, R. R., Jr.: Benzazepines as novel antihypertensive agents. U.S. Patent, Issued 1986.

79. Ruffolo, R. R., Jr. and Messick, K.: Systemic hemodynamic effects of dopamine, (+)-dobutamine and the (+)- and (-)-enantiomers of dobutamine in anesthetized normotensive rat. Eur. J. Pharmacol. 109: 173-181, 1985.

80. Ruffolo, R. R., Jr. and Messick, K.: Inotropic selectivity of dobutamine enantiomers in pithed rat. J. Pharmacol. Exp. Ther. 235: 344-348, 1985.

81. Ruffolo, R. R., Jr. and Morgan, E. L.: Interaction of the enantiomers of dobutamine in pithed rat. Eur. J. Pharmacol. 109: 173-181, 1985.

82. Ruffolo, R.R., Jr., Kurz, K. and Paget, C.J.: Evaluation of a novel antihypertensive agent, LY127210, in anesthetized and conscious spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 232: 134-138, 1985.

83. Ruffolo, R.R., Jr., Messick, K., and Horng, J.S.: Interaction of the enantiomers of 3-0-methyldobutamine with alpha- and beta-adrenoceptors in vitro. Naunyn-Schmeideberg's Arch. Pharmacol. 329: 244-252, 1985.

84. Ruffolo, R. R., Jr.: Distribution and function of peripheral alpha adrenoceptors in the cardiovascular system. Pharmacol. Biochem. Behav. 22: 827-833, 1985.

85. Ruffolo, R. R., Jr.: Pharmacology of adrenoceptors. Trends Pharmacol. Sci. 6: 5-8, 1985.

86. Majerus, T.C., Dasta, J.F., Bauman, J.L., Danziger, L.H. and Ruffolo, R.R., Jr. Dobutamine Ten Years Later. Pharmacotherapy 9: 245-259, 1989.

87. Ruffolo, R. R., Jr. and Hieble, J. P.: Neurohumoral regulation of cardiovascular function. In: Oxygen Transport in the Critically Ill, ed. by J. V. Snyder, Year Book Medical Publishers, Chicago, pp. 67-86, 1987.

88. Ruffolo, R. R., Jr., Fondacaro, J. D., Levitt, B., Edwards, R.M. and Kinter, L. B.: Pharmacologic manipulation of regional blood flow. In: Oxygen Transport in the Critically Ill, ed. by J. V. Snyder, Year Book Medical Publishers, Chicago, pp. 450-474, 1987.

Publications (Continued)

89. Hamada, A., Yaden, E. L., Horng, J. S., Ruffolo, R. R., Jr., Patil, P. N. and Miller, D. D.: N-Substituted imidazolines and ethylenediamines and their action on α- and β-adrenergic receptors. J. Med. Chem. 28: 1269-1273, 1985.

90. Ruffolo, R. R., Jr. and Zeid, R. L.: Relationship between α_2-adrenoceptor occupancy and response for B-HT 933 in canine saphenous vein. Eur. J. Pharmacol. 111: 267-271, 1985.

91. Zeid, R. L. and Ruffolo, R. R., Jr.: Role of prostacyclin, thromboxane A_2 and leukotrienes in cardiovascular function and disease. In: Oxygen Transport in the Critically Ill., ed. by J. V. Snyder, Year Book Medical Publishers, Chicago, pp. 138-150, 1987.

92. Ruffolo, R. R., Jr. and Zeid, R. L.: Relationship between α-adrenoceptor occupancy and response for the α_1-adrenoceptor agonist, cirazoline, and the α_2-adrenoceptor agonist, B-HT 933, in canine saphenous vein. J. Pharmacol. Exp. Ther. 235: 636-643, 1985.

93. Ruffolo, R. R., Jr. and Messick, K.: Effects of dopamine, (±)-dobutamine and the (+)- and (-)-enantiomers of dobutamine on cardiac function in pithed rats. J. Pharmacol. Exp. Ther. 235: 558-565, 1985.

94. DeMarinis, R., Wise, M., Hieble, J. P. and Ruffolo, R. R., Jr.: Structure-activity relationships for α_1-adrenergic receptor agonists and antagonists. In: The Alpha$_1$-Adrenergic Receptor, edited by R. R. Ruffolo, Jr., Humana Press (Clifton, N.J.), pp 211-265, 1987.

95. Ruffolo, R. R., Jr.: The Alpha$_1$-Adrenoceptors, edited by R. R. Ruffolo, Jr., Humana Press (Clifton, N.J.), pp. 1-543, 1987.

96. Hieble, J. P., Matthews, W. D., DeMarinis, R. M. and Ruffolo, R. R., Jr.: Heterogeneity of α_1-adrenergic receptors. In: The Alpha$_1$-Adrenoceptors, edited by R. R. Ruffolo, Jr., Humana Press (Clifton, N.J.), pp. 325-349, 1987.

97. Ruffolo, R. R., Jr. and Kopia, G. A.: The importance of receptor regulation in the pathophysiology and therapy of congestive heart failure. Am. J. Med. 80: (suppl 2B) 67-72, 1986.

98. Ruffolo, R. R., Jr. and Nichols, A. J.: Alpha-adrenoceptor coupling to vasoconstriction in the peripheral circulation. In: Brain Epinephrine Neuronal Functions, edited by J.M. Stolk, D.C. U'Prichard and K. Fuxe, Oxford University Press, New York, pp. 339-346, 1988.

99. Ruffolo, R. R., Jr., DeMarinis, R. M., Wise, M. and Hieble, J. P.: Structure-activity relationships for α_2-adrenoceptor agonists and antagonists. In: The α_2-Adrenoceptors, edited by L. Limbird, Humana Press (Clifton, NJ), pp. 115-186, 1988.

Publications (Continued)

100. Hieble, J. P. and Ruffolo, R. R., Jr.: Therapeutic applications of agents interacting with alpha-1 adrenergic receptors. In: The Alpha$_1$-Adrenoceptors, edited by R. R. Ruffolo, Jr., Humana Press (Clifton, NJ), pp. 477-500, 1987.

101. Ruffolo, R. R., Jr.: Pharmacological basis of drug-receptor interaction. Proceedings from the American Statistical Association Annual Symposium, Biopharmaceutical Section, pp. 54-59, 1985.

102. Ruffolo, R. R., Jr.: Spare α-adrenoceptors in the peripheral circulation: excitation contraction coupling. Fed. Proc. 45: 2341-2346, 1986.

103. Kruse, L. I., Kaiser, C., DeWolf, W. E., Frazee, J. S., Berkowitz, B. A., Erickson, R. W., Ezekiel, M., Ohlstein, E. H., and Ruffolo, R. R., Jr.: Substituted 1-benzylimidazole-2-thiols as potent and orally active inhibitors of dopamine β-hydroxylase. J. Med. Chem. 29: 887-889, 1986.

104. Kopia, G. A., Kopaciewicz, L. J. and Ruffolo, R. R., Jr.: α-Adrenoceptor regulation of coronary blood flow in normal and stenotic canine coronary artery. J. Pharmacol. Exp. Ther. 239: 641-647, 1986.

105. Gellai, M. and Ruffolo, R. R., Jr.: Renal effects of selective α_1- and α_2-adrenoceptor agonists in conscious, normotensive rats. J. Pharmacol. Exp. Ther. 240: 723-728, 1987.

106. Shebuski, R. J., Fujita, T. and Ruffolo, R. R., Jr.: Evaluation of α_1- and α_2-adrenoceptor mediated responses in the in situ, autoperfused pulmonary circulation of the anesthetized dog. J. Pharmacol. Exp. Ther. 238: 217-223, 1986.

107. Nichols, A. J., Hieble, J. P. and Ruffolo, R. R., Jr.: The pharmacology of peripheral α_1- and α_2-adrenoceptors. Reviews in Clinical and Basic Pharmacology 7: 129-205, 1988.

108. Shebuski, R. J., Fujita, T., Smith, J. M. and Ruffolo, R. R., Jr.: Comparison of the α-adrenoceptor activity of dopamine, ibopamine and epinine in the pulmonary circulation of the dog. J. Pharmacol. Exp. Ther. 241: 6-12, 1987.

109. Ruffolo, R. R., Jr., Sulpizio, A. C., Nichols, A. J., DeMarinis, R. M. and Hieble, J. P.: Pharmacologic differentiation between pre- and postjunctional α_2-adrenoceptors by SK&F 104078. Naunyn-Schmiedeberg's Arch. Pharmacol. 336: 415-418, 1987.

110. Nichols, A. J. and Ruffolo, R. R. Jr.: The relationship between alterations in α_1-adrenoceptor reserve by phenoxybenzamine and benextramine and the sensitivity of cirazoline-induced pressor responses to inhibition by nifedipine. Eur. J. Pharmacol. 126: 297-301, 1986.

Publications (Continued)

111. Applefeld, M. M., Sutton, F. J., Achuff, S., Fisher, M. L., Ruffolo, R. R., Jr., Dembo, D., Moulton, A. and Weber, R.: Contemporary issues in the management of chronic congestive heart failure, panel discussion II. Am. J. Med. 80: (suppl. 2B) 78-80, 1986.

112. Shebuski, R. J., Fujita, T. and Ruffolo, R. R., Jr.: Interaction of dopamine, (+)-dobutamine and the (-)-enantiomer of dobutamine with α- and β-adrenoceptors in the pulmonary circulation of the dog. Pharmacology 34: 201-212, 1987.

113. Kopia, G. A., Kopaciewicz, L. J. and Ruffolo, R. R., Jr.: Coronary thrombolysis with intravenous streptokinase in the anesthetized dog: a dose-response study. J. Pharmacol. Exp. Ther. 244:956-962, 1988.

114. Kopia, G. A., Ohlstein, E. H. and Ruffolo, R. R., Jr.: Systemic hemodynamic and coronary vascular actions of the novel inotropic agent, ibopamine, and the de-esterified derivative and active form, epinine: relationship to left ventricular performance in the dog. J. Pharmacol. Exp. Ther. 246: 434-440, 1988.

115. Shebuski, R.J., Ohlstein, E.H., Smith, J.M., Jr., and Ruffolo, R.R., Jr.: Enhanced pulmonary alpha-2 adrenoceptor responsiveness under conditions of elevated pulmonary vascular tone. J. Pharmacol. Exp. Ther. 242: 158-165, 1987.

116. Nichols, A.J., Smith, J.M., Jr., Shebuski, R.J. and Ruffolo, R.R., Jr.: Comparison of the effects of the novel inotropic agent, ibopamine, with epinine, dopamine and fenoldopam on vascular dopamine receptors in the anesthetized dog. J. Pharmacol. Exp. Ther. 242: 573-578, 1987.

117. Hieble, J.P., Sulpizio, A.C., DeMarinis, R.M. and Ruffolo, R.R., Jr.: α_2-Adrenoceptor antagonists: A new approach to Cardiovascular Therapy. In: Vasodilation: Vascular Smooth-Muscle, Peptides, Autonomic Nerves and Endothelium, ed. by P. Vanhoutte, Raven Press Ltd., New York, pp. 217-222, 1988.

118. Ruffolo, R.R., Jr., Sulpizio, A.C., Nichols, A.J., DeMarinis, R.M. and Hieble, J.P.: Arterial α_2-adrenoceptor blockade: a potentially new approach to antihypertensive therapy. J. Cardiovascular Pharmacol. 10 (Suppl. 4): S100-S103, 1987.

119. Hieble, J.P., Sulpizio, A.C., Nichols, A.J., DeMarinis, R.M., Pfeiffer, F.R., Lavanchy, P.G. and Ruffolo, R.R., Jr.: Pharmacological differentiation of pre- and postjunctional α_2-adrenoceptors. J. Hypertension 4 (Suppl. 6): S189-S192, 1987.

Publications (Continued)

120. Ruffolo, R.R., Jr.: Mode of action and structure-activity relationships among imidazoline-like compounds acting at the α-adrenoceptor. In: Recent Advances in Receptor Chemistry, ed. by C. Melchiorre and M. Giannella, Elsevier Science Publishers, Pharmacochemistry Library Series, B.V. Amsterdam, pp. 77-84, 1988.

121. Ruffolo, R.R., Jr. and Nichols, A.J.: Recent experimental and conceptual advances in drug receptor research in the cardiovascular system. In: Advances in Drug Research, Vol. 17, ed. by B. Testa, Academic Press (London), pp. 235-348, 1988.

122. Kopia, G.A., Kopaciewicz, L.J., Fong, K.L., Crysler, C.S., Boyle, K. and Ruffolo, R.R., Jr.: Evaluation of the acute hemodynamic, cardioprotective and pharmacokinetic effects of coronary thrombolysis with intravenous tissue plasminogen activator in the anesthetized dog. J. Cardiovasc. Pharmacol. 12: 308-316, 1988.

123. Ruffolo, R.R., Jr. and Nichols, A.J.: Drugs with combined α-adrenoceptor and β-adrenoceptor blocking properties. In: ISI Atlas of Science 1: 241-245, 1987.

124. Daly, R.N., Sulpizio, A.C., Levitt, B., DeMarinis, R.M., Regan, J.W., Ruffolo, R.R., Jr. and Hieble, J.P.: Evidence for heterogeneity between pre- and postjunctional α_2-adrenoceptors using novel 9-substituted 3-benzazepines. J. Pharmacol. Exp. Ther. 247: 122-128, 1988.

125. Shebuski, R.J., Smith, J.M., Jr. and Ruffolo, R.R., Jr.: Effect of dopamine, ibopamine and epinine on α- and β-adrenoceptors in canine pulmonary circulation. Fundam. Clin. Pharmacol. 3: 211-221, 1989.

126. Shebuski, R.J., Smith, J.M., Jr. and Ruffolo, R.R., Jr.: Comparison of the renal and pulmonary hemodynamic effects of fenoldopam, dobutamine, dopamine and norepinephrine in the anesthetized dog. Pharmacology 36: 35-43, 1988.

127. Bylund, D.B. and Ruffolo, R.R., Jr.: Alpha-1 Adrenergic Receptors: Summary and Future Vistas. In: The Alpha-1 Adrenergic Receptors (ed. by R. Ruffolo). Humana Press, Clifton, N.J., pp 503-507, 1987.

128. Ruffolo, R.R., Jr.: Cardiovascular Adrenoceptors - Physiology- Critical Care Implications. In: The Pharmacologic Approach to the Critically Ill Patient. Edited by B. Chernow, Williams & Wilkins, Baltimore, MD, pp. 166-183, 1988.

129. Ruffolo, R.R., Jr. and Bylund, D.B.: The Alpha-1 Adrenergic Receptors. (Preface) In: The Alpha-1 Adrenergic Receptors, edited by R.R. Ruffolo, Jr., Humana Press, Clifton, N.J. pp. v-vii, 1987.

Publications (Continued)

130. Lin, T.M., Evans, D.C., Warrick, M.W. and Ruffolo, R.R., Jr.: Actions of nizatidine on the rat uterus, dog stomach and experimentally-induced gastric lesions. J. Pharmacol. Exp. Ther. 239: 400-405, 1986.

131. Ruffolo, R.R., Jr. and Nichols, A.J.: The relationship of receptor reserve and agonist efficacy to the sensitivity of α-adrenoceptor-mediated vasopressor responses to inhibition by calcium channel antagonists. Ann. N.Y. Acad. Sci. 522: 361-376, 1988.

132. Nichols, A.J. and Ruffolo, R.R., Jr.: Evaluation of the alpha- and beta-adrenoceptor mediated activities of the novel, orally active inotropic agent, ibopamine, in the cardiovascular system of the pithed rat: comparison with epinine and dopamine. J. Pharmacol. Exp. Ther. 242: 455-463, 1987.

133. Ruffolo, R.R., Jr.: The role of intracellular and extracellular calcium in α-adrenoceptor mediated vasoconstriction: A model to explain the relationship between agonist efficacy and the sensitivity to inhibition by calcium channel antagonists. In: Vascular Neuroeffector Mechanisms, ed. by J.A. Bevan, H. Majewski, R.A. Maxwell and D.F. Story, IRL Press, Oxford, pp. 41-48, 1987.

134. Ruffolo, R.R., Jr., Nichols, A.J. and Hieble, J.P.: Functions mediated by α_2-adrenergic receptors. In: The α_2-Adrenergic Receptors, edited by L. Limbird, Humana Press (Clifton, N.J.), pp. 187-280, 1988.

135. Ruffolo, R.R., Jr.: Molecular Biology, Biochemistry and Pharmacology of the β-adrenoceptors: Preface: In: Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. x-xii, 1991.

136. Hieble, J.P., Kopia, G.A., Shebuski, R.J. and Ruffolo, R.R., Jr.: The role of postjunctional α_2-adrenoceptor in the control of vascular resistance. In: Vascular Neuroeffector Mechanisms, edited by J.A. Bevan, H. Majewski, R.A. Maxwell, and D.F. Story, IRL Press, Oxford, pp. 49-56, 1988.

137. Ruffolo, R.R., Jr.: The Pharmacology of dobutamine. The American Journal of the Medical Sciences 294: 244-248, 1987.

138. Ohlstein, E.H., Horohonich, S., Shebuski, R.J. and Ruffolo, R.R., Jr.: Localization and characterization of the canine pulmonary α_2-adrenoceptor. J. Pharmacol. Exp. Ther. 248: 233-239, 1989.

139. Nichols, A.J. and Ruffolo, R.R., Jr.: The relationship of α-adrenoceptor reserve and agonist intrinsic efficacy to calcium utilization in the vasculature. Trends in Pharmacological Sciences 9: 236-241, 1988.

Publications (Continued)

140. Nichols, A.J., Shebuski, R.J. and Ruffolo, R.R., Jr.: Inhibition of plasma cholinesterase prevents the dopamine DA-1 receptor mediated renal vasodilation produced by ibopamine. Eur. J. Pharmacol. 141: 515-518, 1987.

141. Medgett, I.C. and Ruffolo, R.R., Jr. Characterization of α-adrenoceptors mediating sympathetic vasoconstriction in rat autoperfused hindlimb: Effects of SK&F 104078. Eur. J. Pharmacol. 144: 393-397, 1987.

142. Ruffolo, R.R., Jr.: Physiology and biochemistry of the peripheral autonomic nervous system. In: Human Pharmacology: Molecular to Clinical, edited by L.B. Wingard, Jr. and T.M. Brody, J. Larner and A. Schwartz, The C.V. Mosby Co., St. Louis, pp. 77-94, 1991.

143. Medgett, I.C. and Ruffolo, R.R. Jr.: α-Adrenoceptor-mediated vasoconstriction in the rat hindlimb: innervated α_2-adrenoceptors in the saphenous arterial bed. J. Pharmacol. Exp. Ther. 246: 249-254, 1988.

144. Gellai, M., DeWolf, R.E. and Ruffolo, R.R., Jr.: Effect of dopamine β-hydroxylase inhibition on systemic hemodynamics in conscious spontaneously hypertensive rats. Pharmacology 41: 82-90, 1990.

145. Ruffolo, R.R., Jr.: Adrenergic receptors in man. Trends in Pharmacological Sciences 8: 515, 1987.

146. Ruffolo, R.R., Jr. Enantioselectivity: Its biological basis and pharmacological consequences. In: Excerpta Medica International Congress Series, ed. by M.J. Rand and C. Raper, Elsevier Science Publishers, pp. 787-790, 1987.

147. Ruffolo, R.R., Jr. and Nichols, A.J.: Relationship between α-adrenoceptor agonist efficacy and the sensitivity to inhibition by calcium channel antagonists. Proceedings of 6th Vascular Neuroeffector Meeting. In: Vascular Neuroeffector Mechanisms, edited by J.A. Bevan, H. Majewski, R.A. Maxwell, and D.F. Story, IRL Press, Oxford, pp. 41-48, 1988.

148. Nichols, A.J., Motley, E.D. and Ruffolo, R.R., Jr.: Differential effect of pertussis toxin on pre- and postjunctional α_2-adrenoceptors in the cardiovascular system of the pithed rat. Eur. J. Pharmacol. 145: 345-349, 1988.

149. Ohlstein, E.H., Kopia, G.A. and Ruffolo, R.R., Jr.: Coronary vascular activity of the novel inotropic pro-drug, ibopamine, and the de-esterified active form, epinine. Arzneimittel Forschung (Drug Research) 38: 1790-1792, 1988.

150. Kopia, G.A. and Ruffolo, R.R., Jr.: Mechanism of action of adrenergic agents used in acute heart failure. In: Update in Intensive Care and Emergency Medicine, Volume 6. Acute Heart Failure, ed. by C. Perret and J.L. Vincent, Springer-Verlag, Berlin, pp. 244-265, 1988.

Publications (Continued)

151. Kinter, L.B., Horner, E., Mann, W.A., Weinstock, J., and Ruffolo, R.R., Jr.: Characterization of the hemodynamic activities of fenoldopam and its enantiomers in the dog. Chirality 2: 219-225, 1990.

152. Nichols, A.J., Hamada, A., Adejare, A., Miller, D.D., Patil, P.N. and Ruffolo, R.R., Jr.: Effect of aromatic flourine substitution on the α- and β-adrenoceptor-mediated effects of 3,4-dihydroxytolazoline in the pithed rat. J. Pharmacol. Exp. Ther. 248: 671-676, 1989.

153. Docherty, J.R. and Ruffolo, R.R., Jr.: Canine pulmonary artery contains a homogeneous population of α_1-adrenoceptors. J. Pharmacol. Exp. Ther. 248: 479-483, 1989.

154. Nichols, A.J., Motley, E.D. and Ruffolo, R.R., Jr.: The effect of pertussis toxin treatment on postjunctional α_1- and α_2-adrenoceptor function in the cardiovascular system of the pithed rat. J. Pharmacol. Exp. Ther. 249: 203-209, 1989.

155. Hieble, J.P., Sulpizio, A.C., Nichols, A.J., Willette, R.N. and Ruffolo, R.R., Jr.: Pharmacologic characterization of SK&F 104078, a novel α-adrenoceptor antagonist that discriminates between pre- and postjunctional α_2-adrenoceptors. J. Pharmacol. Exp. Ther. 247: 645-652, 1988.

156. Daly, R.N., Roberts, M.I., Ruffolo, R.R., Jr. and Hieble, J.P.: The contribution of endogenous neuropeptide Y to contraction during field-stimulation of the rabbit central ear artery and canine saphenous vein. J. Hypertension 6 (Suppl. 4): S529-S534, 1988.

157. Daly, R.N., Roberts, M.I., Ruffolo, R.R., Jr. and Hieble, J.P.: The role of neuropeptide Y in vascular sympathetic neurotransmission may be enhanced in hypertension. J. Hypertension 6 (Suppl. 4): S535-S538, 1988.

158. Hieble, J.P., Ruffolo, R.R., Jr. and Daly, R.N.: Involvement of the vascular endothelium in the potentiation of vasoconstrictor responses by neuropeptide Y. J. Hypertension 6 (Suppl. 4): S239-S242, 1988.

159. Ruffolo, R.R., Jr., Nichols, A.J., Patil, P.N., Hamada, A., Clark, M. and Miller, D.D.: The effects of benzylic hydroxyl substitution on the α_1- and α_2-adrenoceptor blocking activity of tolazoline. Eur. J. Pharmacol. 157: 235-239, 1988.

160. Hieble, J.P. and Ruffolo, R.R., Jr.: Structure-activity relationships of β-adrenoceptor agonists and antagonists. In: β-adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger A.G., Basel, pp. 105-172, 1991.

Publications (Continued)

161. Ruffolo, R.R., Jr., Motley, E.D. and Nichols, A.J.: The effect of pertussis toxin treatment on postjunctional α_1-adrenoceptor mediated vasopressor effects of the full agonist, cirazoline, and the partial agonist, (-)-dobutamine, in pithed rats. Fundam. Clin. Pharmacol. 5: 11-23, 1991.

162. Nichols, A.J., Gellai, M. and Ruffolo, R.R., Jr.: Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol. Fundam. Clin. Pharmacol. 5: 25-38, 1991.

163. Nichols, A.J. and Ruffolo, R.R., Jr.: The coupling of α_1- and α_2-adrenoceptors to G-proteins and second messengers in vascular smooth muscle. Trends Pharmacol. Sci. In preparation.

164. Ruffolo, R.R., Jr. and Nichols, A.J.: Signal transduction processes involved in α-adrenoceptor vasoconstriction. Drug News and Perspectives 2:150-156, 1989.

165. Nichols, A.J., Sung, C.P., Roberts, M.I., Price, C.A. and Ruffolo, R.R., Jr.: Studies on the mode of interaction of SK&F 94482, a novel H_2-receptor antagonist, with H_2-receptors in vitro: A correlation of functional and binding studies. J. Pharmacol. Exp. Ther. Submitted for publication.

166. Nichols, A.J., Sulpizio, A.C., Ashton, D., Hieble, J.P. and Ruffolo, R.R., Jr.: In vitro pharmacological profile of the novel β-adrenoceptor antagonist/vasodilator, carvedilol. Pharmacology 39:327-336, 1989.

167. Smith, E.F. III, Kopia, G.A., Ohlstein, E.H., Crooke, S.T. and Ruffolo, R.R., Jr.: Newer strategies in anti-thrombotic pathways - Thromboxane receptor antagonists. In: Thrombolysis. The Dawn of a New Era. Ed. by P. Sleight and D.A. Chamberlain, IBC Technical Services Ltd., London, 1989.

168. Ruffolo, R.R., Jr.: Physiology and Pharmacology of cardiovascular dopamine receptors. L'Information Cardioloqique 7: 142-147, 1989.

169. Nichols, A.J., Sulpizio, A.C., Ashton, D.J., Hieble, J.P. and Ruffolo, R.R., Jr.: The interaction of the enantiomers of carvedilol with α_1- and β_1-adrenoceptors. Chirality 1: 265-270, 1989.

170. Ruffolo, R.R., Jr.: The use of animal model systems in the development of cardiovascular drugs. Proceedings from the symposium entitled "Modeling in Biomedical Research: An Assessment of Current and Potential Approaches, pp. 35-41, 1989.

171. Ruffolo, R.R., Jr. and Nichols, A.J.: The role of α_1- and α_2-adrenoceptors in the regulation of the cardiovascular system. In: Update in Intensive Care and Emergency Medicine, Volume 10. Ed. by J.L. Vincent, Springer-Verlag, pp. 321-331, 1990.

Publications (Continued)

172. Ruffolo, R.R., Jr.: Structure-activity relationships of α-adrenoceptor agonists and antagonists. In: Adrenoceptors: Structure, Mechanisms, Functions, Ed. by E. Szabadi, C.M. Bradshaw, Birkhauser Verlag, Berlin, pp. 5-14, 1991.

173. Ruffolo, R.R., Jr., Gellai, M., Hieble, J.P., Willette, R.N. and Nichols, A.J.: The Pharmacology of Carvedilol. Eur. J. Clin. Pharmacol. 38: S82-S88, 1990.

174. Willette, R.N., Sauermelch, C. and Ruffolo, R.R., Jr.: Systemic and local cutaneous hemodynamic effects of carvedilol and labetalol in the anesthetized rat. Eur. J. Pharmacol. 176: 237-240, 1990.

175. Oriowo, M.A., Nichols, A.J. and Ruffolo, R.R., Jr.: Receptor protection studies with phenoxybenzamine indicate that a single α_1-adrenoceptor may be coupled to two signal transduction processes in vascular smooth muscle. Pharmacology 45: 17-26, 1992.

176. Gellai, M., DeWolf, R. and Ruffolo, R.R., Jr.: Effect of carvedilol on renal hemodynamics and renal excretory function in spontaneously hypertensive rats. Pharmacology 41: 200-206, 1990.

177. Nichols, A.J., Brooks, D.P. and Ruffolo, R.R., Jr.: The pharmacology of fenoldopam (Corlopam). Proceedings of the Third International Conference on Peripheral Dopamine. Am. J. Hypertension 3: 116S-119S, 1990.

178. Ruffolo, R.R., Jr., Sauermelch, C.F. and Willette, R.N.: Hemodynamic differences between carvedilol and labetalol in the cutaneous circulation. Eur. J. Clin. Pharmacol. 38: S112-S114, 1990.

179. Ruffolo, R.R., Jr.: SIGNS: A Stereochemically Informative Generic-Name System. Trends Pharmacol. Sci. 11: 61, 1990.

180. Motley, E.D., Ruffolo, R.R., Jr., Hay, D.P. and Nichols, A.J.: Role of phosphatidylinositol turnover in the contraction of rat aorta. Adv. Exp. Med. Biol. 308: 211-216, 1991.

181. Brooks, D.P., Drutz, D.J. and Ruffolo, R.R., Jr.: Prevention and complete reversal of cyclosporine A-induced renal vasoconstriction and nephrotoxicity in the rat by fenoldopam. J. Pharmacol. Exp. Ther. 254: 375-379, 1990.

182. Hieble, J.P. and Ruffolo, R.R., Jr.: Effects of α- and β-adrenoceptors on lipids and lipoproteins. In: Antilipidemic Drugs: Medicinal Chemical and Biochemical Aspects, ed. by D.T. Witiak, H.A.I. Newman and D.R. Feller, Elsevier Publisher, Amsterdam, pp. 301-344, 1991.

183. Ruffolo, R.R., Jr.: Autonomic Pharmacology. In: Year Book of Pharmacology, ed. by M.A. Hollinger, CRC Press, Boca Raton, pp. 203-231, 1990.

184. Ruffolo, R.R., Jr., Brooks, D.P., Huffman, W. and Poste, G.: From vasopressin antagonist to agonist: a saga of surprise. Drug News and Perspectives 4: 217-222, 1991.

Publications (Continued)

185. Nichols, A.J. and Ruffolo, R.R., Jr.: Alpha-adrenoceptors and calcium translocation in vascular smooth muscle. In: Adrenoceptors: Structure, Mechanisms, Functions, Ed. by E. Szabadi and C.M. Bradshaw, Birkhauser Verlag, Berlin, pp. 139-148, 1991.

186. Ruffolo, R.R., Jr.: α_2-Adrenoceptor agonists and antagonists. Neurotransmissions 6: 1-8, 1990.

187. Smith, E.F., Kopia, G.A., Ohlstein, E.H. and Ruffolo, R.R., Jr.: Newer strategies in anti-thrombotic pathways - thromboxane receptor antagonists. In: Thrombolysis: Past, Present and Future, ed. by P. Sleight, pp. 124-133, 1990.

188. Ruffolo, R.R., Jr., Nichols, A.J., Oriowo, M.A.: Interaction of vascular α_1-adrenoceptors with multiple signal transduction pathways. Blood Vessels 28: 122-128, 1991.

189. Nichols, A.J. and Ruffolo, R.R., Jr.: Structure-activity relationships for α-adrenoceptor agonists and antagonists. In: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, ed. by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. 75-114, 1991.

190. Nichols, A.J. and Ruffolo, R.R., Jr.: α-Adrenoceptor subclassification. In: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, ed. by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. 1-23, 1991.

191. Nichols, A.J. and Ruffolo, R.R., Jr.: Functions mediated by peripheral α-adrenoceptors. In: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, ed. by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. 115-179, 1991.

192. Oriowo, M.A. and Ruffolo, R.R., Jr.: Chloroethylclonidine-sensitive α_1-adrenoceptor subtype mediates the contractile effect of norepinephrine and UK-14,304 in the rabbit thoracic aorta: Evidence for a single α_1-adrenoceptor subtype linked to two signal transduction process. In preparation.

193. Nichols, A.J. and Ruffolo, R.R., Jr.: The Pharmacology of Adrenoceptors. Drug News and Perspective 3:566-570, 1990.

194. Ruffolo, R.R., Jr.: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, (editor), S. Karger, A.G., Basel, 1991 (226 pages).

195. Ruffolo, R.R., Jr.: Molecular structure and genetics of adrenoceptors. In: Adrenoceptors: Structure, Mechanisms, Function. Ed. by E. Szabadi and C.M. Bradshaw, Birkhauser Verlag, Berlin, pp. 111-114, 1991.

Publications (Continued)

196. Oriowo, M.A., Hieble, J.P., Villalobos-Molina, R. and Ruffolo, R.R., Jr.: Evidence that (-)-dobutamine interacts with α_{1D}-adrenoceptors to promote extracellular calcium influx in the rat aorta: studies with nifedipine and chlorothylclonidine. Pharmacol. Rev. and Communications 11: 11-18, 2000.

197. Oriowo, M.A., Hieble, J.P. and Ruffolo, R.R., Jr.: Evidence for heterogeneity of prejunctional α_2-adrenoceptors. Pharmacology 43:1-13, 1991.

198. Oriowo, M.A. and Ruffolo, R.R., Jr.: Heterogeneity of postjunctional α_1-adrenoceptors in mammalian aortae: subclassification based on chlorethylclonidine, WB 4101 and nifedipine. J. Vascular Research 29: 33-40, 1992.

199. Ruffolo, R.R., Jr., Nichols, A.J., Stadel, J.M. and Hieble, J.P.: Structure and function of α-adrenoceptors. Pharmacological Reviews 43(4):475-505, 1991.

200. Ruffolo, R.R., Jr., Hieble, J.P., Brooks, D.P., Feuerstein, G.Z. and Nichols, A.J.: Drug receptors and control of the cardiovascular system: Recent advances. Progress in Drug Research, Volume 36, edited by E. Jucker, Birkhauser Verlag, Basel, pp. 117-360, 1991.

201. Motley, E., Ruffolo, R.R., Jr. and Nichols, A.J.: The effect of pertussis toxin treatment on α_1-adrenoceptor mediated pressor responses in the pithed rat: dependence on agonist efficacy but not chemical class. Pharmacology 45: 338-344, 1992.

202. Ruffolo, R.R., Jr.: Trends in Shock Research. Fundamentals of receptor theory: Basics for shock research. Circ. Shock 37: 176-184, 1992.

203. Ruffolo, R.R., Nichols, A.J., Hieble, J.P. and Oriowo, M.A.: SK&F 104078 identifies subtypes of prejunctional α_2-adrenoceptors in rat vas deferens. In: Presynaptic Receptors and Neuronal Transporters, Advances in the Biosciences, edited by S.Z. Langer, A.M. Galzin and J. Costentin, Pergamon Press, Oxford, pp. 315-318, 1991.

204. Brooks, D.P., Mitchell, M., Short, B.G., Ruffolo, R.R., Jr. and Nichols, A.J.: Attenuation of amphotericin B nephrotoxicity by the fenoldopam prodrug, SK&F 105058. J. Pharmacol. Exp. Ther. 257: 1243-1247, 1991.

205. Hieble, J.P. and Ruffolo, R.R., Jr.: Therapeutic applications of agents interacting with α-adrenoceptors. In: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8 ed. by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. 180-220, 1991.

206. Ruffolo, R.R., Jr.: Molecular Biology, Biochemistry and Pharmacology of the α-Adrenoceptors: Preface: In: Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, ed. by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, pp. xi-xiii, 1991.

Publications (Continued)

207. Ruffolo, R.R., Jr.: Stereochemistry at α-Adrenoceptors. Proceedings from the Roussel Scientific Institute Table Ronde on "Chirality and Drug Design", Edited by S. Davies and J. Caldwell, Roussel Scientific Institute, Wiltshire, pp. 25-27, 1991.

208. Ruffolo, R.R., Jr.: Autonomic Pharmacology. In: Year Book of Pharmacology, ed. by M.A. Hollinger, CRC Press, Boca Raton, pp. 219-246, 1991.

209. Ruffolo, R.R., Jr. and Morgan, E.L.: Interaction of the enantiomers of 3-0-methyldobutamine, a metabolite of dobutamine, with α- and β-adrenoreceptors in the cardiovascular system of the pithed rat. J. Auton. Pharmac. 4: 295-302, 1984.

210. Hieble, J.P. and Ruffolo, R.R., Jr.: Functions mediated by ß-adrenoceptor activation. In: ß-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger A.G., Basel, pp. 173-209, 1991.

211. Hieble, J.P. and Ruffolo, R.R., Jr.: Therapeutic applications of agents interacting with the ß-adrenoceptor. In: ß-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger A.G., Basel, pp. 210-235, 1991.

212. Dasta, J.F., Baumann, J.L., Danziger, L.H., Majerus, T.C. and Ruffolo, R.R., Jr.: Dobutamine vs. Dopamine: Another Look. Pharmacotherapy 10: 417-418, 1990.

213. Ruffolo, R.R., Jr., Nichols, A.J. and Hieble, J.P.: Minireview: Metabolic regulation by α_1- and α_2-adrenoceptors. Life Sci. 49: 171-183, 1991.

214. Hieble, J.P., Sulpizio, A.C., Edwards, R., Chapman, H., Young, P., Roberts, S.P., Blackburn, T.P., Shah, D.H., DeMarinis, R.M. and Ruffolo, R.R., Jr.: Additional evidence for functional subclassification of α_2-adrenoceptor based on a new selective antagonist, SK&F 104856. J. Pharmacol. Exp. Ther. 259: 643-652, 1991.

215. Weinstock, J., Keenan, R.M., Samanen, J., Hempel, J., Finkelstein, J.A., Franz, R.G., Giatanopoulos, D.E., Girard, G.R., Gleason, J.G., Hill, D.T., Morgan, T.M., Peishoff, C.E., Aiyar, N., Brooks, D.P., Frederickson, T.A., Ohlstein, E.H., Ruffolo, R.R., Jr., Stack, E.J., Sulpizio, A.C., Weidley, E.F. and Edwards, R.M.: 1-(4-Carboxybenzyl)imidazole-5-acrylic acids: Potent and selective angiotensin II receptor antagonists. J. Med. Chem. 34: 1514-1517, 1991.

216. Brooks, D.P., Frederickson, T.A., Koster, P.F. and Ruffolo, R.R., Jr.: Effect of the dopamine-β-hydroxylase inhibitor, SK&F 102698, on blood pressure in the 1-kidneys, 1-clip hypertensive dog. Pharmacology 43: 90-95, 1991.

Publications (Continued)

217. Hieble, J.P., Nichols, A.J., Fredrickson, T.A., DePalma, P.D., Ruffolo, R.R., Jr. and Brooks, D.P.: Cardiovascular actions of a new, selective postjunctional α-adrenoceptor antagonist, SK&F 104856, in normotensive and hypertensive dogs. Brit. J. Pharmacol. 105: 992-996, 1992.

218. Ruffolo, R.R., Jr., Trendelenburg, U. and Langer, S.Z.: Chemical neurotransmission: Peripheral Autonomic nervous system. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 87-103, 1994.

219. Hieble, J.P. and Ruffolo, R.R., Jr.: Local anesthetics. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 191-200, 1994.

220. Ruffolo, R.R., Jr.: Chirality in α- and β-adrenoceptor agonists and antagonists. Invited review, Tetrahedron Report (Number 302). Tetrahedron 47 (48): 9953-9980, 1991.

221. Edwards, R.M., Aiyar, N., Ohlstein, E.H., Weidley, E.F., Griffin, E., Ezekiel, M., Keenan, R.M., Ruffolo, R.R., Jr. and Weinstock, J.: Pharmacological characterization of the nonpeptide angiotensin II receptor antagonist, SK&F 108566. J. Pharmacol. Exp. Ther. 260: 175-181, 1992.

222. Ruffolo, R.R., Jr., Boyle, D., Venuti, R.P. and Lukas, M.: Carvedilol (Kredex): A novel, multiple action cardiovascular agent (Invited Review). Drugs of Today 27(7): 465-492, 1991.

223. Nichols, A.J., Koster, P.F., Sanford, K., Drutz, D.J., Brooks, D.P. and Ruffolo, R.R., Jr.: The effects of fenoldopam on the acute and sub-acute nephrotoxicity produced by amphotericin-B in the dog. J. Pharmacol. Exp. Ther. 260: 269-274, 1992.

224. Nichols, A.J. and Ruffolo, R.R., Jr.: Uterine Pharmacology. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 201-208, 1994.

225. Hieble, J.P. and Ruffolo, R.R., Jr.: Pharmacology of Neuromuscular Transmission. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 145-159, 1994.

226. Hamburger, S.A., Valocik, R.E., Schnell, M.A., Griswold, D.E., Hillegass, L.M., Feuerstein, G. and Ruffolo, R.R., Jr.: Infarct size reduction by carvedilol in dogs undergoing coronary artery occlusion and reperfusion. J. Int'l. Cardiol. Submitted for publication.

Publications (Continued)

227. Ruffolo, R.R, Jr., Nichols, A.J., Stadel, J.M. and Hieble, J.P.: Pharmacologic and therapeutic application of α_2-adrenoceptor subtypes. Annual Review of Pharmacology and Toxicology 33: 243-279, 1993.

228. Feuerstein, G.Z., Nichols, A.J., Valocik, R.E., Gagnon, R., Sellars, T.S., Fears, R.C., Ferris, H. and Ruffolo, R.R., Jr.: Cardioprotection and thrombolysis by Eminase in the anesthetized dog. J. Cardiovasc. Pharmacol. 25: 625-633, 1995.

229. Hamburger, S.A., Barone, F., Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol (Kredex®) reduces infarct size in a canine model of acute myocardial infarction. Pharmacology 43: 113-120, 1991.

230. Ruffolo, R.R., Jr., Boyle, D.A., Venuti, R.P. and Lukas, M.A.: Preclinical and clinical pharmacology of carvedilol. Journal of Human Hypertension 7(1): S2-S15, 1993.

231. McFalls, E.O., Duncker, D.J., Sassen, L.M.A., Krams, R., Man in't Veld, A.J., Ruffolo, R.R., Jr. and Verdouw, P.D.: Altered coronary flow reserve in the hypertrophied heart: implications for theory. Journal of Human Hypertension 7(1): S29-S36, 1993.

232. Feuerstein, G.Z., Hamburger, S.A., Smith, E.F., Bril, A. and Ruffolo, R.R., Jr.: Myocardial protection by carvedilol. J. Cardiovasc. Pharmacology 19 (Suppl I): S138-S141, 1992.

233. Nichols, A.J., Ruffolo, R.R., Jr., Huffman, W.F., Poste, G. and Samanen, J.: Development of GPIIb/IIIa receptor antagonists as antithrombotic drugs. Trends Pharmacol. Sci. 13: 413-417, 1992.

234. Ruffolo, R.R., Jr., Bril, A. and Feuerstein, G.Z.; Cardioprotective potential of carvedilol. Proceedings of the International Cardiological Institute for Therapeutic Research Symposium entitled: "Carvedilol: Wider Therapeutic Potential in Cardiovascular Syndrome". Cardiology 82 (Suppl. 3): 24-28, 1993.

235. Brooks, D.P., Feuerstein, T.A., Weinstock, J., Ruffolo, R.R., Jr., Edwards, R.M. and Gellai, M.: Antihypertensive activity of the non-peptide angiotensin II receptor antagonist, SK&F 108566, in rats and dogs. Naunyn-Schmiedeberg's Arch. Pharmacol. 345: 673-678, 1992.

236. Hieble, J.P. and Ruffolo, R.R., Jr.: Subclassification of β-adrenoceptors. In: Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger A.G., Basel, pp. 1-25, 1991.

237. Oriowo, M.A. and Ruffolo, R.R., Jr.: Activation of a single α_1-adrenoceptor subtype in rat aorta mobilizes intracellular and extracellular pools of calcium. Pharmacology 44: 139-149, 1992.

Publications (Continued)

238. Feuerstein, G.Z., Langer, S.Z. and Ruffolo, R.R., Jr.: Adrenal Medulla. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 161-167, 1994.

239. Hieble, J.P., Nichols, A.J., Langer, S.Z. and Ruffolo, R.R., Jr.: Pharmacology of the sympathetic nervous system. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 121-144, 1994.

240. Hieble, J.P. and Ruffolo, R.R., Jr.: Cholinergic Drugs. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 105-120 1994.

241. Ohlstein, E.O., Gellai, M., Brooks, D.P., Vickery, L., Jugus, J., Sulpizio, A., Ruffolo, R.R., Jr., Weinstock, J. and Edwards, R.M.: The antihypertensive effect of the angiotensin II receptor antagonist, DuP 753, may not be due solely to angiotensin II receptor antagonism. J. Pharmacol. Exp. Ther. 262: 595-601, 1992.

242. Bril, A., Slivjak, M.J., DiMartino, M.J., Feuerstein, G.Z., Linee, P., Poyser, R.H., Ruffolo, R.R., Jr. and Smith, E.F. III: Cardioprotective effects of carvedilol, a novel β-adrenoceptor antagonist with vasodilating properties, in anesthetized minipigs: comparison with propranolol. Cardiovascular Research 26: 518-525, 1992.

243. Clancy, A. and Ruffolo, R.R., Jr.: Cardioprotective use of carbazolyl-(y)-oxypropranolamine compounds. U.S. Patent, Filed October 2, 1991.

244. Samanen, J., Huffman, W., Ruffolo, R.R., Jr., and Nichols, A.J.: The first class of cell adhesion inhibitors: fibrinogen receptor antagonists, small potent antithrombotic peptides. Biotechnology. In preparation.

245. Ruffolo, R.R., Jr. and Nichols, A.J.: Signal transduction mechanisms for vascular α-adrenoceptors. Receptors. Submitted for publication.

246. Ruffolo, R.R., Jr.: Molecular Biology, Biochemistry and Pharmacology of the β-Adrenoceptors: Preface. In: Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, vol. 7, ed. R.R. Ruffolo, Jr., S. Karger AG, Basel, pp. x-xii, 1991.

247. Ruffolo, R.R., Jr.: Autonomic Pharmacology. In: Year Book of Pharmacology, edited by M.A. Hollinger, CRC Press, Boca Raton, pp. 209-242, 1992.

248. Ruffolo, R.R., Jr.: Drug Receptor Theory: Important concepts and practical applications. Drug News and Perspectives 6: 12-23, 1993.

Publications (Continued)

249. Feuerstein, G., Smith, E.F., III, Yue, T-L., Cheng, H-Y., and Ruffolo, R.R., Jr.: Myocardial protection by the novel vasodilating β-blocker, carvedilol: potential relevance of antioxidant activity. Hypertension 214: 277-280, 1992.

250. Yue, T-L., McKenna, P.J., Ruffolo, R.R., Jr., and Feuerstein, G.Z.: Carvedilol, a new β-adrenoceptor antagonist and vasodilator, inhibits superoxide release from human neutrophils. Eur. J. Pharmacol. 214: 277-280, 1992.

251. Motley, E.P., Ruffolo, R.R., Jr. and Nichols, A.J.: The effect of pertussis toxin treatment on α_1-adrenoceptor-mediated pressor responses in the pithed rat: dependence on agonist efficacy but not chemical class. Pharmacology 43: 338-344, 1992.

252. Ruffolo, R.R., Jr. and Hieble, J.P.: Alpha-Adrenoceptors. In: International Encyclopedia of Pharmacology and Therapeutics, edited by W.C. Bowman, A.M. Breckenridge, A.C. Sartorelli, and F. Mitchelson, Pergamon Press, New York. In press.

253. Ruffolo, R.R., Jr. and Hieble, J.P.: Alpha-Adrenoceptors (Invited Review). Pharmacology and Therapeutics 61: 1-64, 1994.

254. Ruffolo, R.R., Jr.: Cardiovascular Adrenoceptors: Physiology and Critical Care Implications. Chapter 9. In: The Pharmacologic Approach to the Critically Ill Patient, Third Edition, edited by B. Chernow, Williams and Wilkins, Baltimore, pp. 167-181, 1994.

255. Ruffolo, R.R., Jr., Boyle, D.A., Brooks, D.P., Feuerstein, G.Z., Venuti, R.P., Lukas, M.A. and Poste, G.: Carvedilol: A novel cardiovascular drug with multiple actions. Cardiovasc. Drug Rev. 10: 127-157, 1992.

256. Hieble, J.P. and Ruffolo, R.R., Jr.: Imidazoline receptors: Historical perspective. Fundamen. Clin. Pharmacol 6 (Supplement 1): 7S-13S, 1992.

257. Brooks, D.P., Short, B.G., Cyronak, M.J., Contino, L.C., DiCristo, M., Wang, Y-X., and Ruffolo, R.R., Jr.: Comparison between carvedilol and captopril in rats with partial ablation-induced chronic renal failure. Br. J. Pharmacol. 109: 581-586, 1993.

258. Brooks, D.P., DePalma, D., Ruffolo, R.R., Jr.: Effect of captopril and the nonpeptide angiotensin II antagonists, SK&F 108566 and EXP 3174, on renal function in dogs with a renal artery stenosis. J. Pharmacol. Exp. Ther. 263: 422-427, 1992.

259. Ruffolo, R.R., Jr.: Medicinal chemistry of adrenoceptor agonists. Drug Design and Discovery 9: 351-367, 1993.

Publications (Continued)

260. Hieble, J.P. and Ruffolo, R.R., Jr.: Pharmacology and cholinergic transmission. In: Principles of Pharmacology: Basic Concepts and Clinical Applications, edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, pp. 105-111, 1994.

261. Yue, T.-L., McKenna, P.J., Lysko, P.G., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a new antihypertensive agent, prevents oxidation of human low density lipoprotein by macrophages and copper. Atherosclerosis 97: 209-216, 1992.

262. Ruffolo, R.R., Jr.: Angiotensin II Receptors Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspective (editor), CRC Press, Boca Raton, 1994 (170 pages).

263. Ruffolo, R.R., Jr.: Physiology and Biochemistry of the Peripheral Autonomic Nervous System. In: Human Pharmacology-Molecular to Clinical, edited by T.M. Brody, J. Larner, K.P. Minneman and H. Neu, Mosby Year Book, St. Louis, pp. 81-96, 1994.

264. Ruffolo, R.R., Jr.: The antiarrhythmic, UK-68,798 (Dofetilide; Pfizer), and the specific bradycardic agent, UL-FS 49 (Zatabradine; Thomae). Current Opinion in Investigational Drugs 1: 59-61, 1992.

265. Brooks, D.P., Frederickson, T.A., Kissinger, J.T., Jenkins, E.L. and Ruffolo, R.R., Jr.: Blood pressure lowering activity of the nonpeptide angiotensin II receptor antagonist, SK&F 108566, in furosemide-treated conscious cynomolgus monkeys. Pharmacol. Comm. 2: 331-337, 1993.

266. Yue, T.-L., McKenna, P.J., Gu, J.-L., Cheng, H.-Y., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a new antihypertensive agent, prevents lipid peroxidation and oxidative injury to endothelial cells. Hypertension 22: 922-928, 1993.

267. Ohlstein, E.O., Douglas, S.A., Sung, C.P., Yue, T.-L., Louden, C., Arleth, A., Poste, G., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a novel cardiovascular drug, prevents vascular smooth muscle cell proliferation, migration and neointimal formation following vascular injury. Proc. Natl. Acad. Sci. 90: 6189-6193, 1993.

268. Brooks, D.P. and Ruffolo, R.R., Jr.: Introduction: Angiotensin II Receptors. In: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 1-10, 1994.

269. Brooks, D.P. and Ruffolo, R.R., Jr.: Functions mediated by angiotensin II receptors in the brain. In: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 103-119, 1994.

Publications (Continued)

270. Edwards, R.M. and Ruffolo, R.R., Jr.: Angiotensin II receptor signal transduction mechanisms. In: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 53-70, 1994.

271. Edwards, R.M. and Ruffolo, R.R., Jr.: Angiotensin II receptor subclassification. In: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 11-31, 1994.

272. Bylund, D., Eikenburg, D., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K., Molinoff, P.B., Ruffolo, R.R., Jr. and Trendelenburg, U.: Adrenergic Receptor Subtypes: International Union of Pharmacology Nomenclature of Adrenoceptors. Pharmacol. Rev. 46: 121-136, 1994.

273. Brooks, D.P. and Ruffolo, R.R., Jr.: Functions mediated by peripheral angiotensin II receptors. In: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 71-102, 1994.

274. Ruffolo, R.R., Jr., Stadel, J.M. and Hieble, J.P.: α-Adrenoceptors: Recent Developments. Medicinal Research Reviews 2(4): 229-270, 1994.

275. Brooks, D.P., DePalma, D., Fredrickson, T.A., Spielman, W.S. and Ruffolo, R.R., Jr.: Nonpeptide angiotensin II receptor antagonists: potential advantages over ACE inhibitors. Proceedings of the Western Pharmacological Society 36: 25-28, 1993.

276. Brooks, D.P., Fredrickson, T.A. and Ruffolo, R.R., Jr.: Angiotensin converting enzyme inhibitors, but not angiotensin II receptor antagonists, induce coughing in the dog. Pharmacol. Commun. 3: 209-214, 1993.

277. Ruffolo, R.R., Jr.: The antiarrhythmic, UK-68,798 (Dofetilide; Pfizer), and the specific bradycardic agent, UL-FS 49 (Zatebradine; Thomae). Current Opinion in Investigational Drugs 1: 59-61, 1992.

278. Ruffolo, R.R., Jr.: Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspectives, Preface. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. i-iii, 1994.

279. Kinter, L.B., Mann, W.A., Weinstock, J. and Ruffolo, R.R., Jr.: Effects of catechol ring fluorination on cardiovascular and renal activities of fenoldopam enantiomers. Chirality 6: 446-455, 1994.

280. Feuerstein, G.Z., Vonhof, S., Cruickshank, J.M. and Ruffolo, R.R., Jr.: Molecular pharmacology of ischemic injury of the heart. In: Molecular Cell Biology of Cardiovascular Diseases, ed. by J. Diez, Doyma S.A., Barcelona, in press.

Publications (Continued)

281. Yue, T.-L., McKenna, P.J., Gu, J.-L., Cheng, H-Y., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a new vasodilating β-adrenoceptor blocker antihypertensive drug, protects endothelial cells from xanthine-xanthine oxidase - and neutrophil-initiated damage. Cardiovascular Research 28: 400-406, 1994.

282. Yue, T.-L., McKenna, P.J., Lysko, P.G., Gu, J.-L., Lysko, K.A., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: SB 211475, a metabolite of carvedilol, the novel antihypertensive agent, is a potent antioxidant. Eur. J. Pharmacol. 251: 237-243, 1994.

283. Hieble, J.P., Bondinell, W.E. and Ruffolo, R.R., Jr. : Alpha- and Beta-Adrenoceptors: From the Gene to the Clinic. I: Molecular Biology and Adrenoceptor Subclassification. J. Med. Chem. 38: 3415-3444, 1995.

284. Ruffolo, R.R., Jr., Bondinell, W.E. and Hieble, J.P.: Alpha- and Beta-Adrenoceptors: From the Gene to the Clinic. II: Structure-Activity Relationships and Therapeutic Applications. J. Med. Chem. 38: 3681-3716, 1995.

285. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol, a novel antihypertensive drug that offers the potential for cardiovascular organ protection. Current Opinion in Investigational Drugs 2: 661-671, 1993.

286. Oriowo, A.M., Dennitt, M.V., Smith, S.A., Ruffolo, R.R., Jr. and Cawthorne, M.A.: The β-adrenoceptor selectivity profile of BRL 37344 in the pithed rat. J. Auton. Pharmacol. 14: 337-344, 1994.

287. Feuerstein, G.Z., Yue, T.-L. and Ruffolo, R.R., Jr.: Carvedilol update: a multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. Drugs of Today 29: 401-419, 1993.

288. Fan, D., Poste, G., Ruffolo, R.R., Jr., Dong, Z., Seid, C., Earnest, L.E., Campbell, T.E., Clyne, R.K., Belltran, P.J. and Fidler, I.J.: Circumvention of multidrug resistance in murine fibrisarcoma and colon carcinoma cells by treatment with the α-adrenoceptor antagonist, furobenzazepine. Int'l. J. Oncology 4: 789-798, 1994.

289. Feuerstein, G.Z., Yue, T.-L., Cheng, H-Y. and Ruffolo, R.R., Jr.: Myocardial protection by the novel vasodilating beta-blocker, carvedilol: potential relevance of antioxidant activity. J. Hypertension 11 (Suppl. 4): S41-S48, 1993.

290. Ohlstein, E.H., Nambi, P., Douglas, S.A., Edwards, R., Gellai, M., Lago, A., Leber, J., Cousins, R.D., Gao, A., Frazee, J.S., Peishoff, C., Bean, J.W., Eggleston, D.S., Elshourbagy, N.A., Kumar, C., Lee, J.A., Brooks, D., Weinstock, J., Feuerstein, G., Poste, G., Ruffolo, R.R., Jr., Gleason, J. and Elliot, J.D.: SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist. Proc. Nat. Acad. Sci. 91: 8052-8056, 1994.

Publications (Continued)

291. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol, a novel multiple action antihypertensive drug with antioxidant activity and the potential for myocardial and vascular protection. Eur. Heart J. 16: 38-42, 1995.

292. Hieble, J.P. and Ruffolo, R.R., Jr.: α-Adrenoceptors. In: G-Protein Coupled Transmembrane Signaling Mechanisms. Edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, pp. 1-34, 1994.

293. Yue, T.L., Lysko, P.G., Barone, F.C., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a new antihypertensive agent with antioxidant activity: Potential role in cerebroprotection. Ann. N.Y. Acad. Sci. 738: 230-242, 1994.

294. Ruffolo, R.R., Jr.: Angiotensin II Receptor, Volume 2: Medicinal Chemistry (editor), CRC Press, Boca Raton, 225 pages, 1994.

295. Gleason, J. and Ruffolo, R.R., Jr.: Introduction: Medicinal Chemistry of Angiotensin II Receptors. In: Angiotensin II Receptors, Volume 2. Medicinal Chemistry. Edited by R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 1-10, 1994.

296. Ruffolo, R.R., Jr.: Angiotensin II Receptors, Volume 2: Medicinal Chemistry, Preface. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. i-iii, 1994.

297. Hieble, J.P. and Ruffolo, R.R., Jr.: α_1-Adrenoceptors: Pharmacological subclassification and therapeutic applications. Proceedings of the Western Pharmacology Society 37: 163-167, 1994.

298. Ruffolo, R.R., Jr.: Endothelin Receptors: From the Gene to the Human. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 285 pages, 1995.

299. Ohlstein, E. and Ruffolo, R.R., Jr.: Introduction: Endothelin Receptors. In: Endothelin Receptors: From the Gene to the Human. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 1-14, 1995.

300. Ruffolo, R.R., Jr.: Endothelin Receptors: From the Gene to the Human. Preface. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. i-iii, 1995.

301. Ohlstein, E., Nambi, P. and Ruffolo, R.R., Jr.: Endothelin receptor subclassification. In: Endothelin Receptors: From the Gene to the Human. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 15-36, 1995.

302. Ohlstein, E., Douglas, S., Brooks, D., Hay, D., Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Functions mediated by peripheral endothelin receptors. In: Endothelin Receptors: From the Gene to the Human. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 109-185, 1995.

303. Willette, R., Feuerstein, G.Z., Barone, F., Ohlstein, E. and Ruffolo, R.R., Jr.: Functions mediated by endothelin receptors in the brain. In: Endothelin Receptors: From the Gene to the Human. Edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, pp. 187-213, 1995.

Publications (Continued)

304. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol, a novel vasodilating β-blocker with the potential for cardiovascular organ protection. Eur. Heart J. 17 (Supplement B): 24-29, 1996.

305. Brooks, D.P., DePalma, P.D., Gellai, M., Nambi, P., Ohlstein, E.H., Elliott, J.P., Gleason, J. and Ruffolo, R.R., Jr.: Effect of the endothelin receptor antagonists, (±)-SB 209670 and BQ 123, on acute renal failure in anesthetized dogs. J. Pharmacol. Exp. Ther. Submitted.

306. Oriowo, M.A., Chapman, H., Kirkham, D.M., Sennitt, M.V., Ruffolo, R.R., Jr. and Cawthorne, M.A.: The selectivity in vitro of the stereoisomers of the β3-adrenoceptor agonist, BRL 37344. J. Pharmacol. Exp. Ther. 277: 22-27, 1996.

307. Jobe, P.C., Adams-Curit, L.E., Burks, T.F., Fuller, R.W., Peck, C.C., Ruffolo, R.R., Jr., Snead, C. and Woosley, R.W.: The essential role of integrative biomedical sciences in protecting and contributing to the health and well being of our nation. The Physiologist 37: 79-86, 1994.

308. Elliott, J.D., Lago, M.A., Cousins, R.D., Gao, A., Leber, J.D., Erhard, K.F., Lee, J.A., Bean, J.W., De Brosse, C.W., Eggleston, D.S., Nambi, P., Brooks, D.P., Feuerstein, G.Z., Ruffolo, R.R., Jr., Weinstock, J., Gleason, J.G., Peishoff, C.E. and Ohlstein, E.H.: (15,2R, 3S)-3-(2-Carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indone-2-carboxylic acid (SB 209670), a potent and selective nonpeptide endothelin receptor antagonist. J. Med. Chem. 37: 1153-1157, 1994.

309. Ohlstein, E.H., Beck, G., Douglas, S.A., Nambi, P., Gleason, J., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Elliott, J.D.: Nonpeptide endothelin receptor antagonists. II. Pharmacological characterization of SB 209670. J. Pharmacol. Exp. Ther. 271: 762-768, 1994.

310. Edwards, R.M., Ruffolo, R.R., Jr. and Brooks, D.P.: Angiotensin II receptors. In: Receptors: Recent Findings. Edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, pp. 153-176, 1995.

311. Feuerstein, G.Z., Yue, T-L., Ma X-L. and Ruffolo, R.R., Jr.: Carvedilol: A novel multiple action antihypertensive drug that provides major organ protection. Cardiovascular Drug Reviews 12: 85-104, 1994.

312. Brooks, D.P., DePalma, P.D., Gellai, M., Nambi, P., Ohlstein, E.H., Elliott, J.D., Gleason, J. and Ruffolo, R.R., Jr.: Nonpeptide endothelin receptor antagonists. III. Effect of SB 209670 and BQ123 on acute renal failure in anesthetized dogs. J. Pharmacol. Exp. Ther. 271: 769-775, 1994.

313. Nambi, P., Pullen, M., Wu, H-L., Lee, D., Saunders, D., Heys, R., Aiyar, N., Leber, J., Elliott, J.D., Brooks, D.P., Ohlstein, E.H. and Ruffolo, R.R., Jr.: Nonpeptide endothelin receptor antagonists. VII. Binding characteristics of 3H-SB 209670, a novel nonpeptide antagonist of endothelin receptors. J. Pharmacol. Exp. Ther. 277: 1567-1571, 1996.

Publications (Continued)

314. Ruffolo, R.R., Jr.: Adrenoceptors: Structure, Function and Pharmacology. Edited by R.R. Ruffolo, Jr., Harwood Academic Press, London, pp. 1-287, 1995.

315. Douglas, S.A., Vickery-Clark, L.M., Louden, C., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Ohlstein, E.O.: Acute pretreatment with carvedilol is sufficient for inhibition of neointima formation following rat carotid artery balloon angioplasty. Pharmacol. Communications 5: 65-72, 1994.

316. Autieri, M.V., Feuerstein, G.Z., Yue, T.L., Ruffolo, R.R., Jr., Ohlstein, E.H. and Douglas, S.A.: Differential gene expression associated with neointima formation induced by carotid artery balloon angioplasty in the rat. Circ. Res., submitted.

317. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Comparison of the ability of two vasodilating, β-blockers, carvedilol and celiprolol, to reduce infarct size in a pig model of acute myocardial infarction. Pharmacol. Communications 5: 57-63, 1994.

318. Feuerstein, G.Z., Yue, T.-L. and Ruffolo, R.R., Jr.: Carvedilol Update: A multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection (Japanese Translation). Drugs of Today 30 (Suppl. 1): 1-20, 1994.

319. Bril, A., Faivre, J.F., Forest, M.C., Cheval, B., Gout, B., Linee, Ph., Ruffolo, R.R., Jr. and Poyser, R.H.: Electrophysiological characteristics of BRL 32872, a novel antiarrhythmic agent with potassium and calcium channel blocking properties, in guinea pig cardiac isolated preparations. J. Pharmacol. Exp. Ther. 273: 1264-1272, 1995.

320. Ruffolo, R.R., Jr. and Poste, G.: A prescription for better prescriptions: A response. Issues in Science and Technology, Summer Supplement, page 9, 1994.

321. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: The antiplatelet GPIIb/IIIa antibody, 7E3: A pioneer drug for the treatment of arterial thrombotic disorders. Expert Opinions in Investigational Drugs 3: 745-752, 1994.

322. Wang, X., Yue, T.-L., Barone, F.C., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Differential display of gene expression in rat focal brain ischemia. Neuroscience Protocols 95-020-02: 1-9, 1995.

323. The Contributors (Ruffolo, R.R., Jr. and 36 Other Contributors). The RBI Handbook of Receptor Classification. Edited by J.W. Kebabian and J.L. Neumeyer, Research Biochemicals International, Natick, 1994.

324. Ruffolo, R.R., Jr. and Hollinger, M.: G-Protein Coupled Transmembrane Signaling Mechanisms, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, pp. 1-204, 1995.

Publications (Continued)

325. Li, Y-O., Hieble, J.P., Bergma, D.J., Swift, A.M., Ganguli, S. and Ruffolo, R.R., Jr.: The β-hydroxyl group of catecholamines may interact with Ser^{90} of the second transmembrane helix of the α_{2A}-adrenoceptor. Pharmacol. Commun. 6: 125-131, 1995.

326. Wang, X., Yue, T.-L., Barone, F.C., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Identification of novel gene expression induced by focal ischemia using mRNA differential display. In: Pharmacology of Cerebral Ischemia, J. Krieglstein and H. Oberpichler-Schwenk, Editors, pp. 473-478, 1994.

327. Hieble, J.P. and Ruffolo, R.R., Jr.: Possible structural and functional relationships between imidazoline receptors and α_2-adrenoceptors. Ann. N.Y. Acad. Sci. 763: 8-21, 1995.

328. Ruffolo, R.R., Jr.: G-Protein Coupled Transmembrane Signaling Mechanisms; Preface. in: G-Protein Coupled Transmembrane Signaling Mechanisms, edited by R.R. Ruffolo, Jr. and M. Hollinger. CRC Press, Boca Raton, pp. i-iii, 1995.

329. Bylund, D.B., Regan, J.W., Faber, J.E., Hieble, J.P., Triggle, C. and Ruffolo, R.R., Jr.: Vascular α-adrenoceptors: From the gene to the human. Can. J. Physiol. Pharmacol. 73: 533-543, 1995.

330. Testa, R., Taddei, C., Poggesi, E., Destefani, C., Cotecchia, S., Hieble, J.P., Sulpizio, A.C., Naselsky, D., Bergsma, D., Ellis, C., Swift, A., Ganguly, S., Ruffolo, R.R., Jr. and Leonardi, A.: REC 15/2739 (SB 216469): A novel prostate selective α_1-adrenoceptor antagonist. Pharmacol. Commun. 6: 79-86, 1995.

331. Ruffolo, R.R., Jr. and Hieble, J.P.: The Adrenoceptors: Historical perspectives, current status and future directions. Pharmacol. Commun. 6: 1-7, 1995.

332. Yue, T.L., Wang, X., Gu, J.L., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol: A new vasodilating β-adrenoceptor blocker, inhibits oxidation of low density lipoproteins by vascular smooth muscle cells and prevents leukocyte adhesion to smooth muscle cells. J. Pharmacol. Exp. Ther. 273: 1442-1449, 1995.

333. Hieble, J.P., Ruffolo, R.R., Jr., Sulpizio, A.C., Naselsky, D.P., Conway, T.M., Ellis, C., Swift, A.M., Ganguly, S. and Bergsma, D.J.: Functional subclassification of α_2-adrenoceptors. Pharmacol. Commun. 6: 91-97, 1995.

334. Li, Y-O., Hieble, J.P., Bergma, D.J., Swift, A.M., Ganguli, S. and Ruffolo, R.R., Jr.: The β-hydroxyl group of catecholamines may interact with Ser^{90} of the second transmembrane helix of the α_{2A}-adrenoceptor. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. 125-131, 1995.

Publications (Continued)

335. Testa, R., Taddei, C., Poggesi, E., Destefani, C., Cotecchia, S., Hieble, J.P., Sulpizio, A.C., Naselsky, D., Bergsma, D., Ellis, C., Swift, A., Ganguly, S., Ruffolo, R.R., Jr. and Leonardi, A.: REC 15/2739 (SB 216469): A novel prostate selective α_1-adrenoceptor antagonist. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. 79-86, 1995.

336. Ruffolo, R.R., Jr. and Hieble, J.P.: The Adrenoceptors: Historical perspectives, current status and future directions. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. 1-7, 1995.

337. Hieble, J.P., Ruffolo, R.R., Jr., Sulpizio, A.C., Naselsky, D.P., Conway, T.M., Ellis, C., Swift, A.M., Ganguly, S. and Bergsma, D.J.: Functional subclassification of α_2-adrenoceptors. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. 91-97, 1995.

338. Christopher, T.A., Yue, T.-L., Feuerstein, G.Z., Ruffolo, R.R., Jr., Lopez, B.L. and Ma, X-L.: Carvedilol, a new β-adrenoceptor blocker, vasodilator and free radical scavenger, exerts an anti-shock and endothelial protective effect in rat splanchnic ischemia and reperfusion. J. Pharmacol. Exp. Ther. 273: 64-71, 1995.

339. Hieble, J.P., McCafferty, G.P., Naselsky, D.P., Bergsma, D.J. and Ruffolo, R.R., Jr.: Recent progress in the pharmacotherapy of diseases of the lower urinary tract. European Journal of Medicinal Chemistry, Proceedings of the XIIIth International Symposium on Medicinal Chemistry, edited by J.C. Muller, pp. 2705-2985, 1995.

340. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Ticlopidine: A novel antiplatelet drug for prevention of thrombotic disorders. Expert Opinion in Investigational Drugs 3: 1163-1169, 1994.

341. Hieble, J.P. and Ruffolo, R.R., Jr.: Subclassification of the β-adrenoceptors. Pharmacol. Commun. 6: 183-193, 1995.

342. Ruffolo, R.R., Jr.: Introduction. Adrenoceptors: Structure, Function and Pharmacology. Pharmacol. Commun., page v, Volume 6 (No. 1-3), 1995.

343. Ruffolo, R.R., Jr.: Adrenoceptors: Structure, Function and Pharmacology; Preface. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. vii-ix, 1995.

344. Hieble, J.P. and Ruffolo, R.R., Jr.: Subclassification of the β-adrenoceptors. In: Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, Reading, pp. 183-193, 1995.

Publications (Continued)

345. Hieble, J.P., Bylund, D.B., Clarke, D.E., Eikenberg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P. and Ruffolo, R.R., Jr.: International Union of Pharmacology Nomenclature of Adrenoceptors. Recommendation for Nomenclature of α_1-adrenoceptors: Consensus Update. Pharmacol. Rev. 47: 267-270, 1995.

346. Ruffolo, R.R., Jr.: Inflammation: Mediators and Pathways; Preface. In: Inflammation: Mediators and Pathways, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, pp. i-iii, 1995.

347. Lysko, P.G., Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol: A novel multiple action antihypertensive drug with multiple organ protective actions. Pharmaceutical News 2: 12-16, 1995.

348. Ruffolo, R.R., Jr., Bondinell, W., Ku, T., Naselsky, D.P. and Hieble, J.P.: α_1-Adrenoceptors: Pharmacological classification and newer therapeutic applications. Proc. West. Pharmacol. Soc. 38: 121-126, 1995.

349. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Vascular restenosis: a disease in search of therapy. Expert Opinion in Investigational Drugs 4(3): 237-242, 1995.

350. Ruffolo, R.R., Jr.: Cardiovascular Drugs: Past, Present and Future. Pharmaceutical News 2(2): 13-15, 1995.

351. Yue, T-L., Gu, J-L., Lysko, P.M., Sponer, G., Cheng, H.Y., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Antioxidant activity of a ß-adrenoceptor antagonist, Carazolol: Comparison with Propranolol and Carvedilol. Pharmacol. Commun. 7: 11-19, 1995.

352. Feuerstein, G.Z., Poste, G.P. and Ruffolo, R.R., Jr.: Carvedilol Update III. Rationale for Use in Congestive Heart Failure. Drugs of Today 31: 307-326, 1995.

353. Feuerstein, G.Z., Nichols, A.J. and Ruffolo, R.R., Jr.: Clopidogrel: A novel antiplatelet drug for prevention and treatment of thrombotic disorders. Expert Opinion on Investigational New Drugs 4: 425-430, 1995.

354. Bril, A., Gout, B., Bonhomme, M., Landais, L., Faiure, J.F., Linee, P., Poyser, R.P. and Ruffolo, R.R., Jr.: Combined potassium and calcium channel blocking activities as a basis for antiarrhythmic efficacy with low proarrhythmic risk: experimental profile of BRL 32872. J. Pharmacol. Exp. Ther., in press.

355. Ruffolo, R.R., Jr.: Editor, Special Issue. Pharmacology of Adrenoceptors. Pharmacol. Comm., Volume 6, Numbers 1-3, pp. 1-279, 1995.

356. Ruffolo, R.R., Jr. and Hollinger, M.A.: Inflammation: Mediators and Pathways. Edited by R.R. Ruffolo, Jr. and M.A. Hollinger, CRC Press, Boca Raton, pp. 1-206, 1995.

Publications (Continued)

357. Yue, T.L., Wang, X., Gu, J.L., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol prevents low-density lipoprotein (LDL)-enhanced monocyte adhesion to endothelial cells by inhibition of LDL oxidation. Eur. J. Pharmacol. 294: 585-591, 1995.

358. Ma, X.L., Yue, T.L., Lopez, B.L., Barone, F.C., Christopher, T.A., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol, a new β-adrenoceptor blocker and free radical scavenger, attenuates myocardial ischemia-reperfusion injury in hypercholesterolemic rabbits. J. Pharmacol. Exp. Ther. 227: 128-136, 1996.

359. Ruffolo, R.R., Jr. and Feuerstein, G.Z.: The Pharmacology of Carvedilol: Rationale for use in hypertension, coronary artery disease and congestive heart failure. Cardiovasc. Drugs Ther. 11: 247-256, 1997.

360. Hieble, J.P. and Ruffolo, R.R., Jr.: Subclassification and nomenclature of α_1- and α_2-adrenoceptors. In: Progress in Drug Research, Volume 47, edited by E. Jucker, Birkhauser Verlag, Basel, pp. 81-130, 1996.

361. Li, Y.-O., Hieble, J.P., Bergsma, D.J., Swift, A.M., Ganguly, S. and Ruffolo, R.R., Jr.: Identification of potential sites for interactions of catecholamines with the α_{2A}-adrenoceptor by site-directed mutagenesis: The molecular basis for the Easson-Stedman Hypothesis. Submitted for publication.

362. Ohlstein, E.H., Elliott, J.P., Feuerstein, G.Z., and Ruffolo, R.R., Jr.: Endothelin receptors: Receptor classification, novel receptor antagonists and potential therapeutic targets. Med. Res. Rev. 16: 365-390, 1996.

363. Hieble, J.P., Ruffolo, R.R., Jr. and Starke, K. Identification, characterization and subclassification of α_2-adrenoceptors: An overview. In: α_2-Adrenergic Receptors: Structure, Function and Therapeutic Implications, Edited by S.M. Lanier and L. Limbird, Harwood Academic Publishers, Reading, pp. 1-18-, 1996.

364. Feuerstein, G.Z., Fisher, M., Nunnari, M. and Ruffolo, R.R., Jr.: Carvedilol inhibits aortic lipid deposition in the hypercholesterolemic rat. Pharmacology 54: 24-32, 1997.

365. Lukas, M.A., Ruffolo, R.R., Jr., Shusterman, N.H., Sponer, G. and Strein, K.: Method of treatment for decreasing mortality resulting from congestive heart failure. United States Patent #5,760,069, June 2, 1998.

366. Lopez, B.L., Christopher, T.A., Yue, T.L., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Ma, X.L. Carvedilol, a new β-adrenoceptor blocker antihypertensive drug, protects against free-radical-induced endothelial dysfunction. Pharmacology 51: 165-173, 1995.

Publications (Continued)

367. Hieble, J.P., Hehr, A., Li, Y-O., Naselsky, D.P. and Ruffolo, R.R., Jr.: Characterization of stereoselective interactions of catecholamines with the α_{2a}-adrenoceptor via site-directed mutagenesis. In: α_2-Adrenergic Receptors: Structure, Function and Therapeutic Implications, Edited by S.M. Lanier and L. Limbird, Harwood Academic Publishers, Reading, pp. 43-51, 1996.

368. Feuerstein, G.Z, and Ruffolo, R.R., Jr.: Carvedilol: Preclinical profile and rationale for its use in hypertension, coronary syndromes and congestive heart failure. Cardiovascular Reviews and Reports 17: 27-38, 1996.

369. Wang, X., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: mRNA Differential Display: A new technology for discovery of novel pharmacological targets. Trends Pharmacol. Sci. 17: 276-279, 1996.

370. Wang, X., Yue, T-L., Barone, F.C., White, R.F., Clark, R.K., Willette, R.N., Sulpizio, A.C., Aiyar, N.V., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Discovery of adrenomedullin in rat ischemic cortex and evidence for its role in exacerbating focal brain ischemic damage. Proc. Natl. Acad. Sci. 92: 11480-11484, 1995.

371. Hieble, J.P. and Ruffolo, R.R., Jr.: The use of α-adrenoceptor antagonists in the pharmacological management of benign prostatic hypertrophy: an overview. Pharmacol. Res. 33: 145-160, 1996.

372. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Gene therapy for restenosis: status, issues and future directions. In: Coronary Restenosis: From Genetics and Therapeutics, edited by G.Z. Feuerstein, Marcel Decker, New York, pp. 455-468, 1996.

373. Hehr, A., Hieble, J.P., Li, Y-O., Bergsma, D.J., Swift, A.M., Ganguly, S. and Ruffolo, R.R., Jr.: Serine^{165} of transmembrane helix IV is not involved in the interaction of catecholamines with the α_{2A}-adrenoceptor. Pharmacology 55:18-24, 1997.

374. Hieble, J.P., Naselsky, D.P., Arch, J.R.S., Chapman, H., Smith, S.A., Poyser, R.H. and Ruffolo, R.R., Jr.: Affinity of carvedilol for recombinant human adrenoceptors. Pharmacol. Rev. Commun. 10: 43-50, 1998.

375. Ruffolo, R.R., Jr., Trendelenburg, U. and Langer, S.Z.: Chemical Neurotransmission: Peripheral Autonomic Nervous System. In: Principles of Pharmacology. Edited by P.L. Munson, R.A. Mueller and G.R. Breese, Chapman and Hall, New York, in press.

376. Feuerstein, G.Z., Bril, A. and Ruffolo, R.R., Jr.: Sotalol and d-Sotalol: Apparent benefits of β-blocking activity for ventricular arrhythmias. Expert Opinion on Investigational Drugs 5: 575-580, 1996.

Publications (Continued)

377. Hieble, J.P. and Ruffolo, R.R., Jr.: Adrenergic pharmacology of carvedilol. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

378. Gellai, M., Brooks, D.P., Bril, A. and Ruffolo, R.R., Jr.: Hemodynamic effects of carvedilol. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

379. Feuerstein, G.Z., Yue, T-L. and Ruffolo, R.R., Jr.: Antioxidant activity of carvedilol. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

380. Feuerstein, G.Z., Bril, A., Ohlstein, E.H., Brooks, D.P. and Ruffolo, R.R., Jr.: Major organ protection by carvedilol (heart/cardioprotection, kidneys/ renoprotection, de-emphasis blood vessels/remodeling, brain). In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

381. Feuerstein, G.Z., Ohlstein, E.H., Poste, G., Ma, X, Yue, T-L., Ruffolo, R.R., Jr.: Apoptosis, adhesion molecules, endothelial function, foam cells, gene expression - Additional actions of carvedilol. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

382. Feuerstein, G.Z., Yue, T-L. and Ruffolo, R.R., Jr.: Emerging concepts in heart failure: Apoptosis in CHF. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

383. Ruffolo, R.R., Jr., Feuerstein, G.Z., Poste, G. and Ma, X.: Introduction: Rationale for use of carvedilol in hypertension, coronary artery disease and congestive heart failure. In: Carvedilol: A Multiple Action Neurohormonal Antagonist. Edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in press.

384. Feuerstein, G.Z., Shusterman, N.H., Poste, G. and Ruffolo, R.R., Jr.: β-Blockers and heart failure: Rationale and highlights fro the Carvedilol Heart Failure Trials. Drug News and Perspectives 3: 18-22, 1996.

385. Ruffolo, R.R., Jr.: Carvedilol: an α- and β-adrenoceptor antagonist that inhibits LDL oxidation. Proceedings from the International Symposium on Lipoprotein Oxidation and Atherosclerosis: Biological and Clinical Aspects, edited by G. Finardi, G. Bellomo and E. Maggi, Richelieu Press, pp. 19-23, 1996.

Publications (Continued)

386. Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol: Preclinical profile and mechanisms of action in preventing the progression of congestive heart failure. Eur. Heart J. 19 (Suppl. B): B19-B24, 1998.

387. Hieble, J.P. and Ruffolo, R.R., Jr.: Experimental and emerging drugs: α-Adrenoceptor Agonists and Antagonists. In: Receptor-Based Drug Design, edited by P. Leff, Marcel Dekker, New York, pp. 231-252, 1998.

388. Ruffolo, R.R., Jr.: The role of α- and β-adrenoceptors in cardiovascular diseases: Recent developments. In: Proceedings of Euro-Conference Symposium on the Role of Drug Receptors in Cardiovascular Disease. Edited by B. Verstig, 1996.

389. Bril, A., Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Carvedilol, a new paradigm for the treatment of congestive heart failure. Expert Opinion on Investigational Drugs 5: 1523-1529, 1996.

390. Feuerstein, G. and Ruffolo, R.R., Jr.: β-Blockers in congestive heart failure. The pharmacology of carvedilol, a vasodilating β-blocker and antioxidant and its therapeutic utility in congestive heart failure. In: Catecholamines, Bridging Basic Science with Clinical Medicine. Edited by D.S. Goldstein, G. Eisenhofer and R. McCarthy, Academic Press, London. Adv. Pharmacol. 42: 611-615, 1998.

391. Feuerstein, G.Z., Poste, G., Shusterman, N.H. and Ruffolo, R.R., Jr.: The changing paradigm for pharmacotherapy of congestive heart failure. Cardiovasc. Drugs Ther. 11: 9S-14S, 1998.

392. Ruffolo, R.R., Jr., Feuerstein, G.Z. and Ohlstein, E.H.: Recent observations with β-adrenoceptor blockade: Beneficial effects in hypertension and heart failure. Am. J. Hypertension 11 (Suppl.):9S-14S, 1998.

393. Hieble, J.P. and Ruffolo, R.R., Jr.: Pharmacotherapy of benign prostatic hypertrophy with α_1-adrenoceptor agonists. Pharmacol. Rev. Commun. 8:251-256, 1996.

394. Feuerstein, G.Z., Ruffolo, R.R., Jr. and Samanen, J.: The integrin αII β_3 (GPIIb/IIIa). A target for novel antiplatelet drugs. Pharmacol. Rev. Commun. 8: 257-265, 1996.

395. Hieble, J.P. and Ruffolo, R.R., Jr.: Recent advances in the identification of α_1- and α_2-adrenoceptor subtypes: Therapeutic implications. Expert Opinion on Investigational Drugs 6: 367-387, 1997.

396. Feuerstein, G.Z., Bril, A. and Ruffolo, R.R., Jr.: Pharmacology of β-Blockers: Protective effects of carvedilol in the myocardium. Am. J. Cardiol. 80(11A): 41L-45L, 1997.

Publications (Continued)

397. Feuerstein, G.Z., Ruffolo, R.R., Jr. and Yue, T-L.: Apoptosis and congestive heart failure. Cardiovascular Medicine 7: 249-255, 1997.

398. Feuerstein, G.Z., Shusterman, N., Ruffolo, R.R., Jr.: Carvedilol Update IV: Prevention of oxidative stress, cardiac remodeling and progression of congestive heart failure. Drugs of Today 33: 453-473, 1997.

399. Ruffolo, R.R., Jr.: Pharmacology of β-adrenoceptor blockade. Perspectives in Cardiology (Supplement, August 1997); Proceedings of the International Meeting of the Canadian Cardiac Society Symposium on Evolution of Heart Failure Management: Emerging role of β-blockers, pp. 11-14, 1997.

400. Ma, X.L., Lopez, B.L., Liu, G.L., Christopher, T.A., Gao, F., Guo, Y., Feuerstein, G.Z., Ruffolo, R.R., Jr., Barone, F.C. and Yue, T.L.: Hypercholesterolemia impairs a detoxification mechanism against peroxynitrite and renders the vascular tissue more susceptible to oxidative injury. Circ. Res. 80: 894-901, 1997.

401. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: The Pharmacology of Carvedilol. Trends Pharmacol. Sci., in press.

402. Gurbanov, K., Callanan, E., Feuerstein, G.Z., Ruffolo, R.R., Jr., Brodski, S., Hoffman, A., Haramati, A. and Winaver, J.: Effects of carvedilol on renal function in rats with experimental congestive heart failure. International J. Science Medicine, 2005.

403. Griswold, D., Ruffolo, R.R., Jr., Poste, G. and Torphy, T.J.: Prostaglandin H2 synthase isoforms and their relationship to gastrointestinal side effects. Trends in Pharmacol. Sci. 18: 311-313, 1997.

404. Yue, T-L., Ma, X-L., Wang, X., Romanic, A.M., Liu, G-L., Londen, C., Gu, J-L., Kumar, S., Poste, G., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Possible involvement of the stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion induced cardiomyocyte apoptosis by carvedilol. Circ. Res. 82: 166-174, 1998.

405. Ruffolo, R.R., Jr., Poste, G.P. and Metcalf, B.W.: Preface to the Series. In: Cell Cycle Regulation. Edited by R. Ruffolo, Jr., G. Poste and B. Metcalf, Harwood Academic Publishers, London, pp. xi-xii, 1997.

406. Ruffolo, R.R., Jr., Poste, G. and Metcalf, B.W.: Preface to the Volume. In: Cell Cycle Regulation. Edited by R. Ruffolo, Jr., G. Poste and B. Metcalf, Harwood Academic Publishers, London, pp. xiii-xiv, 1997.

407. Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Neurohormonal activation, oxygen free radicals and apoptosis in the pathogenesis of congestive heart failure. J. Cardiovasc. Pharmacol. 32 (Suppl. 1): S22-S30, 1998.

Publications (Continued)

408. Ohlstein, E.H., Brooks, D.P., Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Inhibition of sympathetic outflow by the angiotensin II receptor antagonist, eprosartan, but not by losartan, valsartan and irbesartan: Possible relationship to differences in prejunctional angiotensin II receptor blockade. Pharmacology 55: 244-251, 1997.

409. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Congestive Heart Failure and genomic medicine: A look into the 21st Century. Cardiovasc. Drugs Ther. 11: 713-717, 1997.

410. Willette, R.N., Mitchell, M.P., Ohlstein, E.H., Lukas, M.A. and Ruffolo, R.R., Jr.: Evaluation of intrinsic sympathomimetic activity of bucindolol and carvedilol in rat heart. Pharmacology 56: 30-36, 1998.

411. Ruffolo, R.R., Jr., Poste, G. and Metcalf, B.W. Cell Cycle Regulation. Edited by R.R. Ruffolo, Jr., G. Poste and B.W. Metcalf, Harwood Academic Publishers, London, pp. 1-174, 1997.

412. Feuerstein, G.Z., Liu, G.L., Yue, T.L., Cheng, H.Y., Hieble, J.P., Arch, J., Ruffolo, R.R., Jr. and Ma, X.L.: Comparison of metoprolol and carvedilol pharmacology and cardioprotection in rabbit ischemia and reperfusion model. Proceedings from the XIIIth World Congress of Cardiology, Monduzzi Editore S.p.A., Bologna, Italy, pp. 959-963, 1998.

413. Yue, T.L., Ma, X.L., Gu, J.L., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol inhibits activation of stress-activated protein kinase and reduces reperfusion injury in perfused rabbit heart. Eur. J. Pharmacol. 345: 61-65, 1998.

414. Brodsky, S., Gurbanov, K., Abassi, Z., Hoffman, A., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Winaver, J.: Effect of eprosartan on renal function and cardiac hypertrophy in rats with experimental heart failure. Hypertension 32: 746-752, 1998.

415. Gellai, M., Schreiner, G.T., Ruffolo, R.R., Jr., Fletcher, T., DeWolf, R. and Brooks, D.P.: CVT-124, a novel adenosine A1 receptor antagonist with unique diuretic activities. J. Pharmacol. Exp. Ther. 286: 1191-1196, 1998.

416. Brunvand, H., Liu, G., Ma, X.L., Yue, T.L., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: SB 211475, a metabolite of carvedilol, reduces infarct size after myocardial ischemic and reperfusion injury in rabbits. Eur. J. Pharmacol. 356: 193-198, 1998.

417. Ruffolo, R.R., Jr., Poste, G. and Metcalf, B.W.: Inflammatory cells and mediators in central nervous system diseases; Preface to the Series. In: Inflammatory Cells and Mediators in Central Nervous System Diseases. Edited by R.R. Ruffolo, Jr., G. Poste and B.W. Metcalf, Harwood Academic Publishers, London, pp. XI-XII, 1999.

Publications (Continued)

418. Edwards, R.M., Ruffolo, R.R., Jr. and Brooks, D.P.: Pharmacology of the angiotensin II receptor antagonist, eprosartan. Expert Opinion on Investigational Drugs 7: 463-469, 1998.

419. Christopher, T.A., Lopez, B.L., Ma, X.L., Feuerstein, G.Z., Ruffolo, R.R., Jr. and Yue, T.L.: Effects of a hydroxylated metabolite of the β-adrenoceptor antagonist, carvedilol, on post-ischemic splanchnic tissue injury. Br. J. Pharmacol. 123: 292-298, 1998.

420. Ruffolo, R.R., Jr.: Pharmacology of Carvedilol. Proceedings of the Canadian Cardiac Society Symposium on Congestive Heart Failure. New Advances in the Treatment of Congestive Heart Failure, pp. 9-10, 1998.

421. Humphrey, P.P.A. and Ruffolo, R.R., Jr.: The IUPHAR Compendium on Receptor Characterization and Classification: Preface. In: The IUPHAR Compendium of Receptor Characterization and Classification. Edited by P. Godfraind, P.M. Vanhoutte, R.R. Ruffolo, Jr. and P. Humphrey, IUPHAR Media, Burlington Press, Cambridge, pp. vii, 1998.

422. Hieble, J.P., Hehr, A., Li, Y.O. and Ruffolo, R.R., Jr.: Molecular basis for the stereoselective interactions of catecholamines with α-adrenoceptors. Proc. West. Pharmacol. Soc. 41: 225-228, 1998.

423. Godfraind, T., Vanhoutte, P.M., Ruffolo, R.R., Jr. and Humphrey, P.: The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media, Burlington Press, Cambridge, 267 pages, 1998.

424. Hieble, J.P., Kolpak, D.C., McCafferty, G.P., Ruffolo, R.R., Jr., Testa, R. and Leonardi, A.: Effects of α_1-adrenoceptor antagonists on agonist and tilt-induced changes in blood pressure: relationships to uroselectivity. Eur. J. Pharmacol. 373: 51-62, 1999.

425. Feuerstein, G.Z., Yue, T.L. and Ruffolo, R.R., Jr.: Novel mechanisms in the treatment of heart failure: Inhibition of oxygen radicals and apoptosis by carvedilol. Prog. Cardiovasc. Dis. 41(Suppl. 1), 17-24, 1998.

426. Gurbanov, K.G., Chandra, S., Schaffer, T., Freed, T., Sarkar, S.K., Ohlstein, E.H., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Use of serial cardiac-gated magnetic resonance imaging for analysis of cardiac function and structure in experimental congestive heart failure: studies with carvedilol. In press.

427. Brooks, D.P., Feuerstein, G.Z., Ohlstein, E.O. and Ruffolo, R.R., Jr.: Method for treating isolated systolic hypertension. United States Patent, P50667. 9.13.99.

428. Jobe, P.C., Adams-Curtis, L., Burks, T.F., Fuller, R.W., Peck, C.C., Ruffolo, R.R., Snead, O.C., Woosley, R.L.: The essential role of integrative biomedical sciences in protecting and contributing to the Health and Well Being of our Nation. The Pharmacologist 40: 32-37, 1998.

Publications (Continued)

429. Brodsky, S., Gurbanov, K., Abassi, Z., Hoffman, A., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Winaver, J.: Effects of eprosartan on renal function and cardiac hypertrophy in rats with experimental heart failure. Hypertension 32: 746-752, 1998.

430. Ruffolo, R.R., Jr. and Hieble, J.P.: Adrenoceptor Pharmacology: Urogenital Applications. Eur. Urology 36 (Suppl. 1): 17-22, 1999.

431. Brooks, D.P. and Ruffolo, R.R., Jr.: Pharmacological mechanisms of angiotensin II receptor antagonists: implication for the treatment of elevated systolic blood pressure. J. Hypertension 17 (Suppl. 2): S27-S32, 1999.

432. Bylund, D.B., Bond, R.P., Clarke, D.E., Eikenburg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffolo, R.R., Strosberg, A.D. and Trendelenburg, U.G.: Adrenoceptors. In: The IUPHAR Compendium of Receptor Characterization and Classification. Edited by T. Godfraind, P.M. Vanhoutte, R.R. Ruffolo, Jr. and P. Humphrey. Published by IUPHAR Media, Burlington Press, Cambridge, pp. 58-74, 1998.

433. Douglas, S.A., Nambi, P., Gellai, M., Luengo, J.I., Xiang, J.N., Brooks, D.P., Ruffolo, R.R., Jr., Elliott, J.D. and Ohlstein, E.O.: Pharmacologic characterization of the novel, orally available endothelin-A-selective antagonist, SB 247083. J. Cardiovasc. Pharmacol. 31: S273-S276, 1998.

434. Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Recent developments in the use of beta-blockers in the treatment of heart failure. Pharmacol. and Toxicol. 83 (Suppl. 1): 42-43, 1998.

435. Feuerstein, G., Liu, G.L., Yue, T.L., Cheng, H.Y., Hieble, J.P., Arch, J.R.S., Ruffolo, R.R., Jr. and Ma, X.L.: Comparison of metoprolol and carvedilol pharmacology and cardioprotection in rabbit ischemia and reperfusion model. Eur. J. Pharmacol. 351: 341-350, 1998.

436. Feuerstein, G.Z., Shusterman, N.H., Poste, G. and Ruffolo, R.R., Jr.: The changing paradigm for pharmacotherapy of congestive heart failure. Internat. J. Cardiovasc. Med. Sci. 1/2: 171-189, 1998.

437. Willette, R.N., Aiyar, N., Yue, T.L., Mitchell, M.P., Disa, J., Storer, B.L., Naselsky, D.P., Stadel, J.M., Ohlstein, E.O. and Ruffolo, R.R., Jr.: In vitro and in vitro characterization of intrinsic sympathomimetic activity in normal and heart failure rats. J. Pharmacol. Exp. Ther. 289: 48-53, 1999.

438. Gubanov, K.G., Schaffer, C.S., Freed, T., Ruffolo, R.R., Jr., Sarkar, S.K., Ohlstein, E.O. and Feuerstein, G.Z.: Utilization of Magnetic Resonance Imaging (MRI) for analysis of cardiac structure and function in rat: Studies with the cardioprotective drug carvedilol. J. Cardiovasc. Med. Sci., submitted.

Publications (Continued)

439. Yue, T.L., Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Antioxidant action of carvedilol. A potential role in the treatment of heart failure. Heart Failure Reviews 4: 39-51, 1999.

440. Ruffolo, R.R., Jr.: Inflammatory Cells and Mediators in CNS Diseases. Preface to the volume. In: Inflammatory Cells and Mediators in CNS Diseases, edited by, R.R. Ruffolo, Jr., G.Z. Feuerstein, A.J. Hunter, G. Poste and B.W. Metcalf. Harwood Academic Publishers, London, pp. XIII-XV, 1999.

441. Ruffolo, R.R., Jr., Feuerstein, G.Z., Hunter, A.J., Poste, G. and Metcalf, B.W.: Inflammatory Cells and Mediators in CNS Diseases. Harwood Academic Publishers, London, pp. 1-518, 1999.

442. Yue, T.L., Ohlstein, E.H. and Ruffolo, R.R., Jr.: Apoptosis: A potential target for discovering novel therapies for cardiovascular diseases. Current Opinion in Chemical Biology 3: 474-480, 1999.

443. Ruffolo, R.R., Jr.: Use of carbazole compounds for the treatment of congestive heart failure. U.S. Patent Number 5,902,821, May 11, 1999.

444. Ohlstein, E.H., Ruffolo, R.R., Jr. and Elliott, J.D.: Drug discovery in the next millennium. Ann. Rev. Pharmacol. Toxicol. 40: 177-191, 2000.

445. Ruffolo, R.R., Jr.: Apoptosis in Health and Disease; Preface to the Series. In: Apoptosis in Health and Disease, edited by R.R. Ruffolo, Jr. and F. Walsh, Harwood Academic Publishers, London, IX-X, 2000.

446. Ruffolo, R.R., Jr.: Apoptosis in Health and Disease; Preface to the Volume. In: Apoptosis in Health and Disease, edited by R.R. Ruffolo, Jr. and F. Walsh, Harwood Academic Publishers, London, XI-XII, 2000.

447. Brooks, D.P., Ohlstein, E.H. and Ruffolo, R.R., Jr.: Pharmacology of eprosartan, an angiotensin II receptor antagonist: Exploring hypotheses from clinical data. Am. Heart J. 138: S246-S251, 1999.

448. Ruffolo, R.R., Jr. and Walsh, F.: Apoptosis in Health and Disease. Harwood Academic Publishers, London, 2000 (249 pages).

449. Gao, F., Chen, J., Lopez, B.L., Christopher, T.A., Gu, J., Lysko, P., Ruffolo, R.R., Jr., Ohlstein, E.O., Ma, X.L. and Yue, T.L.: Comparison of Bisoprolol and Carvedilol cardioprotection in a rabbit ischemia and reperfusion model. Eur. J. Pharmacol. 406: 109-116, 2000.

450. Spedding, M. and Ruffolo, R.R., Jr.: Current Status of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR). In: The IUPHAR Compendium of Receptor Characterization and Classification, edited by R.R. Ruffolo and NC-IUPHAR, IUPHAR Media, London, pp. 1-8, 2000.

Publications (Continued)

451. Humphrey, P.P.A., Barnard, E.A., Bonner, T.I., Catterall, W., Dollery, C.T., Fredholm, B.B., Godfraind, T., Han, Q., Harmar, T.J., Langer, S.Z., Laudet, V., Limbird, L., Ruffolo, R.R., Jr., Spedding, M., Vanhoutte, P. and Watson, S.: The IUPHAR Receptor Code. In: The IUPHAR Compendium of Receptor Characterization and Classification, edited by R.R. Ruffolo and NC-IUPHAR, IUPHAR Media, London, pp. 10-23, 2000.

452. Ruffolo, R.R., Jr., et al.: The IUPHAR Compendium of Receptor Characterization and Classification. Edited by R.R. Ruffolo and NC-IUPHAR, IUPHAR Media, London, 396 pages, 2000.

453. Torphy, T. and Ruffolo, R.R., Jr.: Biology: The new rate-limiting step in Drug Discovery. Pharmaceutical News 8: 50-53, 2001.

454. Bylund, D.B., Bond, R.A., Bouvier, M., Clarke, D.E., Eikenburg, D.C., Hieble, J.P., Kobilka, B.K., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo, R.R., Jr. and Strosberg, A.D.: Adrenoceptor: In: The IUPHAR Compendium of Receptor Characterization and Classification, edited by R.R. Ruffolo and NC-IUPHAR, IUPHAR Media, London, pp. 88-103, 2000.

455. Humphrey, P.P.A., Spedding, M. and Ruffolo, R.R., Jr., Spedding, M. and Watson, S.P.: Preface: The Second IUPHAR Compendium on Receptor Characterization and Classification. In: The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media, London, p. vii, 2000.

456. Lysko, P.G., Webb, C.L., Gu, J-L., Ohlstein, E.O., Ruffolo, R.R., Jr. and Yue, T.L.: A comparison of carvedilol and metoprolol antioxidant activities in vitro. J. Cardiovasc. Pharmacol. 36: 277-281, 2000.

457. Bril, A., Rouanet, S., Berrebi-Bertrand, I., Ohlstein, E.H. and Ruffolo, R.R., Jr.: Anti-hypertrophic effect of Carvedilol in neonatal rat ventricular myocytes: Role of p42/44 and p38 mitogen-activated protein kinases. J. Cardiovasc. Pharmacol. Submitted for publication.

458. Naselsky, D.P., Ashton, D., Ruffolo, R.R., Jr. and Hieble, J.P.: Rabbit α_2-adrenoceptors: Both platelets and adipocytes have α_{2A}-pharmacology. J. Pharmacol. Exp. Ther. 298: 219-225, 2001.

459. Davenport, A.P., Godfraind, T., Luscher, T.F., Ohlstein, E.O., Rubanyi, G.M. and Ruffolo, R.R., Jr.: Endothelin Receptors In: The IUPHAR Compendium of Receptor Characterization and Classification. Edited by R.R. Ruffolo and NC IUPHAR, IUPHAR Media, London, pp. 182-188, 2000.

460. Hieble, J.P. and Ruffolo, R.R., Jr.: Adrenergic Receptors. In: Understanding G-Protein Coupled Receptors and their role in the Central Nervous System. Edited by M. Pangalos and C. Davies. Oxford University Press, Oxford, pp. 205-220, 2002.

Publications (Continued)

461. Spedding, M. and Ruffolo, R.R., Jr.: Receptor Closure? The effects of publishing the human genome on receptor nomenclature, pharmacology and drug discovery. Nature Pharmacology, 2002.

462. Abernethy, D.R. and Ruffolo, R.R., Jr.: International Union of Pharmacology Nomenclature and Pharmacological Reviews. Pharmacological Reviews 54: 159, 2002.

463. Redmond, C.M. and Ruffolo, R.R., Jr.: Building Brands through R&D and Commercial Collaboration. Pharmaceutical Executive, September, 2002, p. 46.

464. Ruffolo, R.R., Jr. and Spedding, M.: The International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. In: The IUPHAR Compendium of Voltage Gated Ion Channels. Edited by W.A. Catterall, K.G. Chandy and G.A. Gutman, IUPHAR Media, London, pp. 1-7, 2002.

465. Ruffolo, R.R., Jr. and Boath, D.: Re-engineering R&D: Wyeth's Challenge. Scrip, November 5, 2003, 2899.

466. Ruffolo, R.R., Jr.: Research and Development Productivity: A New Way of Working at Wyeth. American Pharmaceutical Review 7: 2-4, 2004.

467. Ruffolo, R.R., Jr.: World Health is Headed for a Day of Reckoning. In preparation.

468. Ruffolo, R.R., Jr.: Use of an mTOR inhibitor in treatment of uterine leiomyoma. U.S. Patent # 7,528,145, 8 pages, 2009.

469. Ruffolo, R.R., Jr. and Feuerstein, G.Z.: Carvedilol: Historical case study of the first beta-adrenoceptor blocker (β-blocker) approved for treatment of heart failure. In: Comprehensive Medicinal Chemistry II. Edited by D.J. Triggle, J.B. Taylor, Elsevier Ltd., Oxford, pp. 137-147, 2006.

470. Bowery, N., Headley, E. and Ruffolo, R.R., Jr.: Happy 5th Birthday Current Opinion in Pharmacology. Curr. Opin. Pharmacol. 5: 1-4, 2006.

471. Feuerstein, G.Z. and Ruffolo, R.R. Jr.: Translational Medicine. In: Handbook of Translational Medicine, Birkhauser Verlag, Basel. In press.

472. Gurbanov, K., Callanan, E., Feuerstein, G.Z., Ruffolo, R.R., Jr., Brodsky, S., Hoffman, A., Haramati, A. and Winaver, J.: Effects of carvedilol and renal function in rats with experimental congestive heart failure. Intl. J. Cardiovasc. Med. and Sci. 5: 61-67, 2005.

473. Ruffolo, R.R., Jr.: The Rhythm of Drug Development. Drug Discovery Today 9: 631-635, 2004.

Publications (Continued)

474. Gurbanov, K.G., Chandra, S., Strittmatter, R., Schaeffer, T., Freed, T., Ohlstein, E.O., Ruffolo, R.R., Jr., Feuerstein, G.Z. and Sarkar, S.K.: Serial characterization of an experimental model of cardiac dysfunction using magnetic resonance imaging. Intl. J. Cardiovasc. Med. Sci. 5: 97-100, 2005.

475. Ruffolo, R.R. Jr.: Why has R&D productivity declined in the pharmaceutical industry? Expert Opin. Drug Discov. 1: 99-102, 2006.

476. Ruffolo, R.R. Jr. and Feuerstein, G.Z.: Carvedilol Case History. The discovery and development of the first β-blocker for the treatment of congestive heart failure. Expert Opin. Drug Discov. 1: 85-89, 2006.

477. Feuerstein, G.Z., Ruffolo, R.R., Coughlin, C., Wang, J. and Miller, J.: Inflammation. In: Encyclopedia of Stress, 2nd Edition, Editor-in-Chief, George Fink, Academic Press (San Diego), pp. 530-539, 2007.

478. Feuerstein, G.Z., Ruffolo, R.R. and David, B.N.: Viruses and Stress. In: Encyclopedia of Stress, 2nd Edition, Editor-in-Chief, George Fink, Academic Press (San Diego), pp. 850-853, 2007.

479. Feuerstein, G.Z., Ruffolo, R.R. and Rutkowski, J.L.: Neuroinflammation. In: Encyclopedia of Stress, 2nd Edition, Editor-in-Chief, George Fink, Academic Press (San Diego), pp. 889-894, 2007.

480. Feuerstein, G.Z., Rutkowski, J.L., Walsh, F.L. and Ruffolo, R.R. Jr.: The role of translational medicine and biomarker research in drug discovery and development. Am. Drug Disc. 2: 23-28, 2007.

481. Ruffolo, R.R. Jr., Walsh, F.W. and Feuerstein, G.Z.: Drug Discovery. In: Pharmacology. Ed. by H. Majewski, UNESCO EOLSS Publishers, Paris, in press.

482. Feuerstein, G.Z., Zaleska, M.M., Krams, M., Wang, X., Day, M., Rutkowski, J.L., Finkelstein, S.P., Pangalos, M.N., Poole, M., Stiles, G.L., Ruffolo, R.R. Jr. and Walsh, F.L.: Missing Steps in the STAIR Case: A Translational Medicine Perspective on the Development of NXX-059 for Treatment of Acute Ischemic Stroke. J. Cerebral Blood Flow and Metab. In press.

483. Feuerstein, G.Z., Dormer, C., Ruffolo, R.R., Jr., Stiles, G., Walsh, F.W. and Rutkowski, J.L.: Translational medicine perspectives of biomarkers in drug discovery and development. Part I: Target selection and validation-Biomarkers take center stage. In: Training and Education in Translational Medicine. In press.

Publications (Continued)

484. Brooks, D., Feuerstein, G, Ohlstein, E.H. and Ruffolo, R. R, Jr.: Method of Treating Isolated Systolic Hypertension. U. S. Patent Application No. 20070129415 Issued June 7, 2007

485. Brooks, D., Feuerstein, G., Ohlstein, E.O. and Ruffolo, R. R., Jr: Method of Treating Isolated Systolic Hypertension. U. S. Patent Application No. 20050113431, Issued May 26, 2004.

486. Lukas-Laskey, Ruffolo, R. R., Jr., Shusterman, N. H., Sponer, G. and Strein, K.: Method of Treatment for Decreasing Mortality Resulting from Congestive Heart Failure. U. S Patent Application No. 20030105138, Issued June 5, 2003,

487. Feuerstein, G.Z. and Ruffolo, R.R. Jr.: Discontinued drugs in 2006: Cardiovascular Drugs Translational Medicine Perspective. Expert Opin. Investig. Drugs 16: 1315-1326, 2007.

488. Feuerstein, G.Z., Gill, D., Dormer, C., Ruffolo, R.R. Jr., Rutkowski, L., Walsh, F.S. and Hurko, O.: Translational Medicine Perspectives in Drug Discovery and Development. Part II: Target compound interaction - The vastly neglected biomarkers contributing to early clinical development failure. American Drug Discovery 3: 48-53, 2008.

489. Feuerstein, G.Z. and Ruffolo, R.R., Jr.: Cardiogenic Shock. In: Encyclopedia of Molecular Mechanisms of Disease, Springer-Verlag, Berlin, 2009

490. Feuerstein, G.Z., Caughlin, C., Ruffolo, R.R., Jr., Dormer, C., Walsh, F.W., Hurko, O. and Rutkowski, J.L. Translational Medicine Perspectives in Drug Discovery and Development. Part III: Disease biomarkers-setting the lexicon straight for drug registration, drug labeling and commercial opportunities. American Drug Discovery, in press.

491. Feuerstein, G.Z. and Ruffolo, R.R, Jr. Hypovolemic Shock. In: Encyclopedia of Molecular Mechanisms of Disease, Springer-Verlag, Berlin, pp. 1011-1012, 2009.

492. Feuerstein, G.Z., Keith, J.C. and Ruffolo, R.R., Jr. Septic Shock. In: Encyclopedia of Molecular Mechanisms of Disease, Springer Verlag, Berlin, p 1920, 2009.

493. Feuerstein, G.Z., Walsh, F.S., Ruffolo, R.R., Jr., Goddard, C., Rutkowski, J.L., Parsons, S., Coughlin, C. Wan, H.I., Alesci, S., Lee, J.H., Wolfgang, K.W. and Smith, M.F.Jr. Translational medicine perspectives in drug discovery and development-Part IV: Surrogate diseases pave the way for successful proof of concept studies towards investment decisions in large morbidity and mortality outcome studies in major diseases. American Drug Discovery 2/4: 36-41, 2008.

Publications (Continued)

494. Lukas-Laskey, Ruffolo, R. R., Jr., Shusterman, N. H., Sponer, G. and Strein, K.: Method of Treatment for Decreasing Mortality Resulting from Congestive Heart Failure. U. S. Patent No. 5760069, Issued June 2, 1998.

495. Lukas-Laskey, Ruffolo, R. R., Jr., Shusterman, N. H., Sponer, G and Strein, K: Method of Treatment for Decreasing Mortality Resulting from Congestive Heart Failure. U. S. Patent. No. RE400000, Issued January 8, 2008.

496. Lukas-Laskey, Ruffolo, R. R., Jr., Shusterman, N. H., Sponer, G and Strein, K.: Use of Carbazole Compounds for the Treatment of Congestive Heart Failure. U. S. Patent. No. RE40707, Issued May 5, 2009.

497. Feuerstein, G.Z., Alesci, S., Walsh, F.W., Rutkowski, J.L. and Ruffolo, R.R., Jr.: Translational Medicine - A Paradigm Shift in Modern Drug Discovery and Development: The Role of Biomarkers. In: Biomarkers in Drug Development: A Handbood of Practice, Application and Strategy, Ed. By M. R. Bleavins, C. Carini, M. Jurimar-Romet and Ramin Rahbari, John Wiley and Sons, pp. 361-373, 2010.

Abstracts

Over 200 Abstracts Published

Trials@uspto.gov
571-272-7822

Paper 79
Date: June 24, 2014

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE SCOTTS COMPANY LLC
Petitioner

v.

ENCAP, LLC
Patent Owner

Case IPR2013-00110
Patent 6,209,259

Before MICHAEL P. TIERNEY, LORA M. GREEN, and RAMA G. ELLURU, *Administrative Patent Judges.*[1]

PER CURIAM.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

---

[1] Floyd, Administrative Patent Judge, who participated in the oral hearing held on January 30, 2014, has left the Board; accordingly, Tierney, Administrative Patent Judge, has been added to the panel.

I. BACKGROUND

Petitioner, The Scotts Company LLC ("Scotts Company"), filed a Petition on January 10, 2013, for an *inter partes* review of claims 1-5, 7-11, 13, and 14 ("the challenged claims") of U.S. Patent No. 6,209,259 ("the '259 patent") pursuant to 35 U.S.C. §§ 311-319. Paper 2. On April 15, 2013, Patent Owner, Encap, LLC ("Encap"), filed a Preliminary Response. Paper 9. On July 3, 2013, the Board granted an *inter partes* review for all challenged claims on less than all of the grounds of unpatentability alleged in the Petition. Paper 12, ("Dec."). The Board also stayed concurrent reexamination of the '259 patent. Paper 10.

After institution of trial, Encap filed a Corrected Patent Owner's Response. Paper 48. Encap also filed a Corrected Contingent Motion to Amend Claims that requests substituting proposed new claims 15-24 for claims 2-5, 8-11, 13, and 14, respectively—contingent upon a determination of unpatentability. Paper 47. Scotts Company filed a Reply to Patent Owner's Response (Paper 30), and an Opposition to Encap's Motion to Amend Claims (Paper 33). Encap then filed a Corrected Reply to Scotts Company's Opposition to Encap's Motion to Amend Claims. Paper 49.

Additionally, Scotts Company filed a Motion to Exclude Evidence (Paper 52), to which Encap responded (Paper 64) and submitted supplemental evidence (Paper 58). Scotts Company filed a Reply in further support of its Motion to Exclude. Paper 68.

Encap also filed a Motion to Exclude Evidence (Paper 54) to which Scotts Company responded (Paper 60). Encap, with authorization (Paper 70), filed a Supplement to its Motion to Exclude (Paper 66), as well as a Reply (Paper 67).

Oral hearing was held on January 30, 2014.[2]

The Board has jurisdiction under 35 U.S.C. § 6(c). This Final Written Decision is issued pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73.

Scotts Company has shown by a preponderance of the evidence that claims 1-5, 7-11, 13, and 14 of the '259 patent are unpatentable. Encap's Motion to Amend Claims is denied.

A. The '259 Patent

The '259 patent is directed to a combination seed capsule, comprising at least one viable seed, a coating of a composition comprising a soil conditioning material mounted proximate and disposed outwardly of the outer surface of the seed, and optionally including one or more of inorganic chemical fertilizers, growth enhancer, binder, and/or an anti-fungal agent. Ex. 1001, Abstract, 4:5-11. According to the '259 patent Specification, the primary object of the invention is to "provide solid plant seed capsule products that supply both soil conditioning properties and the seed, which can benefit from such conditioned soil, in a given seed capsule particle." *Id.* at 3:28-31.

B. Illustrative Claim

Claims 1 and 7 are the only independent claims in the '259 patent, and are directed to a "[a] combination seed capsule." The only difference between these claims is that claim 7 additionally states that the seed coating is applied by an agglomeration process. The remaining challenged claims depend from either claim 1 or 7. Claim 1 is illustrative of the claimed subject matter, and is reproduced below.

---

[2] A transcript of the oral hearing is included in the record as Paper 78.

1. A combination seed capsule comprising:

one viable seed;

said seed acting as a core or pseudo core of said combination seed capsule;

a coating of a composition comprising soil conditioning materials;

said soil conditioning materials being in a solid state at time of coating.

C. Prior Art Supporting the Instituted Challenges

Name	Reference	Issue or Publication	Exhibit
Schreiber	U.S. Patent No. 3,698,133	Oct. 17, 1972	Ex. 1002
Roth	U.S. Patent No. 4,065,287	Dec. 27, 1977	Ex. 1003
Lowe	U.S. Patent No. 5,019,564	May 28, 1991	Ex. 1004
Matthews	GB670,461	Apr. 16, 1952	Ex. 1007

D. The Instituted Challenges of Unpatentability

References	Grounds	Claims
Schreiber	§ 102(b)	Claims 1, 7, and 13
Schreiber and Roth	§ 103(a)	Claims 2, 5, 8, 11, and 14
Schreiber and Lowe	§ 103(a)	Claims 3, 4, 9, and 10
Matthews	§ 102(b)	Claims 1, 2, 7, 8, 13, and 14
Roth	§ 102(b)	Claims 1, 2, 5, 7, 8, 11, 13, and 14
Roth and Lowe	§ 103(a)	Claims 1-5, 7-11, 13, and 14

II. DISCUSSION

A. Evidentiary Matters

1. Scotts Company's Reply (Paper 30)

In a conference call held on December 3, 2013, Encap asserted that Scotts Company had raised new arguments and evidence in its Reply to Patent Owner's Response to Decision to Institute. Order (Paper 37), 2. The Board denied Encap's request to file a surreply, or to enlarge the page limit of Encap's Reply in support of its Motion to Amend. *Id.* We indicated, however, that we would determine whether Scotts Company's Reply and supporting evidence contain material exceeding the proper scope of a reply. *Id.*

We find that Scotts Company's Reply, and in particular, the supporting Declarations of Mr. Fredrick Sundstrom (Ex. 1039) and Mr. Krishna Pagilla (Ex. 1040) contain material outside the proper scope of a reply. 37 C.F.R. § 42.23(b) (reply is limited to arguments raised in Patent Owner's Response). Specifically, both Declarations contain materials in support of Scotts Company's Petition, and therefore, untimely filed. For example, Mr. Sundstrom includes analyses of claim construction (e.g., Ex. 1039 ¶¶ 7-9), as well as analyses of the Schreiber (e.g., *id.* at ¶¶ 10-13), Matthews (e.g., *id.* at ¶¶ 28, 29), Roth (e.g., *id.* at ¶ 34), Simmons (*id.* at ¶¶ 36, 38), and Evans (*id.* at ¶¶ 43, 44, 46, 48) references. Likewise, Mr. Pagilla addresses claim construction, as well as the references upon which Scotts Company sought institution. *See, e.g.*, Ex. 1040 ¶¶ 9-13, 23-27, 32, 33, 36-38. Specifically, we hold that the new evidence could have been included with the motion. By waiting to serve this evidence on Encap in Scotts Company's Reply, Encap was denied the opportunity to file responsive evidence. Thus, we

have not considered the untimely Declarations of Mr. Sundstrom and Mr. Pagilla, nor the arguments based thereon.[3]

2. Scotts Company's Motion to Exclude

Scotts Company filed a Motion to exclude: portions of the deposition testimony of Mr. Michael Krysiak taken by Encap on November 6, 2013 (Ex. 2002) and December 23, 2013 (Ex. 1038); and the Second Krysiak Declaration, which includes Attachments A and B (Ex. 2016). Pet. Mot. Excl. (Paper 52), 1. Mr. Krysiak, Encap's witness, submitted a second Declaration (Ex. 2012) in support of its Reply to Petitioner's Opposition to Encap's Motion to Amend (Paper 49). Encap responded to Scotts Company's Motion to Exclude and filed supplemental evidence. PO Resp. Mot. Excl. (Paper 64); PO Supp. Evid. (Paper 58), respectively. Scotts Company filed a Reply. Paper 68. We grant-in-part Scotts Company's Motion to Exclude Evidence.

Scotts Company asserts that Mr. Krysiak's deposition testimony in response to two questions (i.e., Ex. 2002, 207, l. 9; Ex. 1038, 209, ll. 7-8) should be excluded. Pet. Mot. Excl. 9-10. As we did not rely upon this deposition testimony that Scotts Company seeks to exclude, Scotts Company's Motion is moot with respect to such testimony.

Scotts Company also moves to exclude the Second Declaration of Mr. Krysiak (Ex. 2012). Scotts Company's primary objection is that the Declaration is untimely, as it should have been submitted with Encap's Motion to

---

[3] The fact that two declarations may contain some material appropriate for a response does not require our consideration of them, as the Board will not attempt to sort the proper from the improper portions. *See Office Patent Trial Practice Guide*, 77 Fed. Reg. 48,756, 48,767 (Aug. 14, 2012).

Amend (Paper 47). Pet. Mot. Excl., 11-14; *see* 37 C.F.R. § 42.23(b) ("All arguments for the relief requested in a motion must be made in the motion. A reply may only respond to arguments raised in the corresponding opposition or patent owner response."). In support of Scotts Company's Opposition to Encap's Motion to Amend (Paper 33), it relied upon the Declaration of Mr. Sundstrom (Ex. 1039), which was not considered, as discussed above. Encap asserts that Mr. Krysiak's Second Declaration is in rebuttal to Declarations and deposition testimony of Mr. Sundstrom and Mr. Pagilla. PO Resp. Mot. Excl. 10-11. Encap proffers supplemental evidence—a revised Second Declaration of Mr. Krysiak with citations to the Declaration and deposition of Mr. Sundstrom. Paper 58; Ex. 2016.

Reading Mr. Krysiak's Second Declaration, it is clear that the majority of the Declaration is in support of Encap's Motion to Amend rather than in rebuttal to Scotts Company's Opposition to Encap's Motion to Amnd or the Declarations and deposition testimony[4] of Mr. Sundstrom and Mr. Pagilla, and is thus, untimely. For example, paragraphs 2-3 relate to written description and claim construction, which Encap has the burden of proving in its Motion to Amend. Additionally, paragraphs 6-12 describe the background of the technology, which could have been submitted with Encap's Motion to Amend opening brief, and thus, are not in rebuttal to testimony from Mr. Sundstrom or Mr. Pagilla. Likewise, paragraphs 25-53 and Schedule A attempt to distinguish over Matthews and Schreiber, which Encap should have done in Patent Owner's Motion to Amend. Furthermore, to the extent that portions of Mr. Krysiak's Second Declaration are in response to the

[4] While not addressed, we do not suggest that filing a declaration in rebuttal to deposition testimony is appropriate.

Declarations of Mr. Sundstrom and Mr. Pagilla, which were excluded, they should likewise be excluded. Those errors were not corrected in the Supplemental Evidence (i.e., Ex. 2016) submitted by Encap.

In addition, Encap attempts to incorporate Mr. Krysiak's Second Declaration into its Reply to Scott's Opposition to the Motion to Amend by merely stating, "The proposed claims define over the prior art succinctly. *Id.* [Mr. Krysiak's Second Declaration] at ¶¶ 14-53." Reply Mot. Amend 5. In our Order of August 27, 2013, we admonished Encap to refrain from attempting to use an expert declaration in such fashion. We stated, "Encap's motion to amend may be supported by an expert declaration, but that the motion itself should set forth the arguments and explanations with appropriate pinpoint citations to the expert declaration, rather than incorporating by reference the expert declaration." Paper 17, 2-3. Thus, Scotts Company's Motion to Exclude Mr. Krysiak's Second Declaration (Ex. 2012) is granted, as Mr. Krysiak's Corrected Second Declaration (Ex. 2016) did not remedy the issues, it is not considered.

3. Encap's Motion to Exclude

Encap moves to exclude the Declaration of Mr. Sundstrom (Ex. 2014), Scott Company's witness who provided a declaration in support of Scott Company's Reply to Patent Owner's Response to Decision to Institute (Paper 30), on the basis that the declarant refused to answer certain questions during his deposition on the basis of confidentiality, even though a protective order was in place. PO Mot. Excl. (Paper 54), 5. Having found that Mr. Sundstrom's Declaration was untimely submitted, and thus, not considered, Encap's Motion to Exclude is dismissed as moot.

B. Claim Construction

Consistent with the statute and the legislative history of the AIA, the Board interprets claims by applying the broadest reasonable construction in the context of the specification in which the claims reside. 37 C.F.R. § 42.100(b); *see Office Patent Trial Practice Guide*, 77 Fed. Reg. 48,756, 48,766 (Aug. 14, 2012). Claim terms also are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art in the context of the entire disclosure. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007).

Two exceptions to the general rule that a claim term is given its ordinary meaning are: 1) when a patentee sets out a definition and acts as his own lexicographer; or 2) when the patentee disavows the full scope of a claim term either in the specification or during prosecution. *See In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994). If an inventor acts as his or her own lexicographer, the definition must be set forth in the specification with reasonable clarity, deliberateness, and precision. *Id.*

1. "soil conditioning materials"

All of the challenged claims require "a coating of a composition comprising soil conditioning materials." The '259 patent Specification states that "*all soil conditioning materials contemplated herein* beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients." Ex. 1001, col. 8, ll. 41-44 (emphasis added). The Specification further provides specific examples of soil conditioning materials, such as municipal or other sewage sludge, paper mill sludge, fly ash, and dust. *Id.* at col. 7, ll. 21-23. Accordingly, in the Decision to Institute, the Board construed "soil conditioning materials" as "materials that beneficially modify soil

to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients, including for example, municipal or other sewage sludge, paper mill sludge, fly ash, and dust." Dec. 6-7.

Although Scotts Company agrees with the Board's preliminary construction (Pet. Reply, 1-2), Encap asserts the construction is overly broad in view of the Specification (PO Resp., 8-9). Specifically, Encap asserts the construction should be amended to include that the soil conditioner not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated irrespective of the general tilth condition of the growth medium. *Id.* (citing Ex. 1001, col. 8, ll. 42-52,[5] Abstract). Encap does not assert that its construction is the plain and ordinary meaning of "soil conditioning materials," but rather, that the Specification defines the phrase. PO Resp. at 8. Specifically, Encap asserts the following portion of the Specification defines "soil conditioning materials:"

> However, all soil conditioning materials contemplated herein beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients. By use of soil conditioner in intimate association with the seed, this invention not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated, and in intimate association with the seed, irrespective of the general tilth condition of the growth medium into or onto which the seed capsule is applied.

Ex. 1001, col. 8, ll. 42-52.

Through the inclusion of "all soil conditioning materials contemplated herein," the first sentence requires the soil conditioning material to beneficially

---

[5] Encap mistakenly refers to col. 15, l. 29–col. 16, l. 6.

modify the soil in some way, other than directly providing plant nutrients. The second sentence is an observation of benefits provided by "this invention;" it does not *require* the invention provide the observed benefits; much less require *just* the soil conditioning material of the invention provide such benefits.

Encap relies upon its experts, Mr. John Katers, Mr. Daniel Madigan, and Mr. Michael Krysiak, all of whom provide identical claim constructions, in support of its position. Ex. 2007 ¶ 11; Ex. 1020 ¶ 10; Ex. 1022 ¶ 13. The experts provide, however, no credible analysis in support of their claim constructions, and thus, are unpersuasive.

Encap asserts also that the examples included in the Board's preliminary claim construction should be omitted, because not *all* municipal or other sewage sludge, paper mill sludge, fly ash, or dust, necessarily modify the soil in a beneficial manner. PO Resp. 9. The Board's preliminary construction, however, requires the soil conditioning materials "modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients." The inclusion of the examples is intended to clarify, not modify, this requirement.

Accordingly, the Board maintains its construction of "soil conditioning materials" as:

> Materials that beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients, including for example, municipal or other sewage sludge, paper mill sludge, fly ash, and dust.

2. "combination seed capsule"

The phrase "combination seed capsule" appears in the preamble of claims 1 and 7. Encap asserts that the Abstract of the '259 patent defines "combination seed capsule." PO Resp. 10-11. The Abstract reads:

> This invention pertains to combination seed capsules wherein each seed capsule includes both moieties of at least one soil conditioner and at least one seed, and optionally, one or more inorganic chemical fertilizer, growth enhancer, binder, and/or anti-fungal agent. The combination seed capsules are made by physically combining the respective soil conditioner and seed with one other, in the absence of any requirement for chemical reactions in the process of so combining the respective materials. The combination seed capsules *provide cooperative and beneficial effects of the soil conditioner* and the optional inorganic fertilizer, *working together in controlled intimate relation with the seed, to enhance the germination and growth processes of the seed, and the plant emergent therefrom, greater than when the soil conditioner and seed*, and optionally inorganic chemical fertilizer, *are applied to the soil separately; the improvement being a result of the intimate relationship of the respective materials in the combination seed capsule, whereby the respective materials cooperate with each other in support of germination and plant growth*.

Ex. 1001, Abstract (emphases added). Encap asserts that the text that has been italicized is the definition of a "combination seed capsule." PO Resp. 11. Encap also relies upon its technical experts, Messrs. Baker, Madigan, and Krysiak. *Id.* at 11-12. The experts, however, provide no credible analysis in support of their claim constructions and are thus, unpersuasive.

Scotts Company asserts that the term "combination seed capsule" appears in the preamble of both independent claims (i.e., claims 1 and 7), and thus, is not limiting. Pet. Reply 2. Scotts Company also asserts that in 1998, when the application that matured into the '259 patent was filed, the rules prohibited relying

on the Abstract "for interpreting the scope of the claims." *Id.* at 3 (quoting 37 C.F.R. § 1.72(b)). Lastly, Scotts Company asserts that Encap is attempting to improperly import limitations into the claims. *Id.*

First, the Abstract does not provide a definition for a "combination seed capsule," but rather observes the benefits of the combination seed capsule. Second, the preamble term "combination seed capsule" is not limiting because the claim body describes a structurally complete invention. *Catalina Mktg. Int'l v. Coolsavings.com Inc.*, 62 USPQ2d 1781, 1785 (Fed. Cir. 2002). Thus, we need not construe "combination seed capsule," as it does not limit the claim.

3. "being in a solid state at time of coating"

Independent claim 1 recites, "being in a solid state at time of coating." Similarly, independent claim 7 recites, "are in a solid state at time of coating." Additionally, claim 7 recites, "said coating being applied to said viable seed by an agglomeration operation." Due to the inclusion of these three limitations, claims 1 and 7 were determined to be product-by-process claims in the Decision to Institute. Dec. 7-8.

Encap asserts that "in a solid state at time of coating" should be construed as "solid material in the form of particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers." PO Resp. 12-13 (citing Ex. 1001, col. 8, ll. 1-5[6]). Scotts Company points out that the Specification reads, the soil conditioning raw material "*may* be a particulate powder, or *may* be fibrous, or *may* be a suspension of a powder or fibrous material in a liquid carrier, and is preferably coated onto the substrate seed

[6] Encap erroneously cites to col. 14, ll. 24-28.

to form a seed capsule or other agglomeration of particles, fibers, *or the like*," and thus, does not support Encap's construction. Pet. Reply 3 (quoting Ex. 1001, col. 8, ll. 1-5 with emphasis added). We agree that the Specification does not support Encap's proposed construction.

Encap further asserts that during prosecution of the '259 patent application, Mr. Krysiak had discussions with the Examiner relating to "being in a solid state at the time of coating." PO Resp. 12 (citing Ex. 1022 ¶ 15). Encap's description of events does not provide support for its proposed claim construction. That is, it does not follow that adding the limitation to overcome Roth, defines the limitation to require "solid material in the form of particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers." As before, Mr. Krysiak's opinion as to how the phrase should be construed includes no analysis, and thus, is unpersuasive.

Encap does establish that it disavowed claim scope, however, by adding the limitation "in a solid state at time of coating" to overcome Roth. That clear and unambiguous disavowal of claim scope causes us to modify the claim construction from that set forth in the Decision to Institute. Specifically, Encap narrowed the "in a solid state at time of coating" limitation to require the soil conditioning material be in a solid state at the time of coating the seed. Encap did not narrow "in a solid state at time of coating," however, to further require a particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers, as suggested by Encap.

The Federal Circuit has addressed the issue of determining whether a claim has been narrowed in the related context of prosecution history estoppel.

> In order to give due deference to public notice considerations under the *Warner–Jenkinson* framework, a patent holder seeking to establish

> the reason for an amendment must base his arguments solely upon the public record of the patent's prosecution, i.e., the patent's prosecution history. To hold otherwise—that is, to allow a patent holder to rely on evidence not in the public record to establish a reason for an amendment—would undermine the public notice function of the patent record.

Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., 234 F.3d 558, 586 (Fed. Cir. 2000), *vacated on other grounds*, 535 U.S. 722 (2002).

An examination of the prosecution history of record reveals the following events which support our determination that Encap clearly disavowed the full scope of claims 1 and 7. On May 10, 2000, the Examiner issued a rejection to claim 77 as anticipated by Roth, and further rejected claims 77 and 85 as being obvious in view of Roth in combination with two other references. Ex. 1008, 171, 175.[7] On August 8, 2000, the Examiner issued an interview summary, which indicates that a proposed claim amendment was discussed. Specifically, the Examiner stated that adding, "wherein said soil conditioning material, when added to the seed, are in a dry, solid form," to the claims would overcome Roth. The Examiner suggested "that the claims be written in a product by process form to clearly distinguish over Roth." *Id.* at 203. On September 8, 2000, the Examiner issued an Interview Summary indicating that claims 77 and 85 were discussed, and that "[b]ased on the proposed draft amendment and arguments recited therein, the prior art was overcome." *Id.* at 204. The record clearly shows that the only amendment made to claim 77 was the addition of the limitation, "said soil conditioning materials being in a solid state at time of coating." *Id.* at 200. Claim 85 was amended in similar fashion to recite, "wherein said soil conditioning

[7] Claims 77 and 85, ultimately issued as claims 1 and 7, respectively.

materials are in a solid state at time of coating." *Id.* at 201. Claims 77 and 85 ultimately issued as claims 1 and 7, respectively.

Thus, Encap successfully overcame Roth by adding the "in a solid state at the time of coating" limitation to claims 1 and 7. Construing the phrase as a product-by-process limitation would not result in distinguishing over Roth, as no discussion was had, nor evidence provided, to suggest the end product of Roth had different characteristics than the claimed composition. The disavowal of claim scope is clear. The limitation "in a product by process form," therefore, must be construed to require the soil conditioning material be in a solid state at the time of coating. *See Tempo Lighting, Inc. v. Tivoli, LLC*, 742 F.3d 973, 978 (Fed. Cir. 2014).

Furthermore, Roth discloses a spray application of a MAS material that contains 0.1% to 2.5% solids at the time of coating. Ex. 1003, col. 3, ll. 50-51. Thus, the limitation "in a solid state at the time of coating" must further be construed to require more than 2.5% solids. Therefore, we construe "in a solid state at the time of coating" to mean that more than 2.5% of the soil conditioning material must be in a solid state at the time of coating the seed.

4. "agglomeration operation"

Independent claim 7 requires an "agglomeration operation," which we construed in our Decision to Institute to be a product-by-process limitation. Dec. 8. Patent Owner concedes that claim 7 is a product-by-process claim. PO Resp. 16. Patent Owner, however, takes issue with the Board's "holding" that an agglomeration operation means using water and heat to bind a plurality of particles. *Id.* at 13.

We did not construe "agglomeration operation," other than to note that it is a product-by-process limitation. *In re Thorpe*, 777 F.2d 695, 698 (Fed. Cir. 1985). The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art. *See, e.g.*, *In re Garnero*, 412 F.2d 276, 279 (CCPA 1969). That is especially true where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. *Id.* Thus, the issue is not focused on what "agglomeration operation" means, but rather on what properties would be embodied in a product made by an agglomeration operation (i.e., an agglomerate). Here, the parties are in near agreement on the properties of an agglomerate.

Encap states that an agglomerate is an assemblage of particles adhering to each other, and thus, a magnified image of an agglomerate would reveal that the product is comprised of particulate. PO Resp. 13-16. Without credible explanation, Encap in its conclusion limits its final description of an agglomerate to an assemblage of *fine* particles. *Id.* at 16. Evidence cited by Encap that may support this additional limitation is an article by Wolfgang B. Pietsch, titled "The Agglomerative Behavior of Fine Particles." *Id.* at 13-14 (citing Ex. 1020 ¶ 11, Attachment A). As the title suggests, however, the article is specifically directed to agglomerates of fine particles. There is no credible suggestion in Mr. Madigan's Declaration (Ex. 1020) that an "agglomerate" is limited to fine particles. *See* Ex. 1020 ¶¶ 11-17.

Scotts Company appears to accept Encap's description of an agglomerate, but takes exception, as we do, with the limitation to fine particles. Pet. Reply 3-4.

Thus, we determine that an agglomerate is an assemblage of particles adhering to each other. The "agglomeration operation" limitation of claim 7 implies that the claimed "combination seed capsule" has a coating of a composition comprising soil conditioning materials comprised of particulate. As such, to satisfy the limitation of an "agglomeration operation," a reference must disclose a product with the structural limitation of being comprised of particulate, irrespective of the process used to make the product.

C. Anticipation by Roth—Claims 1, 2, 5, 7, 8, 11, 13, and 14

Roth explains that the MAS coating is "solid" after application. Roth, however, does not disclose the soil conditioning materials "being in a solid state at time of coating," because Roth discloses a spray application of a MAS material that is 97.5% to 99.9% liquid with the remainder "solids content." PO Resp. 31-32 (citing Ex. 1003, col. 3, ll. 50-51). While a tiny amount (i.e., 0.1% to 2.5%) of the soil conditioning material is in solid state at the time of coating, as discussed above, this is not enough to satisfy the limitation "in a solid state at time of coating," recited in claims 1 and 7. As such, Scotts Company has not shown, by a preponderance of the evidence, that Roth anticipates 1, 2, 5, 7, 8, 11, 13, and 14.

D. Obviousness over Roth and Lowe—Claims 1-5, 7-11, 13, and 14

Roth teaches the claimed "seed acting as a core or pseudo core" with a "coating of a composition comprising soil conditioning materials," as required by claims 1 and 7. Specifically, Roth describes coating seeds with a methanol treated "sludge" carrier having one or more agricultural chemicals dispersed therein, wherein the source material is "municipal sewage," as required by dependent claims 2, 5, 8, and 11. *See, e.g.*, Ex. 1003, col. 3, ll. 23-26. Roth also discloses that its coating may include a "binder," e.g., polyvinyl alcohol, starch derivatives,

and further may include a fertilizer, as recited in claims 13 and 14. *Id.* at col. 2, ll. 3-5, 48-51; col. 5, ll. 49-52. Thus, we determine that Roth discloses all the limitations of claims 1, 2, 5, 7, 8, 11, 13, and 14 with the exception of "in a solid state at time of coating," as required by independent claims 1 and 7.

Lowe discloses coating a seed with de-inked paper sludge having a "fiber content of the solids in the mixture should exceed at least 10%-15% by weight," thereby teaching "in a solid state at time of coating." Ex. 1004, col. 3, ll. 17-21. Lowe also discloses using "agglomeration" to combine the fibers to form individual granules. *Id.* at Abstract; col. 3, ll. 21-22. Thus, as discussed in greater detail below, Lowe in combination with Roth satisfies the limitations of independent claims 1 and 7 as the combination involves the use of known components for their known purpose to achieve a predictable result.

Lowe further teaches coating a seed with a material that is a byproduct of a "paper making process," and specifically that the byproduct is "paper sludge," as required by dependent claims 3, 4, 9, and 10. Lowe describes an agricultural granule for carrying and releasing agricultural chemicals that resembles a clay-based granule. *Id.* at Abstract. The agricultural granule is made from using waste materials from paper manufacture, referred to as paper sludge. *Id.* at col. 1, l. 68–col. 2, l. 1; col. 2, ll. 40-44.

Scotts Company asserts that because Roth teaches a MAS carrier for agricultural chemicals that can coat a seed, and because Lowe likewise teaches an agricultural carrier consisting of paper sludge, a person of ordinary skill in the art would have had reason to substitute Lowe's paper mill sludge for Roth's MAS coating. Pet. 57.

Encap asserts that the proposed combination runs contrary to the disclosure of Roth. PO Resp. 43. In particular, Encap asserts that Lowe requires the fiber content of the finished particle be above 10%, which means, therefore, that the material is 90% or less filler. *Id.* (citing Ex. 1004, col. 4, ll. 65-66; col. 6, ll. 53-63). On the other hand, Roth discloses MAS that is 97.5%-99.9% liquid. *Id.* (citing Ex. 1003, col. 3, ll. 50-51). Encap asserts that a product that is 97.5% or more liquid could not be replaced by a product with 10% or more fiber content and still be sprayed. *Id.* (citing Ex. 1020 ¶ 22). We do not find Encap's argument persuasive because Roth is not limited to spray-on coatings. The MAS, and presumably Lowe's paper sludge, can be applied to the seeds "by dipping, soaking, spraying, or other conventional mode of application." Ex. 1003, col. 4, ll. 48-50.

Encap also asserts that Roth's disclosure of a coating with 0.1% to 2.5% solids teaches away from using Lowe's coating containing over 10% solids. PO Resp. 43. Roth, however, "does not criticize, discredit, or otherwise discourage" the use of a higher percentage of solids. *In re Fulton*, 391 F.3d 1195, 1201 (Fed. Cir. 2004). Thus, Encap's argument is not persuasive.

Encap further asserts that paper sludge and MAS have very different characteristics. PO Resp. 44-45. In particular, Encap asserts that attempting to coat a seed with paper sludge, using the agglomeration process disclosed in Lowe, would not have a reasonable likelihood of success. *Id.* at 46. In support of its assertion, Encap submits the Declaration of Mr. Madigan (Ex. 1020) who testifies as to the difficulties associated with coating seeds with paper sludge utilizing the agglomeration process of Lowe. *Id.* We do not credit Mr. Madigan's declaration as it fails to provide the underlying basis for his conclusions. For example, Mr. Madigan cites an attachment that purports to show what a final product of Lowe

would look like if seed is introduced into the agglomeration process of Lowe. Ex. 1020, ¶ 23 and Attachment 5. Mr. Madigan, however, does not provide sufficient details regarding the underlying testing upon which he appears to rely. 37 C.F.R. § 42.65. Further, Scotts Company combined the paper sludge of Lowe (not its agglomeration process) with Roth. *See, e.g.*, Pet. 57.

As to Encap's assertion that Roth in view of Lowe does not disclose a "combination seed capsule," as discussed above, the preamble recitation "combination seed capsule" is not an additional structural limitation on the claim. PO Resp. 47.

Lastly, Encap asserts that Lowe's paper sludge is not a "soil conditioning material." *Id.* (citing Ex. 2007 ¶ 19). Paragraph 19 of Mr. Katers' Declaration, however, does not support Encap's contention. Mr. Katers merely states that "[n]ot all paper sludge material would benefit the soil to which it is applied;" he does not state that Lowe's paper sludge is not beneficial to the soil. Ex. 2007 ¶ 19.

We, therefore, conclude that the ordinary artisan would have combined Roth and Lowe to arrive at the claimed composition.

E. Anticipation by Schreiber—Claims 1, 7, and 13

Schreiber discloses the limitations of claims 1 and 7. For example, Schreiber discloses a plant seed having multiple coatings thereon, which satisfies the claimed "seed acting as a core or pseudo core." Ex. 1002, col. 1, ll. 4-6; col. 9, ll. 38-43. Schreiber further discloses the claimed "coating of a composition comprising soil conditioning materials." Specifically, Schreiber describes a seed coating made of a composition comprising solid particulate coating material, such as ground peat moss, thereby satisfying the claimed "being in a solid state at time of coating," of claims 1 and 7. *Id.* at col. 2, ll. 34-49; col. 10, ll. 40-42. Schreiber

explains that its invention permits the tailoring of seed coatings for achieving optimum germination and growth, while allowing early planting within a wide time period. Schreiber also explains that other advantages also accrue from the invention. Schreiber, thus, satisfies our construction of "soil conditioning materials" because its coating provides better root development and drought resistance. *Id.* at col. 2, ll. 15-19; col. 9, ll. 44-49. Schreiber also discloses that the coating is an "agglomeration" of a plurality of types of materials, as Schreiber explains that the coating composition includes a "binder," required by claim 13, or a plasticizer, and that the coating layers may coalesce, thereby satisfying the agglomeration requirement of claim 7. *Id.* at col. 2, ll. 37-39, 55-56; col. 3, ll. 35-42; col. 6, ll. 23-32.

Encap asserts that Schreiber does not disclose a "combination seed capsule." PO Resp. 18-23. For the reasons discussed above, a "combination seed capsule" found in the preamble of claims 1 and 7 does not further limit the claim. Encap also asserts that Schreiber does not disclose a "soil conditioning material." *Id.* at 23-26. Schreiber, however, discloses peat moss, limestone, gypsum, and vermiculite. Ex. 1002, col. 2, ll. 44-49. Those materials are known to beneficially modify the soil in some way other than direct provision of plant nutrients, and are, thus, "soil conditioning materials," as recited in claims 1 and 7. *See, e.g.*, Exs. 1028-1031. Encap's expert, Mr. Baker, acknowledged that peat moss, limestone, gypsum, and vermiculite are all soil conditioning materials. Baker Depo., Ex. 2005, 88, l. 22– 90, l. 9.[8]

[8] We reference page numbers found in the lower right corner of the exhibit.

Encap seeks to distinguish Schreiber on a purported difference in the function of the Schreiber coating and those disclosed in the '259 patent. Specifically, Encap asserts that Schreiber discloses using a water-insoluble coating with a water-soluble binder (e.g., peat moss) to delay germination until growing conditions are favorable, whereas, the soil conditioning materials of the '259 patent enhance germination and plant growth. PO Resp. 25. For the reasons already discussed, the claim limitation "soil conditioning materials" does not require the material also provide soil conditioning value to the seed. Moreover, the '259 patent explicitly discloses that the coating may be used to delay germination. Ex. 1001, col. 4, ll. 12-20; col. 25, ll. 8-17. Just because Schreiber's coating also serves to delay germination does not mean that it is not a "soil conditioning material," so long as it beneficially modifies the soil, in some way other than direct provision of plant nutrients.

In summary, we hold that Scotts Company has shown, by a preponderance of the evidence, that claims 1, 7, and 13 are anticipated by Schreiber, under 35 U.S.C. § 102(b).

F. Obviousness over Schreiber and Roth—Claims 2, 5, 8, 11, and 14[9]

As discussed above, Schreiber discloses the elements of independent claims 1 and 7. Scotts Company proposes using Roth's MAS in place of Schreiber's peat moss. Pet. 38-39. Scotts Company's proposed combination would result in a seed coated with Roth's MAS, and as discussed above, MAS does not satisfy the claim limitation that the soil conditioning material be "in a solid state at the time of coating."

---

[9] In its Response, Encap references claim 15 instead of 14. We have interpreted Encap's reference as intended to be to claim 14. PO Resp. 26-27.

Therefore, we hold that Scotts Company has not shown, by a preponderance of the evidence, that claims 2, 5, 8, 11, and 14 are unpatentable over Schreiber and Roth, under 35 U.S.C. § 103(a).

G. Obviousness over Schreiber and Lowe—Claims 3, 4, 9, and 10

As discussed above, Schreiber discloses the elements of independent claims 1 and 7. Lowe further teaches a material that is a byproduct of a "paper making process," and specifically that the byproduct is "paper sludge" as required by dependent claims 3, 4, 9, and 10. Lowe describes an agricultural granule for carrying and releasing agricultural chemicals that resembles a clay-based granule. Ex. 1004, Abstract. The agricultural granule is made from using waste materials from paper manufacture, referred to as paper sludge. *Id.* at col. 1, l. 68–col. 2, ll. 1, 40-44. Scotts Company asserts that because Lowe teaches an agricultural granule made from paper sludge for carrying and releasing incorporated agricultural chemicals that resembles a clay-based granule (*id.* at Abstract; col. 2, l. 1), a person of ordinary skill would have had reason to substitute Schreiber's water-insoluble, solid, clay-like, agricultural inner coating material with Lowe's paper sludge materials. Pet. 40.

Schreiber discloses that its inner coating controls permeability of water and is typically water insoluble. Ex. 1002, col. 2, ll. 34-39. Encap asserts that there is no evidence that Lowe's material, derived from paper sludge, would operate to control water permeability (i.e., is water insoluble)—a trait important to the teachings of Schreiber. PO Resp. 28. Scotts Company does not respond to Encap's argument, and fails to provide any evidence that Lowe's agricultural granule is water insoluble. If Lowe's material is water soluble, it would not be a

suitable replacement for Schreiber's inner coating, as it would frustrate Schreiber's objective of delayed germination.

In summary, we hold that Scotts Company has failed to show, by a preponderance of the evidence, that claims 3, 4, 9, and 10 are unpatentable over Schreiber and Lowe under 35 U.S.C. § 103(a).

H. Anticipation by Matthews—Claims 1, 2, 7, 8, 13, and 14

Matthews discloses the claimed "seed acting as a core or pseudo core" with a "solid" "coating of a composition comprising soil condition materials," as required by claims 1 and 7. Ex. 1007, 2, ll. 41-89. Specifically, Matthews describes a seed pellet product coated with "fly ash," as required by dependent claims 2 and 8. *Id.* at 2, ll. 10-12, 61-64. Mathews further describes alternatingly spraying and dusting the seed with the coating until the desired thickness is reached, after which the seed pellets are dried. *Id.* at 2, ll. 81-84, 88-89. Matthews also discloses that the coating is an "agglomeration" of a plurality of types of materials, as required by claim 7, because Matthews explains that the coating of dust particles is bound by an adhesive water-soluble plastic, such as polyvinyl alcohol or methyl cellulose, around and about the original seed particle. *Id.* at 2, ll. 42-45, 50-54; 3, ll. 5-9. Matthews describes applying a "binder," as required by dependent claim 13, to the seed capsule, e.g., polyvinyl alcohol, to hold the coating substances firmly on the seed. *Id.* at 2, ll. 42-45; 3, ll. 5-9. Further, the Matthews seed coating may include "fertilizer," thus satisfying dependent claim 14. *Id.* at 5, ll. 25-27.

Encap asserts that Matthews does not disclose a "combination seed capsule." PO Resp. 38. As discussed above, the preamble recitation "combination seed capsule" does not further limit the claim. In addition, Encap unpersuasively asserts

that Matthews' fly ash may not be necessarily beneficial to the seed (*id.*)—a requirement lacking from our claim construction of "soil conditioning material." Relying upon Messrs. Baker and Katers, Encap asserts that Matthews' fly ash does not *necessarily* modify the soil in a beneficial manner, and hence, has not been proved to be a soil conditioning material. *Id.* at 39-42 (citing Ex. 2011 ¶ 21; Ex. 2007 ¶ 24). Essentially, Encap's argument is that while fly ash is specifically identified in the '259 patent as a soil conditioning material (*see, e.g.*, Ex. 1001, col. 7, ll. 21-25), not *all* fly ash is suitable—indeed, some types of fly ash are toxic. *Id.* Matthews, however, states that "[e]ach material must be stable and non-toxic." Ex. 1007, 8, ll. 9-10. Moreover, Mr. Baker also acknowledged that a person of ordinary skill would have understood that a non-toxic fly ash could be used to coat a seed as a soil condition material, and that using toxic materials harmful to the seed should be avoided. Ex. 2005, 150, l. 18–151, l. 20. Lastly, Matthews also discloses that the use of its coating materials "aid in germination" and "growth of the plant." Ex. 1007, 2, ll. 33-39. Thus, we determine that a person of ordinary skill would interpret Matthews as using non-toxic fly ash, beneficial to the soil.

Matthews also discloses using lime (*id.* at 5, ll. 28-35), which Mr. Krysiak admitted was a soil condition material (Ex. 2002, 148, ll. 18-23).

Therefore, we hold that Scotts Company has shown, by a preponderance of the evidence, that claims 1, 2, 7, 8, 13, and 14 are anticipated by Matthews under 35 U.S.C. § 102(b).

I. Secondary Considerations

Before we can determine that the combination of Roth and Lowe (*see* Section D, above), renders the challenged claims unpatentable as obvious, we must consider the evidence of obviousness anew in light of any evidence of secondary

considerations of nonobviousness presented by Encap. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966) ("Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy."); *Transocean Offshore Deepwater Drilling, Inc. v. Maersk Drilling USA, Inc.*, 699 F.3d 1340, 1349 (Fed. Cir. 2012) ("This objective evidence must be 'considered as part of all the evidence, not just when the decisionmaker remains in doubt after reviewing the art.'") (quoting *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538-39 (Fed. Cir. 1983)).

Encap alleges copying by others, long felt need, and commercial success as secondary considerations of non-obviousness. PO Resp. 48-49. Encap, however, fails to provide sufficient credible evidence to support its allegations.

Encap alleges that Scotts Company's Miracle-Gro® Turf Builder Grass Seed with Water Smart® is a copy of the product of the '259 patent. *Id.* at 48. To support its allegations, Encap submits a copy of marketing brochures for EncapSeed™ products (Ex. 1009, 89-97), a copy of the packaging from Scotts Company's Turf Builder Grass Seed with Water Smart® (*id.* at 98-101; Ex. 2013, 342-43, 346-47), a copy of a website print out pertaining to Scotts Company's TurfBuilder (Ex. 2013, 344-45), a Declaration by Mr. Krysiak dated October 31, 2012 and submitted during an *ex parte* reexamination (Ex. 1009, 118-131), and a Declaration by Mr. Krysiak (Ex. 1022 ¶¶ 41, 42). None of the evidence submitted by Encap, however, demonstrates that Scotts Company's Miracle-Gro® Turf Builder Grass Seed with Water Smart® product falls within the scope of any claim of the '259 patent, that Scotts Company was aware of the '259 patent prior to

developing its product, or that Scotts Company developed its product by copying the '259 patent.

Encap also asserts that there was a long-felt need for invention disclosed in the '259 patent. PO Resp. 48-49. Specifically, Encap asserts that many homeowners could not get their grass seed to grow because of inappropriate watering. *Id.* at 48. Encap, however, presents no credible evidence this need was satisfied by the '259 patented invention.

Lastly, Encap asserts commercial success because Meadowland took a license to the '259 patent. *Id.* at 49. Encap, however, does not allege that Meadowland's licensed product was commercially successful, or that any such commercial success was attributable to the patented features of the product. Encap also asserts that Scotts Company's product was commercially successful. *Id.* Encap, however, does not provide persuasive evidence that Scotts Company's product is covered by any claim of the '259 patent, that such product was commercially successful, or that such success was attributable to the patented feature.

After weighing all the evidence of obviousness and nonobviousness of record, on balance, we conclude that the strong evidence of obviousness outweighs the weak evidence of nonobviousness. For the foregoing reasons, we conclude that Scotts Company has shown, by a preponderance of the evidence, that claims 1-5, 7-11, 13, and 14 are unpatentable under 35 U.S.C. § 103(a) over Roth and Lowe.

J. Encap's Corrected Motion to Amend Claims

Encap filed a Motion to Amend Claims (Paper 24), which was later corrected (Paper 47) ("Mot."). In the Corrected Motion, Encap proposes substitute

claims 15-24, to replace claims 2-5, 8-11, 13, and 14,[10] respectively. Mot. 1. The Corrected Motion is contingent, meaning that a proposed substitute claim is at issue and would be considered only if "the original claims of the '259 patent are found unpatentable." *Id.* While somewhat ambiguous, we interpret Encap's motion as proposing a substitute claim if the claim it replaces is found unpatentable, as opposed to being contingent on all of the challenged claims being found unpatentable. Scotts Company has demonstrated the unpatentability of claims 1-5, 7-11, 13, and 14. Therefore, the contingency has materialized, and thus, we consider the Corrected Motion on the merits.

As the moving party, Encap bears the burden of proof to establish that it is entitled to the relief requested. 37 C.F.R. § 42.20(c). The proposed amendment is not entered automatically, but only upon Encap's having demonstrated the patentability of those substitute claims. Here, we find that Encap has failed to demonstrate that the added limitations distinguish over the known prior art, for example, Roth in combination with Lowe. Hence, Encap's Motion to Amend is denied.

In a conference call on August 26, 2013, we provided Encap guidance on filing a motion to amend the claims, and specifically directed the parties to the analysis in *Idle Free Sys. v. Bergstrom, Inc.*, IPR2012-00027, Paper 26 (PTAB June 11, 2013). The summary of the call is reflected in Paper 17 of the record. *Idle Free* holds that a patent owner should specifically identify features added to

---

[10] Encap later identifies the substitution as claims 15-24 in place of claims 2-5 and 11-13. Mot. 2-5. Thus, it is unclear whether claims 23-24 are proposed as replacement for claims 13 and 14, or for claims 12 and 13. However, as we discuss below, the issue is moot.

each substitute claim, and come forward with technical facts and reasoning about those features, including construction of new claim terms. *Idle Free*, slip op. at 7. The patent owner should also discuss the "significance and usefulness" of the added features "from the perspective of one with ordinary skill in the art." *Id.* We agree with the reasoning in *Idle Free*, and conclude that Encap has failed to satisfy its burden to demonstrate the patentability of the proposed substitute claims by a preponderance of the evidence.

While Encap identifies nineteen separate "structural limitations," presumed to be new, it does not identify how each of these structural limitations differs from what is previously recited in the claims. 37 C.F.R. § 42.221(b) ("A motion to amend claims must . . . show the changes clearly . . . ."). Specifically, Encap's listing of proposed claims 15-24 does not show, by redline or discussion, how the claims being replaced have been modified. Mot. 1-5. Moreover, Encap fails to construe any new claim limitation, and also fails to proffer any technical facts and reasoning about the amended features. *Idle Free,* slip op. at 7. Encap's failure to comply with the Board's directive places Scotts Company in the unfair position of having to ascertain the claim amendments and then make assumptions about which of the amendments are considered by Encap to be significant. For amended claims, however, the burden "is not on the petitioner to show unpatentability;" it is "on the patent owner to show patentable distinction over the prior art." *Id.* at 7. Encap has not met its burden.

For example, to determine the differences between original claim 2 and its proposed substitute, claim 15, the following comparison was created, with bracketed text indicating material deleted from claim 2, and underlined text indicating material inserted into claim 2 (paragraphing added).

[2] 15. The combination seed capsule of claim 1 wherein [material of said soil conditioning materials are comprised of sludge or fly ash] said combination seed capsules provides cooperative and beneficial effects of said soil conditioning material working together in controlled intimate relation with said seed, to enhance the germination and growth processes of said seed and the plant emergent therefrom, said effects being greater than when said soil conditioning material and said seed are applied to the soil separately; wherein said effects result from an intimate relationship of said soil conditioning materials in said combination seed capsule, whereby said materials cooperate with each other in support of said germination and growth processes;

said soil conditioning material is a material that beneficially modifies soil in some way other than direct provision of fertilizer, used with said seed to provide soil conditioning value to said seed so coated, irrespective of general tilth condition of the growth medium into or onto which the seed capsule is applied;

said solid state at time of coating comprising materials in form of a particulate material, fibrous material, a suspension of said particulate and/or fibrous material in a liquid suspension, or any combination thereof; said soil conditioning value of said soil conditioning material to said seed comprises the enhanced control of moisture about said seed; said enhanced control consists of absorbing and holding water;

said coating of said combination seed capsule comprises a plurality of particles.

Encap does not explain why each new feature is "significant and useful," does not construe any of the new claim limitations, nor proffer any technical facts and reasoning about the amended features. Instead, Encap provides conclusory statements only, such as "Roth does not provide the cooperative and beneficial

effects of this structural limitation." Mot. 6. Encap does not provide a proposed interpretation of the recited "cooperative and beneficial effects" of proposed substitute claim 15, nor does it explain whether Roth provides some of the "effects of this structural limitation," and not others or why.

Encap asserts that the structural limitations themselves provide the technical facts and reasoning, as well as the significance and usefulness of the limitations. Pet. Reply 3. Encap asserts also that the "[c]laim construction of the structural limitations is found within the limitations themselves." *Id.* We disagree. Providing "cooperative and beneficial effects" is vague and not self-defining, in any meaningful way. Consequently, the usefulness and significance of the limitation is not self-evident. The same can be said of, "working together in controlled intimate relation."

Encap also fails to "provide meaningful reasons" for making additional changes to dependent claims. *Idle Free*, slip op. at 9. For example, claim 18, which depends from claim 15, adds three new limitations. *See* Mot. at 3; *see also id.* at 3-4 (claims 19 and 20 both depend from claim 17, and only differ by inclusion of a fungicide in claim 19). But Encap fails to explain why the additional features were added to these dependent claims. *Idle Free*, slip op. at 9-10 ("Adding features for no meaningful reason is . . . not responsive to an alleged ground of unpatentability.").

In addition, *Idle Free* further instructs patent owners to consider and distinguish "prior art," both "of record" and "not of record but known to the patent owner." *Id.* at 7. Moreover, we specifically explained to Encap that "[a] conclusory statement that no prior art is known to the patent owner . . . is insufficient." IPR2013-00110, Paper 17, 2. On page 1 of its Motion (Paper 47),

Encap states, "No closer art than the prior art cited in the underlying *inter partes* review is known to PO." Encap, however, was aware of additional relevant prior art, including Simmons and Evans, which were cited in Scotts Company's request for *inter partes* review, but which were deemed cumulative of the adopted grounds of rejection. *See* Pet. at 41-49; Prelim. Resp. at 25. While those references may have been cumulative over the original claims, they are not be cumulative in view of Encap's proposed substitute claims, and should be addressed. Encap's proposed claim 15 recites that the soil conditioning material "comprises enhanced control of moisture about said seed" consisting of "absorbing and holding water." Encap distinguishes the prior art in this *inter partes* review by arguing that it does not teach enhancing moisture about the seed. Mot. at 9-10. Simmons and Evans specifically disclose coating a seed with a water-absorbable polymer. Yet, Encap failed to distinguish its proposed claims over those two material prior art references.

Encap attempts to correct some of its errors by filing an expert declaration with its Corrected Reply to Motion to Amend. Paper 49; Ex. 2012. As already addressed, however, we exclude this Declaration as untimely and improperly incorporated by reference into Encap's Motion. In addition, as discussed above, the proffered "corrected" Second Declaration of Mr. Krysiak does not overcome Scotts Company's objections, and is thus, excluded.

For the above reasons, Encap's Corrected Motion to Amend Claims is denied as it fails to distinguish over the prior art, for example, Roth in combination with Lowe.

III. CONCLUSION

Scotts Company has shown by a preponderance of the evidence that: (1) claims 1, 7, and 13 of the '259 patent are unpatentable under 35 U.S.C. § 102(b) as anticipated by Schreiber; (2) claims 1, 2, 7, 8, 13, and 14 are unpatentable under 35 U.S.C. § 102(b) as anticipated by Matthews; and (3) claims 1-5, 7-11, 13, and 14 are unpatentable under 35 U.S.C. § 103(a) as obvious over Roth and Lowe.

Scotts Company has not shown by a preponderance of the evidence that: (1) claims 1, 2, 5, 7, 8, 11, 13, and 14 of the '259 patent are unpatentable under 35 U.S.C. § 102(b) as anticipated by Roth; (2) claims 2, 5, 8, 11, and 14 are unpatentable under 35 U.S.C. § 103(a) as obvious over Schreiber and Roth; or (3) claims 3, 4, 9, and 10 are unpatentable under 35 U.S.C. § 103(a) as obvious over Schreiber and Lowe.

Encap has not shown by a preponderance of the evidence that its proposed substitute claims 15-24 are patentable over the prior art.

IV. ORDER

In consideration of the foregoing, it is hereby ORDERED that:

> Scotts Company's Motion to Exclude Mr. Krysiak's Second Declaration (Ex. 2016) is granted and all other relief requested in the motion is denied;
>
> Encap's Motion to Exclude Mr. Sundstrom's Declaration (Ex. 1039) is dismissed as moot;
>
> Claims 1-5, 7-11, 13, and 14 of the '259 patent are determined to be unpatentable; and
>
> Encap's Corrected Motion to Amend Claims is denied.

This is a final decision. Parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

Petitioner:

Robert Schulman
rschulman@hunton.com

Jeff Vockrodt
jvockrodt@hunton.com

Patent Owner:

Philip Weiss
weissandweiss@aol.com

Aaron Olejniczak
aarono@andruslaw.com

Trials@uspto.gov
571-272-7822

Paper 79
Date: June 24, 2014

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE SCOTTS COMPANY LLC
Petitioner

v.

ENCAP, LLC
Patent Owner

Case IPR2013-00110
Patent 6,209,259

Before MICHAEL P. TIERNEY, LORA M. GREEN, and RAMA G. ELLURU, *Administrative Patent Judges.*[1]

PER CURIAM.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

---

[1] Floyd, Administrative Patent Judge, who participated in the oral hearing held on January 30, 2014, has left the Board; accordingly, Tierney, Administrative Patent Judge, has been added to the panel.

I. BACKGROUND

Petitioner, The Scotts Company LLC ("Scotts Company"), filed a Petition on January 10, 2013, for an *inter partes* review of claims 1-5, 7-11, 13, and 14 ("the challenged claims") of U.S. Patent No. 6,209,259 ("the '259 patent") pursuant to 35 U.S.C. §§ 311-319. Paper 2. On April 15, 2013, Patent Owner, Encap, LLC ("Encap"), filed a Preliminary Response. Paper 9. On July 3, 2013, the Board granted an *inter partes* review for all challenged claims on less than all of the grounds of unpatentability alleged in the Petition. Paper 12, ("Dec."). The Board also stayed concurrent reexamination of the '259 patent. Paper 10.

After institution of trial, Encap filed a Corrected Patent Owner's Response. Paper 48. Encap also filed a Corrected Contingent Motion to Amend Claims that requests substituting proposed new claims 15-24 for claims 2-5, 8-11, 13, and 14, respectively—contingent upon a determination of unpatentability. Paper 47. Scotts Company filed a Reply to Patent Owner's Response (Paper 30), and an Opposition to Encap's Motion to Amend Claims (Paper 33). Encap then filed a Corrected Reply to Scotts Company's Opposition to Encap's Motion to Amend Claims. Paper 49.

Additionally, Scotts Company filed a Motion to Exclude Evidence (Paper 52), to which Encap responded (Paper 64) and submitted supplemental evidence (Paper 58). Scotts Company filed a Reply in further support of its Motion to Exclude. Paper 68.

Encap also filed a Motion to Exclude Evidence (Paper 54) to which Scotts Company responded (Paper 60). Encap, with authorization (Paper 70), filed a Supplement to its Motion to Exclude (Paper 66), as well as a Reply (Paper 67).

Oral hearing was held on January 30, 2014.[2]

The Board has jurisdiction under 35 U.S.C. § 6(c). This Final Written Decision is issued pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73.

Scotts Company has shown by a preponderance of the evidence that claims 1-5, 7-11, 13, and 14 of the '259 patent are unpatentable. Encap's Motion to Amend Claims is denied.

A. The '259 Patent

The '259 patent is directed to a combination seed capsule, comprising at least one viable seed, a coating of a composition comprising a soil conditioning material mounted proximate and disposed outwardly of the outer surface of the seed, and optionally including one or more of inorganic chemical fertilizers, growth enhancer, binder, and/or an anti-fungal agent. Ex. 1001, Abstract, 4:5-11. According to the '259 patent Specification, the primary object of the invention is to "provide solid plant seed capsule products that supply both soil conditioning properties and the seed, which can benefit from such conditioned soil, in a given seed capsule particle." *Id.* at 3:28-31.

B. Illustrative Claim

Claims 1 and 7 are the only independent claims in the '259 patent, and are directed to a "[a] combination seed capsule." The only difference between these claims is that claim 7 additionally states that the seed coating is applied by an agglomeration process. The remaining challenged claims depend from either claim 1 or 7. Claim 1 is illustrative of the claimed subject matter, and is reproduced below.

---

[2] A transcript of the oral hearing is included in the record as Paper 78.

1. A combination seed capsule comprising:

one viable seed;

said seed acting as a core or pseudo core of said combination seed capsule;

a coating of a composition comprising soil conditioning materials;

said soil conditioning materials being in a solid state at time of coating.

C. Prior Art Supporting the Instituted Challenges

Name	Reference	Issue or Publication	Exhibit
Schreiber	U.S. Patent No. 3,698,133	Oct. 17, 1972	Ex. 1002
Roth	U.S. Patent No. 4,065,287	Dec. 27, 1977	Ex. 1003
Lowe	U.S. Patent No. 5,019,564	May 28, 1991	Ex. 1004
Matthews	GB670,461	Apr. 16, 1952	Ex. 1007

D. The Instituted Challenges of Unpatentability

References	Grounds	Claims
Schreiber	§ 102(b)	Claims 1, 7, and 13
Schreiber and Roth	§ 103(a)	Claims 2, 5, 8, 11, and 14
Schreiber and Lowe	§ 103(a)	Claims 3, 4, 9, and 10
Matthews	§ 102(b)	Claims 1, 2, 7, 8, 13, and 14
Roth	§ 102(b)	Claims 1, 2, 5, 7, 8, 11, 13, and 14
Roth and Lowe	§ 103(a)	Claims 1-5, 7-11, 13, and 14

II. DISCUSSION

A. Evidentiary Matters

1. Scotts Company's Reply (Paper 30)

In a conference call held on December 3, 2013, Encap asserted that Scotts Company had raised new arguments and evidence in its Reply to Patent Owner's Response to Decision to Institute. Order (Paper 37), 2. The Board denied Encap's request to file a surreply, or to enlarge the page limit of Encap's Reply in support of its Motion to Amend. *Id.* We indicated, however, that we would determine whether Scotts Company's Reply and supporting evidence contain material exceeding the proper scope of a reply. *Id.*

We find that Scotts Company's Reply, and in particular, the supporting Declarations of Mr. Fredrick Sundstrom (Ex. 1039) and Mr. Krishna Pagilla (Ex. 1040) contain material outside the proper scope of a reply. 37 C.F.R. § 42.23(b) (reply is limited to arguments raised in Patent Owner's Response). Specifically, both Declarations contain materials in support of Scotts Company's Petition, and therefore, untimely filed. For example, Mr. Sundstrom includes analyses of claim construction (e.g., Ex. 1039 ¶¶ 7-9), as well as analyses of the Schreiber (e.g., *id.* at ¶¶ 10-13), Matthews (e.g., *id.* at ¶¶ 28, 29), Roth (e.g., *id.* at ¶ 34), Simmons (*id.* at ¶¶ 36, 38), and Evans (*id.* at ¶¶ 43, 44, 46, 48) references. Likewise, Mr. Pagilla addresses claim construction, as well as the references upon which Scotts Company sought institution. *See, e.g.*, Ex. 1040 ¶¶ 9-13, 23-27, 32, 33, 36-38. Specifically, we hold that the new evidence could have been included with the motion. By waiting to serve this evidence on Encap in Scotts Company's Reply, Encap was denied the opportunity to file responsive evidence. Thus, we

have not considered the untimely Declarations of Mr. Sundstrom and Mr. Pagilla, nor the arguments based thereon.[3]

2. Scotts Company's Motion to Exclude

Scotts Company filed a Motion to exclude: portions of the deposition testimony of Mr. Michael Krysiak taken by Encap on November 6, 2013 (Ex. 2002) and December 23, 2013 (Ex. 1038); and the Second Krysiak Declaration, which includes Attachments A and B (Ex. 2016). Pet. Mot. Excl. (Paper 52), 1. Mr. Krysiak, Encap's witness, submitted a second Declaration (Ex. 2012) in support of its Reply to Petitioner's Opposition to Encap's Motion to Amend (Paper 49). Encap responded to Scotts Company's Motion to Exclude and filed supplemental evidence. PO Resp. Mot. Excl. (Paper 64); PO Supp. Evid. (Paper 58), respectively. Scotts Company filed a Reply. Paper 68. We grant-in-part Scotts Company's Motion to Exclude Evidence.

Scotts Company asserts that Mr. Krysiak's deposition testimony in response to two questions (i.e., Ex. 2002, 207, l. 9; Ex. 1038, 209, ll. 7-8) should be excluded. Pet. Mot. Excl. 9-10. As we did not rely upon this deposition testimony that Scotts Company seeks to exclude, Scotts Company's Motion is moot with respect to such testimony.

Scotts Company also moves to exclude the Second Declaration of Mr. Krysiak (Ex. 2012). Scotts Company's primary objection is that the Declaration is untimely, as it should have been submitted with Encap's Motion to

---

[3] The fact that two declarations may contain some material appropriate for a response does not require our consideration of them, as the Board will not attempt to sort the proper from the improper portions. *See Office Patent Trial Practice Guide*, 77 Fed. Reg. 48,756, 48,767 (Aug. 14, 2012).

Amend (Paper 47). Pet. Mot. Excl., 11-14; *see* 37 C.F.R. § 42.23(b) ("All arguments for the relief requested in a motion must be made in the motion. A reply may only respond to arguments raised in the corresponding opposition or patent owner response."). In support of Scotts Company's Opposition to Encap's Motion to Amend (Paper 33), it relied upon the Declaration of Mr. Sundstrom (Ex. 1039), which was not considered, as discussed above. Encap asserts that Mr. Krysiak's Second Declaration is in rebuttal to Declarations and deposition testimony of Mr. Sundstrom and Mr. Pagilla. PO Resp. Mot. Excl. 10-11. Encap proffers supplemental evidence—a revised Second Declaration of Mr. Krysiak with citations to the Declaration and deposition of Mr. Sundstrom. Paper 58; Ex. 2016.

Reading Mr. Krysiak's Second Declaration, it is clear that the majority of the Declaration is in support of Encap's Motion to Amend rather than in rebuttal to Scotts Company's Opposition to Encap's Motion to Amnd or the Declarations and deposition testimony[4] of Mr. Sundstrom and Mr. Pagilla, and is thus, untimely. For example, paragraphs 2-3 relate to written description and claim construction, which Encap has the burden of proving in its Motion to Amend. Additionally, paragraphs 6-12 describe the background of the technology, which could have been submitted with Encap's Motion to Amend opening brief, and thus, are not in rebuttal to testimony from Mr. Sundstrom or Mr. Pagilla. Likewise, paragraphs 25-53 and Schedule A attempt to distinguish over Matthews and Schreiber, which Encap should have done in Patent Owner's Motion to Amend. Furthermore, to the extent that portions of Mr. Krysiak's Second Declaration are in response to the

[4] While not addressed, we do not suggest that filing a declaration in rebuttal to deposition testimony is appropriate.

Declarations of Mr. Sundstrom and Mr. Pagilla, which were excluded, they should likewise be excluded. Those errors were not corrected in the Supplemental Evidence (i.e., Ex. 2016) submitted by Encap.

In addition, Encap attempts to incorporate Mr. Krysiak's Second Declaration into its Reply to Scott's Opposition to the Motion to Amend by merely stating, "The proposed claims define over the prior art succinctly. *Id.* [Mr. Krysiak's Second Declaration] at ¶¶ 14-53." Reply Mot. Amend 5. In our Order of August 27, 2013, we admonished Encap to refrain from attempting to use an expert declaration in such fashion. We stated, "Encap's motion to amend may be supported by an expert declaration, but that the motion itself should set forth the arguments and explanations with appropriate pinpoint citations to the expert declaration, rather than incorporating by reference the expert declaration." Paper 17, 2-3. Thus, Scotts Company's Motion to Exclude Mr. Krysiak's Second Declaration (Ex. 2012) is granted, as Mr. Krysiak's Corrected Second Declaration (Ex. 2016) did not remedy the issues, it is not considered.

3. Encap's Motion to Exclude

Encap moves to exclude the Declaration of Mr. Sundstrom (Ex. 2014), Scott Company's witness who provided a declaration in support of Scott Company's Reply to Patent Owner's Response to Decision to Institute (Paper 30), on the basis that the declarant refused to answer certain questions during his deposition on the basis of confidentiality, even though a protective order was in place. PO Mot. Excl. (Paper 54), 5. Having found that Mr. Sundstrom's Declaration was untimely submitted, and thus, not considered, Encap's Motion to Exclude is dismissed as moot.

B. Claim Construction

Consistent with the statute and the legislative history of the AIA, the Board interprets claims by applying the broadest reasonable construction in the context of the specification in which the claims reside. 37 C.F.R. § 42.100(b); *see Office Patent Trial Practice Guide*, 77 Fed. Reg. 48,756, 48,766 (Aug. 14, 2012). Claim terms also are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art in the context of the entire disclosure. *In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007).

Two exceptions to the general rule that a claim term is given its ordinary meaning are: 1) when a patentee sets out a definition and acts as his own lexicographer; or 2) when the patentee disavows the full scope of a claim term either in the specification or during prosecution. *See In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994). If an inventor acts as his or her own lexicographer, the definition must be set forth in the specification with reasonable clarity, deliberateness, and precision. *Id.*

1. "soil conditioning materials"

All of the challenged claims require "a coating of a composition comprising soil conditioning materials." The '259 patent Specification states that "*all soil conditioning materials contemplated herein* beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients." Ex. 1001, col. 8, ll. 41-44 (emphasis added). The Specification further provides specific examples of soil conditioning materials, such as municipal or other sewage sludge, paper mill sludge, fly ash, and dust. *Id.* at col. 7, ll. 21-23. Accordingly, in the Decision to Institute, the Board construed "soil conditioning materials" as "materials that beneficially modify soil

to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients, including for example, municipal or other sewage sludge, paper mill sludge, fly ash, and dust." Dec. 6-7.

Although Scotts Company agrees with the Board's preliminary construction (Pet. Reply, 1-2), Encap asserts the construction is overly broad in view of the Specification (PO Resp., 8-9). Specifically, Encap asserts the construction should be amended to include that the soil conditioner not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated irrespective of the general tilth condition of the growth medium. *Id.* (citing Ex. 1001, col. 8, ll. 42-52,[5] Abstract). Encap does not assert that its construction is the plain and ordinary meaning of "soil conditioning materials," but rather, that the Specification defines the phrase. PO Resp. at 8. Specifically, Encap asserts the following portion of the Specification defines "soil conditioning materials:"

> However, all soil conditioning materials contemplated herein beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients. By use of soil conditioner in intimate association with the seed, this invention not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated, and in intimate association with the seed, irrespective of the general tilth condition of the growth medium into or onto which the seed capsule is applied.

Ex. 1001, col. 8, ll. 42-52.

Through the inclusion of "all soil conditioning materials contemplated herein," the first sentence requires the soil conditioning material to beneficially

---

[5] Encap mistakenly refers to col. 15, l. 29–col. 16, l. 6.

modify the soil in some way, other than directly providing plant nutrients. The second sentence is an observation of benefits provided by "this invention;" it does not *require* the invention provide the observed benefits; much less require *just* the soil conditioning material of the invention provide such benefits.

Encap relies upon its experts, Mr. John Katers, Mr. Daniel Madigan, and Mr. Michael Krysiak, all of whom provide identical claim constructions, in support of its position. Ex. 2007 ¶ 11; Ex. 1020 ¶ 10; Ex. 1022 ¶ 13. The experts provide, however, no credible analysis in support of their claim constructions, and thus, are unpersuasive.

Encap asserts also that the examples included in the Board's preliminary claim construction should be omitted, because not *all* municipal or other sewage sludge, paper mill sludge, fly ash, or dust, necessarily modify the soil in a beneficial manner. PO Resp. 9. The Board's preliminary construction, however, requires the soil conditioning materials "modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients." The inclusion of the examples is intended to clarify, not modify, this requirement.

Accordingly, the Board maintains its construction of "soil conditioning materials" as:

> Materials that beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients, including for example, municipal or other sewage sludge, paper mill sludge, fly ash, and dust.

2. "combination seed capsule"

The phrase "combination seed capsule" appears in the preamble of claims 1 and 7. Encap asserts that the Abstract of the '259 patent defines "combination seed capsule." PO Resp. 10-11. The Abstract reads:

> This invention pertains to combination seed capsules wherein each seed capsule includes both moieties of at least one soil conditioner and at least one seed, and optionally, one or more inorganic chemical fertilizer, growth enhancer, binder, and/or anti-fungal agent. The combination seed capsules are made by physically combining the respective soil conditioner and seed with one other, in the absence of any requirement for chemical reactions in the process of so combining the respective materials. The combination seed capsules *provide cooperative and beneficial effects of the soil conditioner* and the optional inorganic fertilizer, *working together in controlled intimate relation with the seed, to enhance the germination and growth processes of the seed, and the plant emergent therefrom, greater than when the soil conditioner and seed*, and optionally inorganic chemical fertilizer, *are applied to the soil separately; the improvement being a result of the intimate relationship of the respective materials in the combination seed capsule, whereby the respective materials cooperate with each other in support of germination and plant growth.*

Ex. 1001, Abstract (emphases added). Encap asserts that the text that has been italicized is the definition of a "combination seed capsule." PO Resp. 11. Encap also relies upon its technical experts, Messrs. Baker, Madigan, and Krysiak. *Id.* at 11-12. The experts, however, provide no credible analysis in support of their claim constructions and are thus, unpersuasive.

Scotts Company asserts that the term "combination seed capsule" appears in the preamble of both independent claims (i.e., claims 1 and 7), and thus, is not limiting. Pet. Reply 2. Scotts Company also asserts that in 1998, when the application that matured into the '259 patent was filed, the rules prohibited relying

on the Abstract "for interpreting the scope of the claims." *Id.* at 3 (quoting 37 C.F.R. § 1.72(b)). Lastly, Scotts Company asserts that Encap is attempting to improperly import limitations into the claims. *Id.*

First, the Abstract does not provide a definition for a "combination seed capsule," but rather observes the benefits of the combination seed capsule. Second, the preamble term "combination seed capsule" is not limiting because the claim body describes a structurally complete invention. *Catalina Mktg. Int'l v. Coolsavings.com Inc.*, 62 USPQ2d 1781, 1785 (Fed. Cir. 2002). Thus, we need not construe "combination seed capsule," as it does not limit the claim.

3. "being in a solid state at time of coating"

Independent claim 1 recites, "being in a solid state at time of coating." Similarly, independent claim 7 recites, "are in a solid state at time of coating." Additionally, claim 7 recites, "said coating being applied to said viable seed by an agglomeration operation." Due to the inclusion of these three limitations, claims 1 and 7 were determined to be product-by-process claims in the Decision to Institute. Dec. 7-8.

Encap asserts that "in a solid state at time of coating" should be construed as "solid material in the form of particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers." PO Resp. 12-13 (citing Ex. 1001, col. 8, ll. 1-5[6]). Scotts Company points out that the Specification reads, the soil conditioning raw material "*may* be a particulate powder, or *may* be fibrous, or *may* be a suspension of a powder or fibrous material in a liquid carrier, and is preferably coated onto the substrate seed

[6] Encap erroneously cites to col. 14, ll. 24-28.

to form a seed capsule or other agglomeration of particles, fibers, *or the like*," and thus, does not support Encap's construction. Pet. Reply 3 (quoting Ex. 1001, col. 8, ll. 1-5 with emphasis added). We agree that the Specification does not support Encap's proposed construction.

Encap further asserts that during prosecution of the '259 patent application, Mr. Krysiak had discussions with the Examiner relating to "being in a solid state at the time of coating." PO Resp. 12 (citing Ex. 1022 ¶ 15). Encap's description of events does not provide support for its proposed claim construction. That is, it does not follow that adding the limitation to overcome Roth, defines the limitation to require "solid material in the form of particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers." As before, Mr. Krysiak's opinion as to how the phrase should be construed includes no analysis, and thus, is unpersuasive.

Encap does establish that it disavowed claim scope, however, by adding the limitation "in a solid state at time of coating" to overcome Roth. That clear and unambiguous disavowal of claim scope causes us to modify the claim construction from that set forth in the Decision to Institute. Specifically, Encap narrowed the "in a solid state at time of coating" limitation to require the soil conditioning material be in a solid state at the time of coating the seed. Encap did not narrow "in a solid state at time of coating," however, to further require a particulate, fibrous, or a suspension of a particulate or fibrous material in a liquid carrier to form an agglomeration of said particulate and/or fibers, as suggested by Encap.

The Federal Circuit has addressed the issue of determining whether a claim has been narrowed in the related context of prosecution history estoppel.

> In order to give due deference to public notice considerations under the *Warner–Jenkinson* framework, a patent holder seeking to establish

> the reason for an amendment must base his arguments solely upon the public record of the patent's prosecution, i.e., the patent's prosecution history. To hold otherwise—that is, to allow a patent holder to rely on evidence not in the public record to establish a reason for an amendment—would undermine the public notice function of the patent record.

Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., 234 F.3d 558, 586 (Fed. Cir. 2000), *vacated on other grounds*, 535 U.S. 722 (2002).

An examination of the prosecution history of record reveals the following events which support our determination that Encap clearly disavowed the full scope of claims 1 and 7. On May 10, 2000, the Examiner issued a rejection to claim 77 as anticipated by Roth, and further rejected claims 77 and 85 as being obvious in view of Roth in combination with two other references. Ex. 1008, 171, 175.[7] On August 8, 2000, the Examiner issued an interview summary, which indicates that a proposed claim amendment was discussed. Specifically, the Examiner stated that adding, "wherein said soil conditioning material, when added to the seed, are in a dry, solid form," to the claims would overcome Roth. The Examiner suggested "that the claims be written in a product by process form to clearly distinguish over Roth." *Id.* at 203. On September 8, 2000, the Examiner issued an Interview Summary indicating that claims 77 and 85 were discussed, and that "[b]ased on the proposed draft amendment and arguments recited therein, the prior art was overcome." *Id.* at 204. The record clearly shows that the only amendment made to claim 77 was the addition of the limitation, "said soil conditioning materials being in a solid state at time of coating." *Id.* at 200. Claim 85 was amended in similar fashion to recite, "wherein said soil conditioning

[7] Claims 77 and 85, ultimately issued as claims 1 and 7, respectively.

materials are in a solid state at time of coating." *Id.* at 201. Claims 77 and 85 ultimately issued as claims 1 and 7, respectively.

Thus, Encap successfully overcame Roth by adding the "in a solid state at the time of coating" limitation to claims 1 and 7. Construing the phrase as a product-by-process limitation would not result in distinguishing over Roth, as no discussion was had, nor evidence provided, to suggest the end product of Roth had different characteristics than the claimed composition. The disavowal of claim scope is clear. The limitation "in a product by process form," therefore, must be construed to require the soil conditioning material be in a solid state at the time of coating. *See Tempo Lighting, Inc. v. Tivoli, LLC*, 742 F.3d 973, 978 (Fed. Cir. 2014).

Furthermore, Roth discloses a spray application of a MAS material that contains 0.1% to 2.5% solids at the time of coating. Ex. 1003, col. 3, ll. 50-51. Thus, the limitation "in a solid state at the time of coating" must further be construed to require more than 2.5% solids. Therefore, we construe "in a solid state at the time of coating" to mean that more than 2.5% of the soil conditioning material must be in a solid state at the time of coating the seed.

4. "agglomeration operation"

Independent claim 7 requires an "agglomeration operation," which we construed in our Decision to Institute to be a product-by-process limitation. Dec. 8. Patent Owner concedes that claim 7 is a product-by-process claim. PO Resp. 16. Patent Owner, however, takes issue with the Board's "holding" that an agglomeration operation means using water and heat to bind a plurality of particles. *Id.* at 13.

We did not construe "agglomeration operation," other than to note that it is a product-by-process limitation. *In re Thorpe*, 777 F.2d 695, 698 (Fed. Cir. 1985). The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art. *See, e.g.*, *In re Garnero*, 412 F.2d 276, 279 (CCPA 1969). That is especially true where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. *Id.* Thus, the issue is not focused on what "agglomeration operation" means, but rather on what properties would be embodied in a product made by an agglomeration operation (i.e., an agglomerate). Here, the parties are in near agreement on the properties of an agglomerate.

Encap states that an agglomerate is an assemblage of particles adhering to each other, and thus, a magnified image of an agglomerate would reveal that the product is comprised of particulate. PO Resp. 13-16. Without credible explanation, Encap in its conclusion limits its final description of an agglomerate to an assemblage of *fine* particles. *Id.* at 16. Evidence cited by Encap that may support this additional limitation is an article by Wolfgang B. Pietsch, titled "The Agglomerative Behavior of Fine Particles." *Id.* at 13-14 (citing Ex. 1020 ¶ 11, Attachment A). As the title suggests, however, the article is specifically directed to agglomerates of fine particles. There is no credible suggestion in Mr. Madigan's Declaration (Ex. 1020) that an "agglomerate" is limited to fine particles. *See* Ex. 1020 ¶¶ 11-17.

Scotts Company appears to accept Encap's description of an agglomerate, but takes exception, as we do, with the limitation to fine particles. Pet. Reply 3-4.

Thus, we determine that an agglomerate is an assemblage of particles adhering to each other. The "agglomeration operation" limitation of claim 7 implies that the claimed "combination seed capsule" has a coating of a composition comprising soil conditioning materials comprised of particulate. As such, to satisfy the limitation of an "agglomeration operation," a reference must disclose a product with the structural limitation of being comprised of particulate, irrespective of the process used to make the product.

C. Anticipation by Roth—Claims 1, 2, 5, 7, 8, 11, 13, and 14

Roth explains that the MAS coating is "solid" after application. Roth, however, does not disclose the soil conditioning materials "being in a solid state at time of coating," because Roth discloses a spray application of a MAS material that is 97.5% to 99.9% liquid with the remainder "solids content." PO Resp. 31-32 (citing Ex. 1003, col. 3, ll. 50-51). While a tiny amount (i.e., 0.1% to 2.5%) of the soil conditioning material is in solid state at the time of coating, as discussed above, this is not enough to satisfy the limitation "in a solid state at time of coating," recited in claims 1 and 7. As such, Scotts Company has not shown, by a preponderance of the evidence, that Roth anticipates 1, 2, 5, 7, 8, 11, 13, and 14.

D. Obviousness over Roth and Lowe—Claims 1-5, 7-11, 13, and 14

Roth teaches the claimed "seed acting as a core or pseudo core" with a "coating of a composition comprising soil conditioning materials," as required by claims 1 and 7. Specifically, Roth describes coating seeds with a methanol treated "sludge" carrier having one or more agricultural chemicals dispersed therein, wherein the source material is "municipal sewage," as required by dependent claims 2, 5, 8, and 11. *See, e.g.*, Ex. 1003, col. 3, ll. 23-26. Roth also discloses that its coating may include a "binder," e.g., polyvinyl alcohol, starch derivatives,

and further may include a fertilizer, as recited in claims 13 and 14. *Id.* at col. 2, ll. 3-5, 48-51; col. 5, ll. 49-52. Thus, we determine that Roth discloses all the limitations of claims 1, 2, 5, 7, 8, 11, 13, and 14 with the exception of "in a solid state at time of coating," as required by independent claims 1 and 7.

Lowe discloses coating a seed with de-inked paper sludge having a "fiber content of the solids in the mixture should exceed at least 10%-15% by weight," thereby teaching "in a solid state at time of coating." Ex. 1004, col. 3, ll. 17-21. Lowe also discloses using "agglomeration" to combine the fibers to form individual granules. *Id.* at Abstract; col. 3, ll. 21-22. Thus, as discussed in greater detail below, Lowe in combination with Roth satisfies the limitations of independent claims 1 and 7 as the combination involves the use of known components for their known purpose to achieve a predictable result.

Lowe further teaches coating a seed with a material that is a byproduct of a "paper making process," and specifically that the byproduct is "paper sludge," as required by dependent claims 3, 4, 9, and 10. Lowe describes an agricultural granule for carrying and releasing agricultural chemicals that resembles a clay-based granule. *Id.* at Abstract. The agricultural granule is made from using waste materials from paper manufacture, referred to as paper sludge. *Id.* at col. 1, l. 68–col. 2, l. 1; col. 2, ll. 40-44.

Scotts Company asserts that because Roth teaches a MAS carrier for agricultural chemicals that can coat a seed, and because Lowe likewise teaches an agricultural carrier consisting of paper sludge, a person of ordinary skill in the art would have had reason to substitute Lowe's paper mill sludge for Roth's MAS coating. Pet. 57.

Encap asserts that the proposed combination runs contrary to the disclosure of Roth. PO Resp. 43. In particular, Encap asserts that Lowe requires the fiber content of the finished particle be above 10%, which means, therefore, that the material is 90% or less filler. *Id.* (citing Ex. 1004, col. 4, ll. 65-66; col. 6, ll. 53-63). On the other hand, Roth discloses MAS that is 97.5%-99.9% liquid. *Id.* (citing Ex. 1003, col. 3, ll. 50-51). Encap asserts that a product that is 97.5% or more liquid could not be replaced by a product with 10% or more fiber content and still be sprayed. *Id.* (citing Ex. 1020 ¶ 22). We do not find Encap's argument persuasive because Roth is not limited to spray-on coatings. The MAS, and presumably Lowe's paper sludge, can be applied to the seeds "by dipping, soaking, spraying, or other conventional mode of application." Ex. 1003, col. 4, ll. 48-50.

Encap also asserts that Roth's disclosure of a coating with 0.1% to 2.5% solids teaches away from using Lowe's coating containing over 10% solids. PO Resp. 43. Roth, however, "does not criticize, discredit, or otherwise discourage" the use of a higher percentage of solids. *In re Fulton*, 391 F.3d 1195, 1201 (Fed. Cir. 2004). Thus, Encap's argument is not persuasive.

Encap further asserts that paper sludge and MAS have very different characteristics. PO Resp. 44-45. In particular, Encap asserts that attempting to coat a seed with paper sludge, using the agglomeration process disclosed in Lowe, would not have a reasonable likelihood of success. *Id.* at 46. In support of its assertion, Encap submits the Declaration of Mr. Madigan (Ex. 1020) who testifies as to the difficulties associated with coating seeds with paper sludge utilizing the agglomeration process of Lowe. *Id.* We do not credit Mr. Madigan's declaration as it fails to provide the underlying basis for his conclusions. For example, Mr. Madigan cites an attachment that purports to show what a final product of Lowe

would look like if seed is introduced into the agglomeration process of Lowe. Ex. 1020, ¶ 23 and Attachment 5. Mr. Madigan, however, does not provide sufficient details regarding the underlying testing upon which he appears to rely. 37 C.F.R. § 42.65. Further, Scotts Company combined the paper sludge of Lowe (not its agglomeration process) with Roth. *See, e.g.*, Pet. 57.

As to Encap's assertion that Roth in view of Lowe does not disclose a "combination seed capsule," as discussed above, the preamble recitation "combination seed capsule" is not an additional structural limitation on the claim. PO Resp. 47.

Lastly, Encap asserts that Lowe's paper sludge is not a "soil conditioning material." *Id.* (citing Ex. 2007 ¶ 19). Paragraph 19 of Mr. Katers' Declaration, however, does not support Encap's contention. Mr. Katers merely states that "[n]ot all paper sludge material would benefit the soil to which it is applied;" he does not state that Lowe's paper sludge is not beneficial to the soil. Ex. 2007 ¶ 19.

We, therefore, conclude that the ordinary artisan would have combined Roth and Lowe to arrive at the claimed composition.

E. Anticipation by Schreiber—Claims 1, 7, and 13

Schreiber discloses the limitations of claims 1 and 7. For example, Schreiber discloses a plant seed having multiple coatings thereon, which satisfies the claimed "seed acting as a core or pseudo core." Ex. 1002, col. 1, ll. 4-6; col. 9, ll. 38-43. Schreiber further discloses the claimed "coating of a composition comprising soil conditioning materials." Specifically, Schreiber describes a seed coating made of a composition comprising solid particulate coating material, such as ground peat moss, thereby satisfying the claimed "being in a solid state at time of coating," of claims 1 and 7. *Id.* at col. 2, ll. 34-49; col. 10, ll. 40-42. Schreiber

explains that its invention permits the tailoring of seed coatings for achieving optimum germination and growth, while allowing early planting within a wide time period. Schreiber also explains that other advantages also accrue from the invention. Schreiber, thus, satisfies our construction of "soil conditioning materials" because its coating provides better root development and drought resistance. *Id.* at col. 2, ll. 15-19; col. 9, ll. 44-49. Schreiber also discloses that the coating is an "agglomeration" of a plurality of types of materials, as Schreiber explains that the coating composition includes a "binder," required by claim 13, or a plasticizer, and that the coating layers may coalesce, thereby satisfying the agglomeration requirement of claim 7. *Id.* at col. 2, ll. 37-39, 55-56; col. 3, ll. 35-42; col. 6, ll. 23-32.

Encap asserts that Schreiber does not disclose a "combination seed capsule." PO Resp. 18-23. For the reasons discussed above, a "combination seed capsule" found in the preamble of claims 1 and 7 does not further limit the claim. Encap also asserts that Schreiber does not disclose a "soil conditioning material." *Id.* at 23-26. Schreiber, however, discloses peat moss, limestone, gypsum, and vermiculite. Ex. 1002, col. 2, ll. 44-49. Those materials are known to beneficially modify the soil in some way other than direct provision of plant nutrients, and are, thus, "soil conditioning materials," as recited in claims 1 and 7. *See, e.g.*, Exs. 1028-1031. Encap's expert, Mr. Baker, acknowledged that peat moss, limestone, gypsum, and vermiculite are all soil conditioning materials. Baker Depo., Ex. 2005, 88, l. 22– 90, l. 9.[8]

[8] We reference page numbers found in the lower right corner of the exhibit.

Encap seeks to distinguish Schreiber on a purported difference in the function of the Schreiber coating and those disclosed in the '259 patent. Specifically, Encap asserts that Schreiber discloses using a water-insoluble coating with a water-soluble binder (e.g., peat moss) to delay germination until growing conditions are favorable, whereas, the soil conditioning materials of the '259 patent enhance germination and plant growth. PO Resp. 25. For the reasons already discussed, the claim limitation "soil conditioning materials" does not require the material also provide soil conditioning value to the seed. Moreover, the '259 patent explicitly discloses that the coating may be used to delay germination. Ex. 1001, col. 4, ll. 12-20; col. 25, ll. 8-17. Just because Schreiber's coating also serves to delay germination does not mean that it is not a "soil conditioning material," so long as it beneficially modifies the soil, in some way other than direct provision of plant nutrients.

In summary, we hold that Scotts Company has shown, by a preponderance of the evidence, that claims 1, 7, and 13 are anticipated by Schreiber, under 35 U.S.C. § 102(b).

F. Obviousness over Schreiber and Roth—Claims 2, 5, 8, 11, and 14[9]

As discussed above, Schreiber discloses the elements of independent claims 1 and 7. Scotts Company proposes using Roth's MAS in place of Schreiber's peat moss. Pet. 38-39. Scotts Company's proposed combination would result in a seed coated with Roth's MAS, and as discussed above, MAS does not satisfy the claim limitation that the soil conditioning material be "in a solid state at the time of coating."

---

[9] In its Response, Encap references claim 15 instead of 14. We have interpreted Encap's reference as intended to be to claim 14. PO Resp. 26-27.

Therefore, we hold that Scotts Company has not shown, by a preponderance of the evidence, that claims 2, 5, 8, 11, and 14 are unpatentable over Schreiber and Roth, under 35 U.S.C. § 103(a).

G. Obviousness over Schreiber and Lowe—Claims 3, 4, 9, and 10

As discussed above, Schreiber discloses the elements of independent claims 1 and 7. Lowe further teaches a material that is a byproduct of a "paper making process," and specifically that the byproduct is "paper sludge" as required by dependent claims 3, 4, 9, and 10. Lowe describes an agricultural granule for carrying and releasing agricultural chemicals that resembles a clay-based granule. Ex. 1004, Abstract. The agricultural granule is made from using waste materials from paper manufacture, referred to as paper sludge. *Id.* at col. 1, l. 68–col. 2, ll. 1, 40-44. Scotts Company asserts that because Lowe teaches an agricultural granule made from paper sludge for carrying and releasing incorporated agricultural chemicals that resembles a clay-based granule (*id.* at Abstract; col. 2, l. 1), a person of ordinary skill would have had reason to substitute Schreiber's water-insoluble, solid, clay-like, agricultural inner coating material with Lowe's paper sludge materials. Pet. 40.

Schreiber discloses that its inner coating controls permeability of water and is typically water insoluble. Ex. 1002, col. 2, ll. 34-39. Encap asserts that there is no evidence that Lowe's material, derived from paper sludge, would operate to control water permeability (i.e., is water insoluble)—a trait important to the teachings of Schreiber. PO Resp. 28. Scotts Company does not respond to Encap's argument, and fails to provide any evidence that Lowe's agricultural granule is water insoluble. If Lowe's material is water soluble, it would not be a

suitable replacement for Schreiber's inner coating, as it would frustrate Schreiber's objective of delayed germination.

In summary, we hold that Scotts Company has failed to show, by a preponderance of the evidence, that claims 3, 4, 9, and 10 are unpatentable over Schreiber and Lowe under 35 U.S.C. § 103(a).

H. Anticipation by Matthews—Claims 1, 2, 7, 8, 13, and 14

Matthews discloses the claimed "seed acting as a core or pseudo core" with a "solid" "coating of a composition comprising soil condition materials," as required by claims 1 and 7. Ex. 1007, 2, ll. 41-89. Specifically, Matthews describes a seed pellet product coated with "fly ash," as required by dependent claims 2 and 8. *Id.* at 2, ll. 10-12, 61-64. Mathews further describes alternatingly spraying and dusting the seed with the coating until the desired thickness is reached, after which the seed pellets are dried. *Id.* at 2, ll. 81-84, 88-89. Matthews also discloses that the coating is an "agglomeration" of a plurality of types of materials, as required by claim 7, because Matthews explains that the coating of dust particles is bound by an adhesive water-soluble plastic, such as polyvinyl alcohol or methyl cellulose, around and about the original seed particle. *Id.* at 2, ll. 42-45, 50-54; 3, ll. 5-9. Matthews describes applying a "binder," as required by dependent claim 13, to the seed capsule, e.g., polyvinyl alcohol, to hold the coating substances firmly on the seed. *Id.* at 2, ll. 42-45; 3, ll. 5-9. Further, the Matthews seed coating may include "fertilizer," thus satisfying dependent claim 14. *Id.* at 5, ll. 25-27.

Encap asserts that Matthews does not disclose a "combination seed capsule." PO Resp. 38. As discussed above, the preamble recitation "combination seed capsule" does not further limit the claim. In addition, Encap unpersuasively asserts

that Matthews' fly ash may not be necessarily beneficial to the seed (*id.*)—a requirement lacking from our claim construction of "soil conditioning material." Relying upon Messrs. Baker and Katers, Encap asserts that Matthews' fly ash does not *necessarily* modify the soil in a beneficial manner, and hence, has not been proved to be a soil conditioning material. *Id.* at 39-42 (citing Ex. 2011 ¶ 21; Ex. 2007 ¶ 24). Essentially, Encap's argument is that while fly ash is specifically identified in the '259 patent as a soil conditioning material (*see, e.g.*, Ex. 1001, col. 7, ll. 21-25), not *all* fly ash is suitable—indeed, some types of fly ash are toxic. *Id.* Matthews, however, states that "[e]ach material must be stable and non-toxic." Ex. 1007, 8, ll. 9-10. Moreover, Mr. Baker also acknowledged that a person of ordinary skill would have understood that a non-toxic fly ash could be used to coat a seed as a soil condition material, and that using toxic materials harmful to the seed should be avoided. Ex. 2005, 150, l. 18–151, l. 20. Lastly, Matthews also discloses that the use of its coating materials "aid in germination" and "growth of the plant." Ex. 1007, 2, ll. 33-39. Thus, we determine that a person of ordinary skill would interpret Matthews as using non-toxic fly ash, beneficial to the soil.

Matthews also discloses using lime (*id.* at 5, ll. 28-35), which Mr. Krysiak admitted was a soil condition material (Ex. 2002, 148, ll. 18-23).

Therefore, we hold that Scotts Company has shown, by a preponderance of the evidence, that claims 1, 2, 7, 8, 13, and 14 are anticipated by Matthews under 35 U.S.C. § 102(b).

I. Secondary Considerations

Before we can determine that the combination of Roth and Lowe (*see* Section D, above), renders the challenged claims unpatentable as obvious, we must consider the evidence of obviousness anew in light of any evidence of secondary

considerations of nonobviousness presented by Encap. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966) ("Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy."); *Transocean Offshore Deepwater Drilling, Inc. v. Maersk Drilling USA, Inc.*, 699 F.3d 1340, 1349 (Fed. Cir. 2012) ("This objective evidence must be 'considered as part of all the evidence, not just when the decisionmaker remains in doubt after reviewing the art.'") (quoting *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538-39 (Fed. Cir. 1983)).

Encap alleges copying by others, long felt need, and commercial success as secondary considerations of non-obviousness. PO Resp. 48-49. Encap, however, fails to provide sufficient credible evidence to support its allegations.

Encap alleges that Scotts Company's Miracle-Gro® Turf Builder Grass Seed with Water Smart® is a copy of the product of the '259 patent. *Id.* at 48. To support its allegations, Encap submits a copy of marketing brochures for EncapSeed™ products (Ex. 1009, 89-97), a copy of the packaging from Scotts Company's Turf Builder Grass Seed with Water Smart® (*id.* at 98-101; Ex. 2013, 342-43, 346-47), a copy of a website print out pertaining to Scotts Company's TurfBuilder (Ex. 2013, 344-45), a Declaration by Mr. Krysiak dated October 31, 2012 and submitted during an *ex parte* reexamination (Ex. 1009, 118-131), and a Declaration by Mr. Krysiak (Ex. 1022 ¶¶ 41, 42). None of the evidence submitted by Encap, however, demonstrates that Scotts Company's Miracle-Gro® Turf Builder Grass Seed with Water Smart® product falls within the scope of any claim of the '259 patent, that Scotts Company was aware of the '259 patent prior to

developing its product, or that Scotts Company developed its product by copying the '259 patent.

Encap also asserts that there was a long-felt need for invention disclosed in the '259 patent. PO Resp. 48-49. Specifically, Encap asserts that many homeowners could not get their grass seed to grow because of inappropriate watering. *Id.* at 48. Encap, however, presents no credible evidence this need was satisfied by the '259 patented invention.

Lastly, Encap asserts commercial success because Meadowland took a license to the '259 patent. *Id.* at 49. Encap, however, does not allege that Meadowland's licensed product was commercially successful, or that any such commercial success was attributable to the patented features of the product. Encap also asserts that Scotts Company's product was commercially successful. *Id.* Encap, however, does not provide persuasive evidence that Scotts Company's product is covered by any claim of the '259 patent, that such product was commercially successful, or that such success was attributable to the patented feature.

After weighing all the evidence of obviousness and nonobviousness of record, on balance, we conclude that the strong evidence of obviousness outweighs the weak evidence of nonobviousness. For the foregoing reasons, we conclude that Scotts Company has shown, by a preponderance of the evidence, that claims 1-5, 7-11, 13, and 14 are unpatentable under 35 U.S.C. § 103(a) over Roth and Lowe.

J. Encap's Corrected Motion to Amend Claims

Encap filed a Motion to Amend Claims (Paper 24), which was later corrected (Paper 47) ("Mot."). In the Corrected Motion, Encap proposes substitute

claims 15-24, to replace claims 2-5, 8-11, 13, and 14,[10] respectively. Mot. 1. The Corrected Motion is contingent, meaning that a proposed substitute claim is at issue and would be considered only if "the original claims of the '259 patent are found unpatentable." *Id.* While somewhat ambiguous, we interpret Encap's motion as proposing a substitute claim if the claim it replaces is found unpatentable, as opposed to being contingent on all of the challenged claims being found unpatentable. Scotts Company has demonstrated the unpatentability of claims 1-5, 7-11, 13, and 14. Therefore, the contingency has materialized, and thus, we consider the Corrected Motion on the merits.

As the moving party, Encap bears the burden of proof to establish that it is entitled to the relief requested. 37 C.F.R. § 42.20(c). The proposed amendment is not entered automatically, but only upon Encap's having demonstrated the patentability of those substitute claims. Here, we find that Encap has failed to demonstrate that the added limitations distinguish over the known prior art, for example, Roth in combination with Lowe. Hence, Encap's Motion to Amend is denied.

In a conference call on August 26, 2013, we provided Encap guidance on filing a motion to amend the claims, and specifically directed the parties to the analysis in *Idle Free Sys. v. Bergstrom, Inc.*, IPR2012-00027, Paper 26 (PTAB June 11, 2013). The summary of the call is reflected in Paper 17 of the record. *Idle Free* holds that a patent owner should specifically identify features added to

---

[10] Encap later identifies the substitution as claims 15-24 in place of claims 2-5 and 11-13. Mot. 2-5. Thus, it is unclear whether claims 23-24 are proposed as replacement for claims 13 and 14, or for claims 12 and 13. However, as we discuss below, the issue is moot.

each substitute claim, and come forward with technical facts and reasoning about those features, including construction of new claim terms. *Idle Free*, slip op. at 7. The patent owner should also discuss the "significance and usefulness" of the added features "from the perspective of one with ordinary skill in the art." *Id.* We agree with the reasoning in *Idle Free*, and conclude that Encap has failed to satisfy its burden to demonstrate the patentability of the proposed substitute claims by a preponderance of the evidence.

While Encap identifies nineteen separate "structural limitations," presumed to be new, it does not identify how each of these structural limitations differs from what is previously recited in the claims. 37 C.F.R. § 42.221(b) ("A motion to amend claims must . . . show the changes clearly . . . ."). Specifically, Encap's listing of proposed claims 15-24 does not show, by redline or discussion, how the claims being replaced have been modified. Mot. 1-5. Moreover, Encap fails to construe any new claim limitation, and also fails to proffer any technical facts and reasoning about the amended features. *Idle Free,* slip op. at 7. Encap's failure to comply with the Board's directive places Scotts Company in the unfair position of having to ascertain the claim amendments and then make assumptions about which of the amendments are considered by Encap to be significant. For amended claims, however, the burden "is not on the petitioner to show unpatentability;" it is "on the patent owner to show patentable distinction over the prior art." *Id.* at 7. Encap has not met its burden.

For example, to determine the differences between original claim 2 and its proposed substitute, claim 15, the following comparison was created, with bracketed text indicating material deleted from claim 2, and underlined text indicating material inserted into claim 2 (paragraphing added).

[2] 15. The combination seed capsule of claim 1 wherein [material of said soil conditioning materials are comprised of sludge or fly ash] said combination seed capsules provides cooperative and beneficial effects of said soil conditioning material working together in controlled intimate relation with said seed, to enhance the germination and growth processes of said seed and the plant emergent therefrom, said effects being greater than when said soil conditioning material and said seed are applied to the soil separately; wherein said effects result from an intimate relationship of said soil conditioning materials in said combination seed capsule, whereby said materials cooperate with each other in support of said germination and growth processes;

said soil conditioning material is a material that beneficially modifies soil in some way other than direct provision of fertilizer, used with said seed to provide soil conditioning value to said seed so coated, irrespective of general tilth condition of the growth medium into or onto which the seed capsule is applied;

said solid state at time of coating comprising materials in form of a particulate material, fibrous material, a suspension of said particulate and/or fibrous material in a liquid suspension, or any combination thereof; said soil conditioning value of said soil conditioning material to said seed comprises the enhanced control of moisture about said seed; said enhanced control consists of absorbing and holding water;

said coating of said combination seed capsule comprises a plurality of particles.

Encap does not explain why each new feature is "significant and useful," does not construe any of the new claim limitations, nor proffer any technical facts and reasoning about the amended features. Instead, Encap provides conclusory statements only, such as "Roth does not provide the cooperative and beneficial

effects of this structural limitation." Mot. 6. Encap does not provide a proposed interpretation of the recited "cooperative and beneficial effects" of proposed substitute claim 15, nor does it explain whether Roth provides some of the "effects of this structural limitation," and not others or why.

Encap asserts that the structural limitations themselves provide the technical facts and reasoning, as well as the significance and usefulness of the limitations. Pet. Reply 3. Encap asserts also that the "[c]laim construction of the structural limitations is found within the limitations themselves." *Id.* We disagree. Providing "cooperative and beneficial effects" is vague and not self-defining, in any meaningful way. Consequently, the usefulness and significance of the limitation is not self-evident. The same can be said of, "working together in controlled intimate relation."

Encap also fails to "provide meaningful reasons" for making additional changes to dependent claims. *Idle Free*, slip op. at 9. For example, claim 18, which depends from claim 15, adds three new limitations. *See* Mot. at 3; *see also id.* at 3-4 (claims 19 and 20 both depend from claim 17, and only differ by inclusion of a fungicide in claim 19). But Encap fails to explain why the additional features were added to these dependent claims. *Idle Free*, slip op. at 9-10 ("Adding features for no meaningful reason is . . . not responsive to an alleged ground of unpatentability.").

In addition, *Idle Free* further instructs patent owners to consider and distinguish "prior art," both "of record" and "not of record but known to the patent owner." *Id.* at 7. Moreover, we specifically explained to Encap that "[a] conclusory statement that no prior art is known to the patent owner . . . is insufficient." IPR2013-00110, Paper 17, 2. On page 1 of its Motion (Paper 47),

Encap states, "No closer art than the prior art cited in the underlying *inter partes* review is known to PO." Encap, however, was aware of additional relevant prior art, including Simmons and Evans, which were cited in Scotts Company's request for *inter partes* review, but which were deemed cumulative of the adopted grounds of rejection. *See* Pet. at 41-49; Prelim. Resp. at 25. While those references may have been cumulative over the original claims, they are not be cumulative in view of Encap's proposed substitute claims, and should be addressed. Encap's proposed claim 15 recites that the soil conditioning material "comprises enhanced control of moisture about said seed" consisting of "absorbing and holding water." Encap distinguishes the prior art in this *inter partes* review by arguing that it does not teach enhancing moisture about the seed. Mot. at 9-10. Simmons and Evans specifically disclose coating a seed with a water-absorbable polymer. Yet, Encap failed to distinguish its proposed claims over those two material prior art references.

Encap attempts to correct some of its errors by filing an expert declaration with its Corrected Reply to Motion to Amend. Paper 49; Ex. 2012. As already addressed, however, we exclude this Declaration as untimely and improperly incorporated by reference into Encap's Motion. In addition, as discussed above, the proffered "corrected" Second Declaration of Mr. Krysiak does not overcome Scotts Company's objections, and is thus, excluded.

For the above reasons, Encap's Corrected Motion to Amend Claims is denied as it fails to distinguish over the prior art, for example, Roth in combination with Lowe.

III. CONCLUSION

Scotts Company has shown by a preponderance of the evidence that: (1) claims 1, 7, and 13 of the '259 patent are unpatentable under 35 U.S.C. § 102(b) as anticipated by Schreiber; (2) claims 1, 2, 7, 8, 13, and 14 are unpatentable under 35 U.S.C. § 102(b) as anticipated by Matthews; and (3) claims 1-5, 7-11, 13, and 14 are unpatentable under 35 U.S.C. § 103(a) as obvious over Roth and Lowe.

Scotts Company has not shown by a preponderance of the evidence that: (1) claims 1, 2, 5, 7, 8, 11, 13, and 14 of the '259 patent are unpatentable under 35 U.S.C. § 102(b) as anticipated by Roth; (2) claims 2, 5, 8, 11, and 14 are unpatentable under 35 U.S.C. § 103(a) as obvious over Schreiber and Roth; or (3) claims 3, 4, 9, and 10 are unpatentable under 35 U.S.C. § 103(a) as obvious over Schreiber and Lowe.

Encap has not shown by a preponderance of the evidence that its proposed substitute claims 15-24 are patentable over the prior art.

IV. ORDER

In consideration of the foregoing, it is hereby ORDERED that:

Scotts Company's Motion to Exclude Mr. Krysiak's Second Declaration (Ex. 2016) is granted and all other relief requested in the motion is denied;

Encap's Motion to Exclude Mr. Sundstrom's Declaration (Ex. 1039) is dismissed as moot;

Claims 1-5, 7-11, 13, and 14 of the '259 patent are determined to be unpatentable; and

Encap's Corrected Motion to Amend Claims is denied.

This is a final decision. Parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

Petitioner:

Robert Schulman
rschulman@hunton.com

Jeff Vockrodt
jvockrodt@hunton.com

Patent Owner:

Philip Weiss
weissandweiss@aol.com

Aaron Olejniczak
aarono@andruslaw.com

PTO/SB/123 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

CHANGE OF CORRESPONDENCE ADDRESS
Patent

Address to:
Mail Stop Post Issue
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Patent Number	6,209,259
Issue Date	4/3/01
Application Number	09/113,254
Filing Date	7/10/98
First Named Inventor	D. Madigan
Attorney Docket Number	P/35-145 RE

Please change the Correspondence Address for the above-identified patent to:

☐ The address associated with Customer Number: ____

OR

☒ **Firm or Individual Name** Philip M. Weiss, WEISS & WEISS

Address 410 Jericho Turnpike
Suite 105

City Jericho **State** NY **ZIP** 11753

Country US

Telephone 516-739-1500 **Email** 516-739-2189

This form cannot be used to change the data associated with a Customer Number. To change the data associated with an existing Customer Number use "Request for Customer Number Data Change" (PTO/SB/124).

This form will not affect any "fee address" provided for the above-identified patent. To change a "fee address" use the "Fee Address Indication Form" (PTO/SB/47).

I am the:

☐ Patentee.

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

☒ Attorney or agent of record. Registration Number 34,751

Signature Philip Weiss

Typed or Printed Name Philip M. Weiss

Date November 14, 2012 Telephone 516-739-1500

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Post Issue, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/92 (07-09)
Approved for use through 07/31/2012. OMB 0561-0031
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Certificate of Mailing under 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on 11/14/12
Date

Debbie Broderick
Signature

Debbie Broderick
Typed or printed name of person signing Certificate

Registration Number, if applicable

516-739-1500
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Patent No.___6,209,259_____

NOTICE OF *EX PARTE* REEXAMINATION

Notice is hereby given that a request for *ex parte* reexamination of U.S. Patent No. ___6,209,259___ was filed on ___03/09/12_____ under 35 U.S.C. § 302 and 37 C.F.R. § 1.510(a).

The reexamination proceeding has been assigned Control No. 90/012,183

This Notice incorporates by reference into the patent file, all papers entered into the reexamination file.

Note: This Notice should be entered into the patent file.

AO 120 (Rev. 08/10)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court Eastern District of Wisconsin on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
11-C-685	7/18/2011	Eastern District of Wisconsin

PLAINTIFF	DEFENDANT
Encap LLC	The Scotts Company LLC et al

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	6,209,259	4/3/2001	ENCAP LLC
2	7,412,878	8/19/2008	ENCAP LLC
3	6,745,513	6/8/2004	ENCAP LLC
4			
5			

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading		
	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1			
2			
3			
4			
5			

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
JON W. SANFILIPPO	A. Wachtendonck	7/19/2011

Copy 1—Upon initiation of action, mail this copy to Director **Copy 3—Upon termination of action, mail this copy to Director**
Copy 2—Upon filing document adding patent(s), mail this copy to Director **Copy 4—Case file copy**

COMPLETED

DSD

P/35-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Michael Krysiak

Serial No.: 09/113,254 Date: July 10, 1998

Patent No.: 6,209,259 Issued: April 3, 2001

For: SEEDING TREATMENTS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

CHANGE OF CORRESPONDENCE ADDRESS

Sir:

Please amend the correspondence address for the above-identified patent application to:

Philip M. Weiss, Esq.
Weiss & Weiss
300 Old Country Road, Suite 251
Mineola, New York 11501
Telephone: (516) 739-1500
Telefax: (516) 739-2189

Applicant requests that all future correspondence be mailed to the above-address.

June 29, 2005

Respectfully submitted,

Philip M. Weiss
Registration No. 34,751
Attorney for Applicant
WEISS AND WEISS
300 Old Country Road, Ste. 251
Mineola, New York 11501
Telephone: (516) 739-1500
Telefax: (516) 739-2189

Certificate of Mailing Under 37 C.F.R. §1.8(a)

I hereby certify that this correspondence and any documents attached herewith is being deposited with the U.S. postal service as first class mail in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 on the date indicated below.
Dated: June 29, 2005

Maureen P. Herbst

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231

PHILIP M WEISS
WEISS & WEISS
500 OLD COUNTRY ROAD
GARDEN CITY NY 11530

Mail Date: February 26, 2001
Serial Number: 09/113254
Applicant: MADIGAN

NOTICE TO PAY BALANCE OF ISSUE FEE

Your issue fee payment filed on 12/21/00 has been received. However, new patent fees went into effect on October 1, 2000. The final rule entitled "Revision of Patent Fees for Fiscal Year 2001" was published in the *Federal Register*/Vol. 65, No. 156/Friday, August 11, 2000 [49193–49199] and in the U.S. Patent and Trademark Office *Official Gazette*, August 29, 2000 [1237 OG 131–138]. As stated in the final rule, "Any fee amount that is paid on or after the effective date of the fee increase will be subject to the new fees then in effect." The Notice of Allowance and Issue Fee Due (Form PTOL-85) that was mailed to you prior to October 1, 2000, stated an issue fee amount that was in effect prior to October 1, 2000. However, inasmuch as your issue fee was paid on or after October 1, 2000, the new issue fee amount was due.

In accordance with 37 CFR 1.317, you are given a time period of **THREE (3) MONTHS** from the mailing date of this notice during which to pay the **BALANCE DUE** indicated below. This three-month time period may not be extended. **If your patent issues before the expiration of the three-month period and if you do not pay the balance due before the expiration of the three-month period, your patent will lapse at the termination of the three-month period.**

TYPE OF ISSUE FEE PAID	Column A ISSUE FEE IN EFFECT AS OF OCT. 1, 2000 large entity / small entity	Column B ISSUE FEE PAID	BALANCE DUE [Col. A minus Col. B]
UTILITY	$1,240.00 / $620.00	$ 605.00	$ 15.00
DESIGN	$440.00 / $220.00	$	$
PLANT	$600.0/0 / $300.00	$	$

Office of Patent Publication
Tel: 703-305-8263

You MUST return a copy of this Notice with your payment.

CERTIFICATE OF MAILING

I hereby certify that this notice and the required additional fee are being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to Box ISSUE FEE, Commissioner for Patents, Washington, D.C. 20231 on the date indicated below.

Printed Name: _____ Signature: _____

Date: _____

P135-5

12-22-00

Best Available Copy

PART B - ISSUE FEE TRANSMITTAL

Complete and mail this form, together with applicable fees, to:

Box ISSUE FEE
Assistant Commissioner for Patents
Washington, D.C. 20231

EL 636894151US

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

HM12/0926

PHILIP M. WEISS
WEISS & WEISS
500 OLD COUNTRY ROAD
GARDEN CITY NY ~~11530~~ 11530

Note: The certificate of mailing below can only be used for domestic mailings of the Issue Fee Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

Express

I hereby certify that this Issue Fee Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

Nelly Bronnberg (Depositor's name)

(Signature)

December 21, 2000 (Date)

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
09/113,254	07/10/98	014	GRUNBERG, A 1661	09/26/00
First Named Applicant: MADIGAN,		35 USC 154(b) term ext. =	0 Days.	

TITLE OF INVENTION: SEEDING TREATMENTS

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
3 29214	047-057.600	S82	UTILITY	~~NO~~ YES	$1210.00	12/26/00

1. Change of correspondence address or indication of " Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 ~~Philip M. Weiss, Esq.~~

2 Weiss and Weiss PC

3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)
PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropiate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a subsititue for filing an assignment.

(A) NAME OF ASSIGNEE

Encap, LLC

(B) RESIDENCE: (CITY & STATE OR COUNTRY)

Green Bay, WI

Please check the appropriate assignee category indicated below (will not be printed on the patent)

☐ individual ☒ corporation or other private group entity ☐ government

4a. The following fees are enclosed (make check payable to Commissioner of Patents and Trademarks):

☒ Issue Fee

☐ Advance Order - # of Copies _____

4b. The following fees or deficiency in these fees should be charged to:

DEPOSIT ACCOUNT NUMBER _____

(ENCLOSE AN EXTRA COPY OF THIS FORM)

☐ Issue Fee

☐ Advance Order - # of Copies _____

The COMMISSIONER OF PATENTS AND TRADEMARKS IS requested to apply the Issue Fee to the application identified above.

(Authorized Signature) Philip M. Weiss

Philip M. Weiss-RN: 34,751

(Date) 12/21/00

NOTE; The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington D.C. 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

12/26/2000 MFANAEI1 00000008 09113254

01 FC:631 605.00 OP

TRANSMIT THIS FORM WITH FEE

PTO/SB/10 (1-99)
Approved for use through 09/30/2000. OMB 0651-0031
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

#14
CA3
1-30-01

STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(c))—SMALL BUSINESS CONCERN

Docket Number (Optional) P/35-5

Applicant, Patentee, or Identifier: Encap LLC.
Application or Patent No.: 09/113,254
Filed or Issued: July 10, 1998
Title: SEEDING TREATMENTS

I hereby state that I am
- [x] the owner of the small business concern identified below:
- [ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Encap LLC.

ADDRESS OF SMALL BUSINESS CONCERN 3921 Algoma Raod
Green Bay, WI 54311

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office. Questions related to size standards for a small business concern may be directed to: Small Business Administration, Size Standards Staff, 409 Third Street, SW, Washington, DC 20416.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- [x] the specification filed herewith with title as listed above.
- [ ] the application identified above.
- [ ] the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:
- [x] no such person, concern, or organization exists.
- [ ] each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Michael Krysiak

TITLE OF PERSON IF OTHER THAN OWNER President

ADDRESS OF PERSON SIGNING 3921 Algoma Road, Green Bay, WI 54311

SIGNATURE Michael Krysiak DATE 12-21-00

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

#15KS

P/35-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Encap LLC.

Serial No.: 09/113,254 **Filing Date: July 10, 1998**

For: SEEDING TREATMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

BOX ISSUE FEE

SUBMISSION OF FORMAL DRAWINGS

Sir:

In response to the Notice of Allowability dated September 26, 2000, please find enclosed six (6) sheets of formal drawings containing Figures 1-8.

Application should now proceed to issuance.

Respectfully Submitted,

Philip Weiss

Philip M. Weiss, Esq.
Attorney for Applicant
Reg. No. 34,751
Weiss & Weiss
500 Old Country Road
Suite 305
Garden City, New York 11530

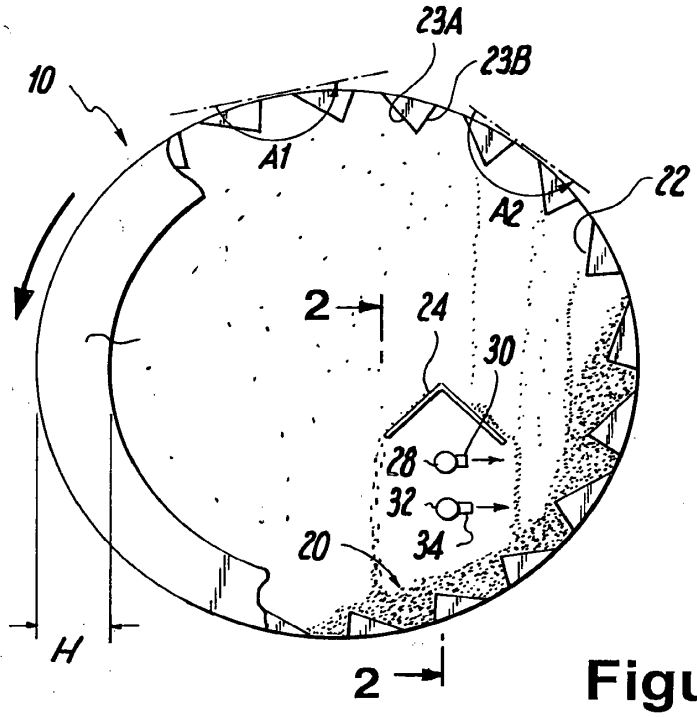


Figure 1

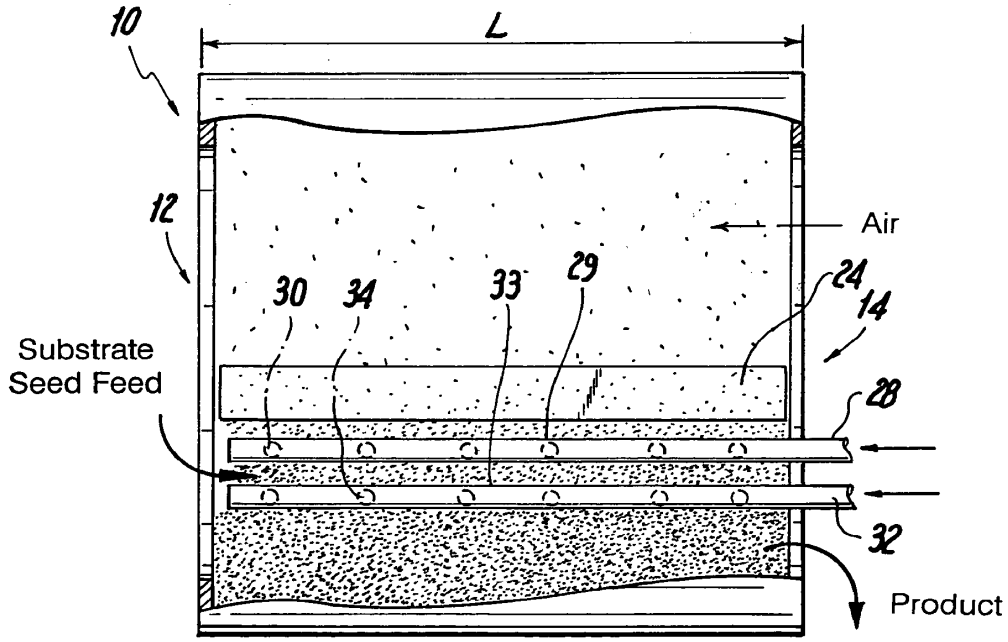
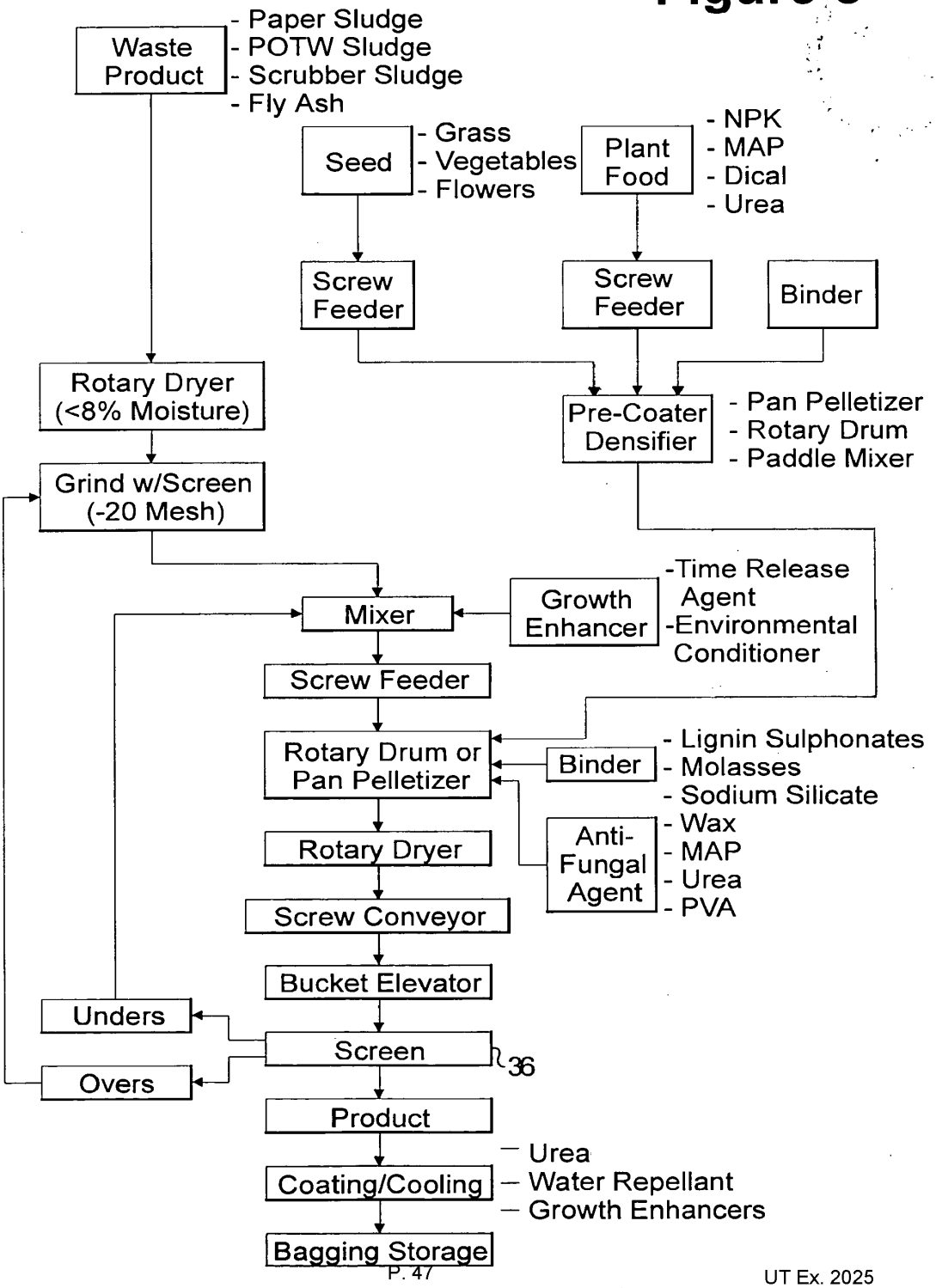


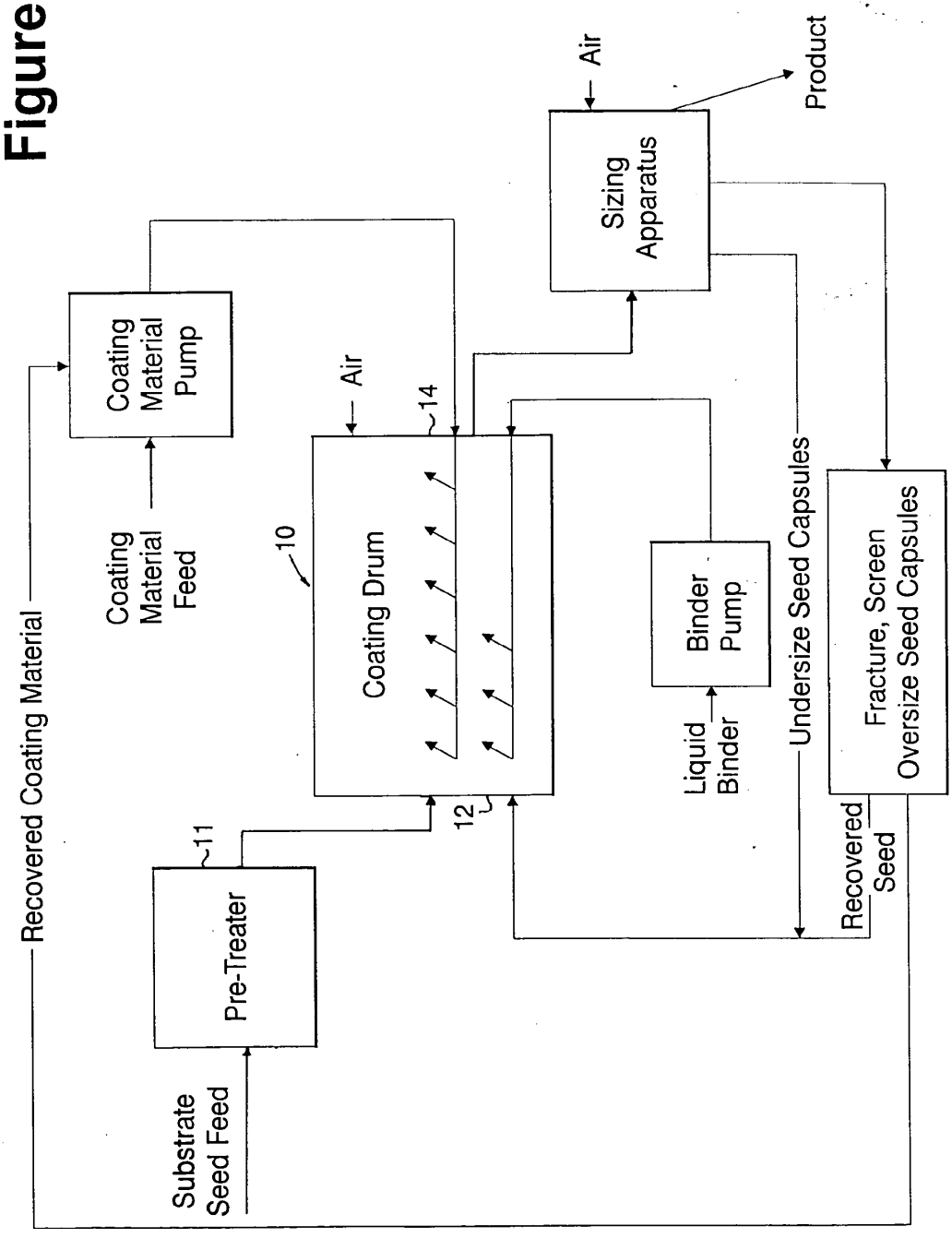
Figure 2

Figure 3



P. 47

Figure 4



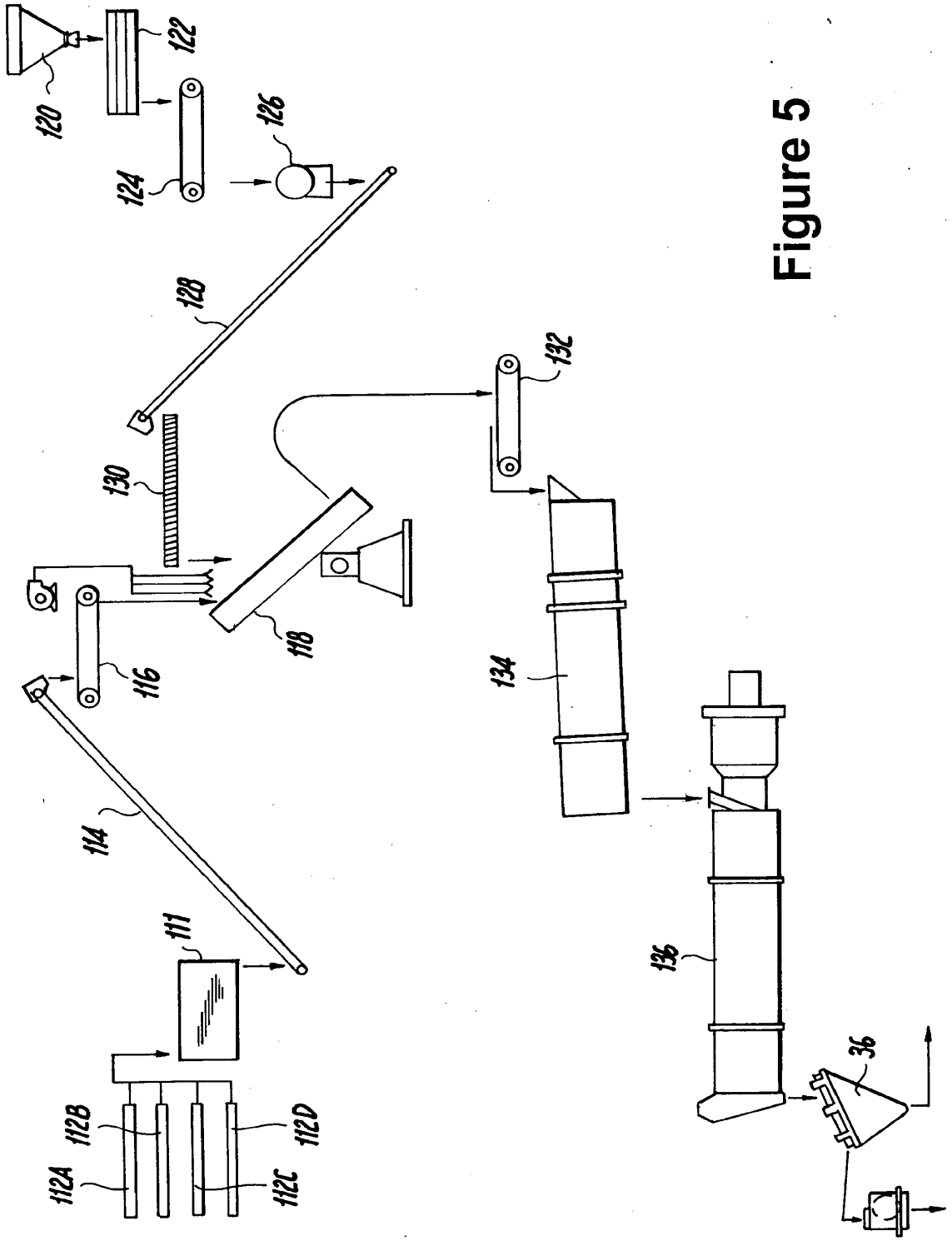


Figure 5

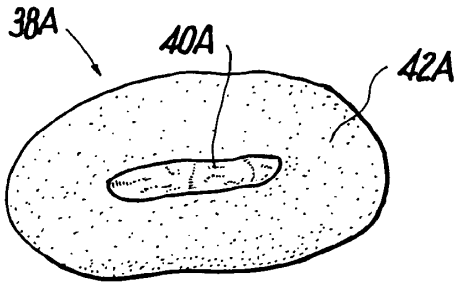


Figure 6A

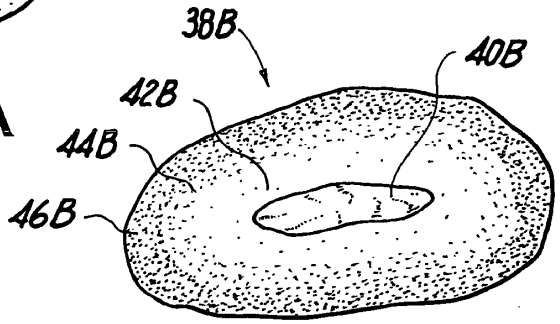


Figure 6B

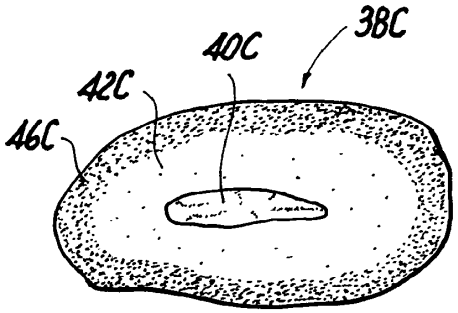


Figure 6C

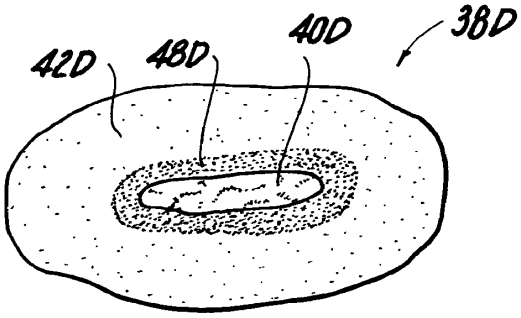


Figure 6D

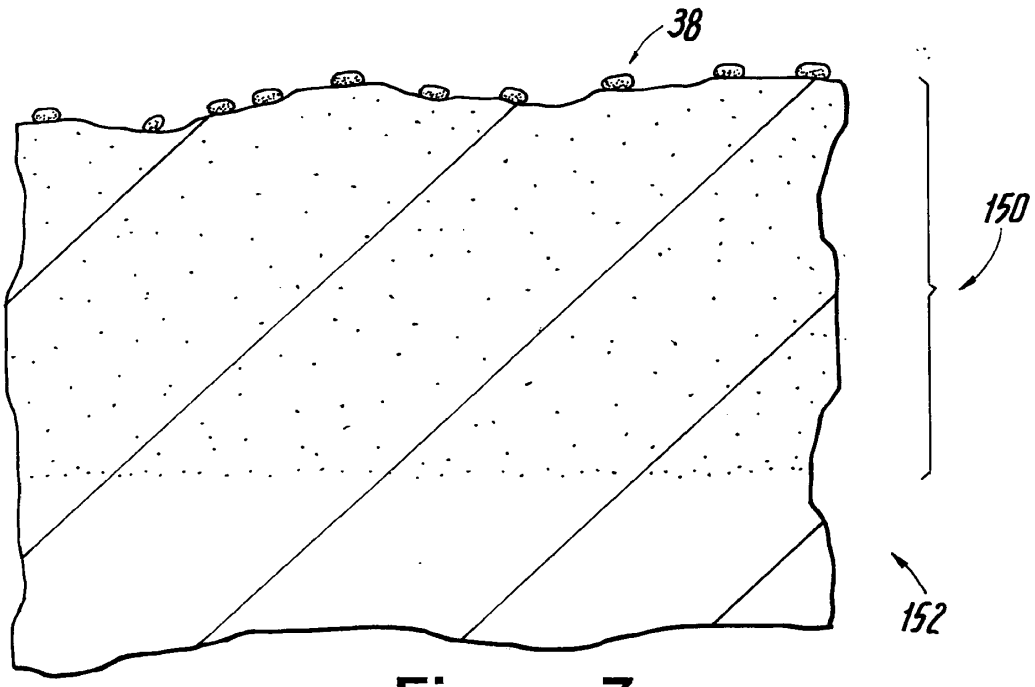


Figure 7

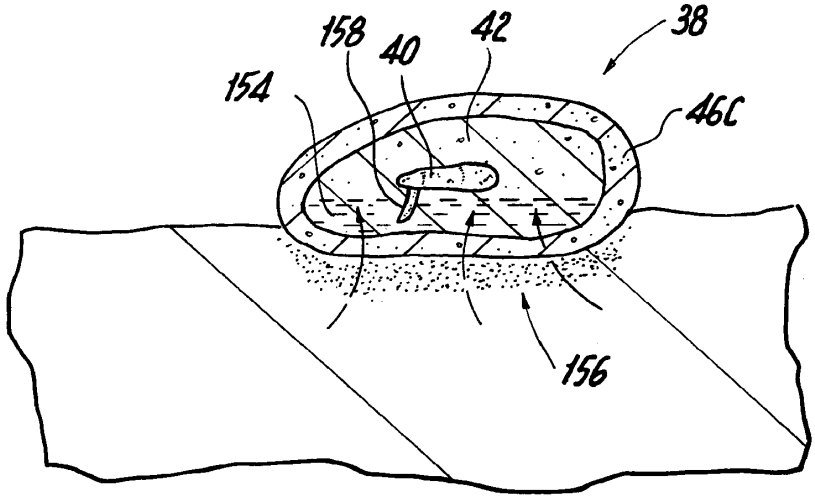


Figure 8

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

V6

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

HM12/0926

PHILIP M. WEISS
WEISS & WEISS
500 OLD COUNTRY ROAD
GARDEN CITY NY 11630

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
09/113,254	07/10/98	014	GRUNBERG, A 1661	09/26/00
First Named Applicant: MADIGAN,			35 USC 154(b) term ext. = 0 Days.	

TITLE OF INVENTION: SEEDING TREATMENTS

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
3 29214	047-057.600	S82	UTILITY	NO	$1210.00	12/26/00

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.
If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
B. If the status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:

A. Pay FEE DUE shown above, or

B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

II. Part B-Issue Fee Transmittal should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B Issue Fee Transmittal should be completed and returned. If you are charging the ISSUE FEE to your deposit account, section "4b" of Part B-Issue Fee Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give application number and batch number.
Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (REV. 10-96) Approved for use through 06/30/99. (0651-0033)

File

Notice of Allowability

Application No.	Applicant(s)
09/113,254	Madigan et al.
Examiner	**Group Art Unit**
Anne Marie Grunberg	1661

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course.

☒ This communication is responsive to *papers faxed 8/21/2000*

☒ The allowed claim(s) is/are *77, 79-81, 83-88, 90-93 (renumbered as 1-14)*

☐ The drawings filed on _____ are acceptable.

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE **THREE MONTHS** FROM THE "DATE MAILED" of this Office action. Failure to timely comply will result in ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

☒ Applicant MUST submit NEW FORMAL DRAWINGS

☐ because the originally filed drawings were declared by applicant to be informal.

☒ including changes required by the Notice of Draftsperson's Patent Drawing Review, PTO-948, attached hereto or to Paper No. 5.

☐ including changes required by the proposed drawing correction filed on _____, which has been approved by the examiner.

☒ including changes required by the attached Examiner's Amendment/Comment.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the reverse side of the drawings. The drawings should be filed as a separate paper with a transmittal lettter addressed to the Official Draftsperson.

☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any response to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☒ Interview Summary, PTO-413

☒ Examiner's Amendment/Comment

☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material

☐ Examiner's Statement of Reasons for Allowance

Bruce Campell

BRUCE R. CAMPELL, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1661.

DETAILED ACTION

Examiner's Amendment

1. An Examiner's Amendment to the record appears below. Should the changes and/or additions be unacceptable to Applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it **MUST** be submitted no later than the payment of the Issue Fee.

IN THE CLAIMS

--7. (Amended) A combination seed capsule comprising:

one viable seed;

said seed acting as a core or pseudo core of said combination seed capsule;

a coating of a composition comprising soil conditioning materials;

said soil conditioning materials being in a solid state at time of coating.--

55

--85. (Amended) A combination seed capsule comprising:

one viable seed;

said seed acting as a core or pseudo core of said combination seed capsule;

a coating of a composition comprising soil conditioning materials;

said coating being applied to said viable seed by an agglomeration operation;

wherein said soil conditioning materials are in a solid state at time of coating.

The above changes were authorized by attorney Phillip Weiss in a telephone interview with Examiner Grünberg on September 8, 2000.

Drawings

2. In order to avoid abandonment, the drawing informalities noted in Paper No. 5, on the Notice of Draftsperson's Patent Drawing Review, and the Office Action, mailed on 18 June, 1999, must now be corrected. Correction can only be effected in the manner set forth in the above noted paper.

2. Any inquiry concerning this or any previous communication from the examiner should be directed to Anne Marie Grünberg whose telephone number is (703) 305-0805. The Examiner can normally be reached Monday through Thursday from 6:30 am to 4:00 pm. The Examiner can also be reached on alternate Fridays from 7:30 am to 4:00 pm.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Bruce Campell, can be reached at (703) 308-4205. The fax phone number for the group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to THE MATRIX CUSTOMER SERVICE CENTER whose telephone number is (703) 308-0196.

Bruce Campell

BRUCE R. CAMPELL, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Anne Marie Grünberg

Interview Summary

Application No.	Applicant(s)
09/113,254	Madigan et al.
Examiner	**Group Art Unit**
Anne Marie Grunberg	1661

All participants (applicant, applicant's representative, PTO personnel):

(1) *Anne Marie Grunberg* (3) _____

(2) *Phillip Weiss* (4) _____

Date of Interview *Aug 8, 2000*

Type: [X] Telephonic [ ] Personal (copy is given to [ ] applicant [ ] applicant's representative).

Exhibit shown or demonstration conducted: [ ] Yes [X] No. If yes, brief description:

_____

_____

Agreement [ ] was reached. [X] was not reached.

Claim(s) discussed: *76-94*

Identification of prior art discussed:
Roth

_____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Claim language was discussed, in particular it was discussed if "wherein said soil conditioning material, when added to the seed, are in a dry, solid form: and," if inserted into the independent claims, would be enough to overcome Roth. It was suggested that the claims be written in a product by process form to clearly distinguish over Roth.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. [ ] It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. [ ] Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

DB Name	Query	Hit Count	Set Name
JPAB,EPAB	l20	0	L21
USPT	l17 and l19	108	L20
USPT	@py<1998	6129483	L19
USPT	l17@py<1998	4294967295	L18
USPT	l16 and l13	120	L17
USPT	((47/57.6)!.CCLS.)	379	L16
USPT	((47/)!.CCLS.)	0	L15
USPT	47.ccls	0	L14
USPT	l8 and l12	1209	L13
USPT	l3 same l4	1298	L12
USPT	l5 and l10	3711	L11
USPT	l3 and l8	5346	L10
USPT	l3 and l8	5346	L9
USPT	l6 or l7	1004442	L8
USPT	powder or powdery or dust or dusty	363204	L7
USPT	dry or solid	889500	L6
USPT	l3 and l4	4196	L5
USPT	soil or earth or ground	568955	L4
USPT	l1 same l2	6208	L3
USPT	coat or coating or coated or agglomerate or agglomeration or agglomerated	533284	L2
USPT	seed	64826	L1

Interview Summary	Application No. 09/113,254	Applicant(s) Madigan et al.
	Examiner Anne Marie Grunberg	Group Art Unit 1661

All participants (applicant, applicant's representative, PTO personnel):

(1) *Anne Marie Grunberg* (3) _____

(2) *Philip Weiss* (4) _____

Date of Interview *Sep 8, 2000*

Type: [X] Telephonic [ ] Personal (copy is given to [ ] applicant [ ] applicant's representative).

Exhibit shown or demonstration conducted: [ ] Yes [X] No. If yes, brief description:

Agreement [X] was reached. [ ] was not reached.

Claim(s) discussed: *77 and 85*

Identification of prior art discussed:
Roth

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Based on the proposed draft amendment and arguments recited therein, the prior art was overcome.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. [X] It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. [X] Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

P/23-3

7/27/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application: Madigan et al.

Serial No.: 09/113,254 **Group Art Unit: 1638**

Filed: July 10, 1998 **Examiner: A. Grunberg**

For: SEEDING TREATMENTS

Box Response
Assistant Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO OFFICE ACTION

The following is in response to the Office Action mailed May 10, 2000.

In the Claims:

Cancel claims 70-73, 76, 78, 82, 89 and 94-100.

Claim 77 (amended) A combination seed capsule comprising:

[at least] one viable seed;

said seed acting as a core or pseudo core of said combination seed capsule;

[coatings] a coating of a composition comprising [a growth enhancer and material fines] soil conditioning materials;

said coating being an integral part of said seed.

Claim 79 lines1-2 change "material fines" to –soil conditioning materials--;

Claim 79 line 2 change "industrial byproduct" to -- sludge or fly ash --;

Claim 80 line 1 change "79" to –77--;

Claim 80 line 2 before "byproduct" insert -- fiber containing --;

Claim 81 line 1 change "79" to –80--;

Claim 83 line 1 change "material fines" to -- soil conditioning materials --;

Claim 84 line 1 change "material fines" to -- soil conditioning materials --;

Claim 84 line 2 change "grassy/woody substances" to -- sawdust --;

Claim 85 line 2 delete "at least";

Claim 85 line 4 change "material fines" to -- soil conditioning materials --;

Claim 85 line 5 change "a lifting and mixing" to -- an --;

Claim 86 lines 1-2 change "material fines" to -- soil conditioning materials --;

Claim 86 line 2 change "industrial byproduct" to -- sludge or fly ash --;

Claim 87 line 2 before "byproduct" insert -- fiber containing --;

Claim 88 line 1 change "86" to –87--;

Claim 90 line 1 change "material fines" to -- soil conditioning materials --;

Claim 91 line 1 change "material fines" to -- soil conditioning materials --;

Claim 91 line 2 "grassy/woody substances" to -- sawdust --;

Claim 93 line 2 change "material fines" to -- soil conditioning materials --;

Claim 93 line 1 change "85" to -- 92 --.

Response

Applicant has canceled claims 70-73, 76, 78, 82, 89 and 94-100. Applicant has amended the claims as requested by the Examiner.

Examiner has rejected claim 77 under 35 U.S.C. §102 as being anticipated by Gerber. Claim 77 has been amended to add the element that the coating being an integral part of said seed. Further, claim 77 has been amended to claim only one viable seed. Gerber teaches a seed capsule having a number of seeds. Paragraph 7 of Krysiak Declaration. Gerber describes a mixture of seeds and loess which are pressed together.

They form a thistle ball. This differs from the encapsulated seed of the present invention because the thistle ball of Gerber includes multiple seeds, loess and the ball is not an integral part of the seed. Further, Gerber does not describe an agglomeration process. Paragraph 8 of Krysiak Declaration.

Examiner has rejected claims 76-78 and 83 under 35 U.S.C. §102 as being anticipated by Roth. Applicant has canceled claim 76 and amended claim 77, 78 and 83. Roth differs from the encapsulated seed of the present invention because Roth does not describe the coating to be an integral part of the seed. Rather, Roth teaches a novel means for releasing sludge into the surrounding soil. In addition, Roth describes the sprayed-on coating as a film with film forming properties. The process described in Roth does not teach the agglomeration process of the present invention. The coating of Roth is described as a thin continuous film. Paragraphs 10 and 11 of Krysiak Declaration.

Examiner has rejected claims 77, 79-81 and 84 under 35 U.S.C. §102 as being anticipated by Nilsson. Nilsson describes the introduction of the seed or seeds into a cover. The cover may be made into halves or parts, at least one part or half of which comprises a suitable recess for the seed or seeds. After introducing the seed into the recess, the capsule parts are secured to each other. Paragraph 13 of the Krysiak Declaration.

Nilsson differs from the present invention because Nilsson does not describe a coating, which is an integral part of the seed. Nilsson describes a shell of paper where the seed is placed within the shell. The shell has spaces which allow gas and liquid to penetrate. Further, Nilsson does not describe an agglomeration process. Paragraph 14 of the Krysiak Declaration.

Examiner has rejected claims 77, 79-81, 84-88 and 91-94 under 35 U.S.C §102 as being anticipated by Loperfido. Loperfido describes coated seeds having a coating comprising non-porous, hydrophobic, non-phytotoxic particles which are adhered to each other and to the seeds by a hydrophilic binder in such a manner that the coating is highly porous and provides facile gas and water exchange between the seed and its environment. Due to the hydrophilic nature of the binder, it will be dissolved readily by soil moisture. Dissolution of the binder destroys the mechanical integrity of the coating. The coating allows the maximum amount of air space in the coated seed. Paragraphs 16-18 of the Krysiak Declaration.

Loperfido differs from the encapsulated seed of the present invention because Loperfido does not teach a coating being an integral part of the seed. Loperfido teaches a binder added to the seed that does not uniformly coat the seed. The coating forms beads that then collect around the seed. The coating formed around the seed is of a highly porous nature. Loperfido describes allowing a maximum amount of air space between the coating and the seed. Paragraph 19 of the Krysiak Declaration.

Examiner has rejected claims 76-94 under 35 U.S.C. §103 as being unpatentable over Loperfido in view of Roth and further in view of Nelson. None of these references describes the coating as being an integral part of these seed.

None of the products described in the prior art patents have ever been made commercially. Paragraph 20 of the Krysiak Declaration. The present invention provides a soil conditioner in intimate association with the seed. The present invention provides a uniformity of coating or coating thickness so that the seed is not on or immediately adjacent an outside surface of the capsule such that the seed may fall out, or be easily

broken out, of the capsule, or easily removed by dissolution of materials at and near the surface of the seed capsule. Paragraph 22 of the Krysiak Declaration.

The present invention applies a seed in a seed capsule wherein the seed is intimately combined with a soil conditioning material in a common particle. This was not taught prior to the present invention. After a review of the prior art provided by the Examiner, this statement is still true. Paragraph 24 of the Krysiak Declaration.

Figures 6A-6D of the present invention illustrate the seed in intimate association with the soil conditioning material. The present invention comprises a combination seed capsule having a viable seed acting as a core or pseudo core. A coating of a composition comprising a soil conditioning material is an integral part of the seed. None of the prior art describes these elements. Further, where the coating is applied in an agglomeration operation is also not described in the prior art. Paragraph 29 and 30 of the Krysiak Declaration.

Enclosed is a sample of EncapSeed which was prepared according to the method described in the present invention. As shown by the enclosed EncapSeed, the coating is an integral part of the seed. The seed (an all-purpose grass seed mixture) comprises 32% of the overall product weight. The blanket that is wrapped around the seed is comprised of dicalcium phosphate (.8%) and dried, ground paper sludge (67.2%). The dried, ground paper fines range in size from approximately 30 mesh to approximately 200 mesh. Of this total material, 68.5% is comprised of inert material. The EncapSeed coating has no visible spaces between the coating and the seed is designed to act as the microenvironment for the seed for the germination process. Field tests by the University

of Wisconsin-Madison's Horticultural Department have shown that the EncapSeed blanket helps to enhance turf establishment. Paragraph 31 of the Krysiak Declaration.

Applicant now believes that the application is in condition for allowance.

Respectfully submitted,

Philip M. Weiss, Esq.
Reg. No.: 34,751

07-14-00 W EAY 1638
Box SEQ

P/23-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application: Madigan et al.

Serial No.: 09/113,254 **Group Art Unit: 1638**

Filed: July 10, 1998 **Examiner: A. Grunberg**

For: SEEDING TREATMENTS

Box Response
Assistant Commissioner for Patents
Washington, D.C. 20231

Enclosed please Response to Office Action, Declaration of Michael Krysiak, Seed Sample. Please stamp postcard and return.

Respectfully submitted,

Philip Weiss

Philip M. Weiss, Esq.
Reg. No.: 34,751

Express Mail mailing label No.: EL636894240US

Date of Deposit: July 13, 2000
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, DC 20231

7/13/00
Date

#12
7-27-00

P/23-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application: Madigan et al.

Serial No.: 09/113,254 Group Art Unit: 1638

Filed: July 10, 1998 Examiner: A. Grunberg

For: SEEDING TREATMENTS

DECLARATION OF MICHAEL KRYSIAK

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Mike Krysiak, residing at 3554 Highland Center Drive, Green Bay, Wisconsin, 54311 declares as follows;

1. I graduated from the University of Wisconsin-Milwaukee majoring in Industrial Engineering.

2. I have given various presentations relating to seed encapsulation, green building, quality and service throughout the United States.

3. I worked for FEECO International, Inc. as Manager of Quality and Service for six years. FEECO designs, builds and installs material processing equipment for companies in the environmental and fertilizer markets. During my last two years at FEECO I worked on the development of the EncapSeed products in our Pilot Lab. Prior to FEECO, I worked at Krueger International (KI) as an Industrial/Quality Engineer.

4. I presently am the President and CEO of Encap. Encap is in the business of encapsulating seeds.

5. I am a named inventor of the 09/113,254 patent application. I have reviewed the Office Action dated May 10, 2000.

6. I have reviewed the Examiner's rejection regarding Gerber and have reviewed the Gerber Patent.

7. Gerber teaches a seed capsule having a number of seeds not more than 4 percent of the total weight of the capsule. Col. 5 lines 23-27.

8. Gerber describes a mixture of seeds and loess which are pressed together. They form a thistle bull. This differs from the encapsulated seed of the present invention because the thistle ball of Gerber includes multiple seeds, loess and the ball is not an integral part of the seed. Further, Gerber does not describe an agglomeration process.

9. I have reviewed the Examiner's rejection over Roth and have reviewed the Roth patent.

10. Roth describes coating crop seeds with an MAS carrier having one or more agricultural chemicals dispersed therein. The process of coating is described as dipping, soaking, spraying, or other conventional mode of application. Col. 4 lines 46-50. Crop seeds described are corn, sorghum and soy. Col. 4 lines 60-62. The coating is described as a thin continuous film. Col. 4 lines 3-5.

11. Roth differs from the encapsulated seed of the present invention because Roth only describes spraying sludge on seeds. The spray does not become an integral part of the seed. Nor does the spraying describe an agglomeration process.

12. I have reviewed the Examiner's rejection over Nilsson and have reviewed the Nilsson patent.

13. Nilsson describes the introduction of the seed or seeds into a cover. The cover may be made into halves or parts, at least one part or half of which comprises a suitable recess for the seed or seeds. After introducing the seed into the recess, the capsule parts are secured to each other. Col. 2 lines 11-25, Col. 3 lines 45-52.

14. Nilsson differs from the present invention because Nilsson does not describe a coating which is an integral part of the seed. Nilsson describes a shell of paper where the seed is placed within the shell. The shell has spaces which allow gas and liquid to penetrate. Further, Nilsson does not describe an agglomeration process.

15. I have reviewed the Examiners rejection over Loperfido and have reviewed the Loperfido patent.

16. Loperfido describes coated seeds having a coating comprising non-porous, hydrophobic, non-phytotoxic particles which are adhered to each other and to the seeds by a hydrophilic binder in such a manner that the coating is highly porous and provides facile gas and water exchange between the seed and its environment. Abstract of the Invention.

17. Due to the hydrophilic nature of the binder, it will be dissolved readily by soil moisture. Dissolution of the binder destroys the mechanical integrity of the coating. Col. 5 lines 4-6.

18. The coating allows the maximum amount of air space in the coated seed. Col. 4 lines 21-22 .

19. Loperfido differs from the encapsulated seed of the present invention because Loperfido does not teach a coating being an integral part of the seed. Loperfido teaches a binder added to the seed that does not uniformly coat the seed. The coating forms beads that then collect around the seed. The coating formed around the seed is of a highly porous nature. Loperfido describes allowing a maximum amount of air space between the coating and the seed.

20 None of the products described in the prior art patents have ever been made commercially.

21. The present invention provides a soil conditioner in intimate association with the seed. Specification Pg. 15 lines 32-33.

22. The present invention provides a uniformity of coating or coating thickness so that the seed is not on or immediately adjacent an outside surface of the capsule such that the seed may fall out, or be easily broken out, of the capsule, or easily removed by dissolution of materials at and near the surface of the seed capsule. Specification Pg. 17 line 31 – Pg. 18 line 3.

23. The present invention prepares a seed that becomes generally uniformly coated with one or more layers of the coating material such that the coating material becomes an integral part of the respective seed capsule. As the coating material solidifies on the seed, the coating material tightly bonds to the respective portions of the seeds. Specification Pg. 22 lines 14-22.

24. The present invention applies a seed in a seed capsule wherein the seed is intimately combined with a soil conditioning material in a common particle. Specification Pg. 35 lines 23-25. This was not taught prior to the present invention. After a review of the prior art provided by the Examiner, this statement is still true.

25. The prior art does not show the soil conditioning material nor the inorganic fertilizer intimately associated in a common capsule or other particle as in the present invention. Specification Pg. 38 lines 30-33.

26. Where the soil conditioning and fertilizer materials are applied separate from the seed, the potential cooperative benefit of the soil conditioning material as relates to solution and up-take of soil moisture and or of the inorganic chemical fertilizer by the seed are not obtained, and/or are not obtained in controlled close association with the seed. Specification Pg. 32 lines 1-10.

27. When applied separately to the soil, the seed and the soil conditioner are not necessarily in intimate contact with each other as they are when both materials are combined into a single combined seed capsule product as in the present invention. Specification Pg. 39 lines 19-23.

28. In the present invention, soil conditioning material and optionally chemical fertilizer, are inherently bound to each other, and to the seed, as by the agglomeration process, and inherently assist the seed in achieving desired germination and strong early growth. Specification Pg. 42 lines 27-31.

29. Figures 6A-6D of the present invention illustrate the seed in intimate association with the soil conditioning material.

30. The present invention comprises a combination seed capsule having a viable seed acting as a core or pseudo core. A coating of a composition comprising a soil conditioning material is an integral part of the seed. None of the prior art describes these elements. Further, where the coating is applied in an agglomeration operation is also not described in the prior art.

31. Enclosed is a sample of EncapSeed which was prepared according to the method described in the present invention. As shown by the enclosed EncapSeed, the coating is an integral part of the seed. The seed (an all-purpose grass seed mixture) comprises 32% of the overall product weight. The blanket that is wrapped around the seed is comprised of dicalcium phosphate (.8%) and dried, ground paper sludge (67.2%). The dried, ground paper fines range in size from approximately 30 mesh to approximately 200 mesh. Of this total material, 68.5% is comprised of inert material. The EncapSeed coating has no visible spaces between the coating and the seed is designed to act as the microenvironment for the seed for the germination process. Field tests by the University of Wisconsin-Madison's Horticultural Department have shown that the EncapSeed blanket helps to enhance turf establishment.

I declare under the penalty of perjury that the foregoing is true and correct.

Date: June 27, 2000

Michael Krysiak

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/113,254	07/10/98	MADIGAN D	29214

HM12/0510

PHILIP M. WEISS
WEISS & WEISS
500 OLD COUNTRY ROAD
GARDEN CITY NY 11630

EXAMINER	
GRUNBERG, A	
ART UNIT	**PAPER NUMBER**
1638	10

DATE MAILED: 05/10/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)

U.S. G.P.O. 1999 460-693

Office Action Summary

Application No.	Applicant(s)
09/113,254	Madigan et al.
Examiner	**Group Art Unit**
Anne Marie Grunberg	1638

☒ Responsive to communication(s) filed on *Dec 14, 1999*

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) *70-73 and 76-100* is/are pending in the applicat

Of the above, claim(s) *70-73 and 95-100* is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *76-94* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☒ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1638.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Originally withdrawn claims 70-73 are still pending. Since they were not elected in response to the original restriction requirement, they should be canceled.

Newly submitted claims 95-100 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: Claims 95-100 are drawn to a method of making capsules; Group II as set forth in the last office action, whereas the elected invention was drawn to a seed capsule and methods of use, Group I as set forth in the last office action.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 95-100 are withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

Applicant's election of Group I in Paper No. 5 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claim Rejections

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 77, 83-86, 90-91 and 93 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 77, 83-86, 90-91 and 93 are indefinite for the terminology "material fines". This term is not defined in the specification and the metes and bounds of the claim can not be readily determined by one skilled in the art. This rejection may be obviated by deletion of "material fines" and substituting the term --soil conditioning materials-- as is described on page 13, lines 16-19 of the specification.

Similarly claims 79 and 86 are vague and indefinite in the use of "industrial byproduct". Changing this term to --sludge-- or --fly ash-- as described on page 13, lines 17-19 of the specification, would obviate this rejection.

In addition, claims 80 and 87 are vague and indefinite in the terminology "byproduct of a paper making process" as a byproduct could be anything, including for example, contaminated water. Insertion of --fiber-containing-- before "byproduct" as described on page 9, line 9 of the specification, would obviate this rejection.

Claim 84 is vague and indefinite in the terminology "grassy/woody substances". This rejection may be obviated by changing the term to state --sawdust--.

Claim 85 is vague and indefinite in the terminology "lifting and mixing agglomeration operation". The phrase is not defined in the specification and it is not clear as to what exactly constitutes a lifting and mixing agglomeration operation. As a result, the metes and bounds of the claim can not be adequately determined. This rejection may be obviated by deleting "a lifting and mixing" and substituting --an-- in its stead to reflect terminology used on page 28, line 20 of the specification.

Claim 94 is vague and indefinite in the terminology "binder contains lignin". This term is not defined in the specification, nor is there support to clarify what the terminology encompasses. As a result, the metes and bounds of the claim can not be determined by one skilled in the art. It is suggested that Applicant cancel this claim.

3. Claim 93 recites the limitation "said binder" in reference to claim 85. There is insufficient antecedent basis for this limitation in the claim. This rejection may be obviated by amending the claim to delete "claim 85" and change it to read --claim 92--.

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 77,83-86, 90-91 and 93-94 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification provides no guidance in identifying "material fines", or a "binder [that] contains lignin". The specification does not contain guidance as to what a material fine would be, nor is it understood what form of lignin would be contained in what type of binder. In contrast, the claims are broadly drawn to any material fine, and any binder that contains lignin. In addition, these phrases are considered to be new matter since the specification as originally filed does not contain these items. The terms "grassy/woody substances" and "lifting and mixing agglomeration operation" are also considered to be new matter since they are not in the specification as originally filed.

The use of any type of material fine, or a binder which contains lignin is unpredictable due to the environmental impacts associated with certain material fines, and the germination characteristics of the seed and sensitivity of the seed to adhesive compounds, as set forth below.

Nelson teaches in column 2, lines 12-16, for example, that certain flue gas desulfurization wastes are not appropriate for soil amendments, owing to their high solubilities in water.

Porter et al teach in column 2, lines 21-30, for example, that selection of a suitable adhesive or binder, must take into account the germination characteristics of the seed and the sensitivity of the seed to damage caused by harsh chemicals that might be present in adhesive compounds.

Given the claim breadth, unpredictability, and lack of guidance as discussed above, undue experimentation would have been required by one skilled in the art to determine what type of material fines, and what type of lignin-containing binder could be used. Undue experimentation would also be required to identify appropriate grassy/woody substances and lifting and mixing agglomeration operations.

This rejection may be obviated by amending the claims as suggested in the section dealing with the second paragraph of 35 U.S.C. 112.

5. New claim 77 is rejected under the previously applied 35 U.S.C. 102(b) as being anticipated by Gerber.

Claim 77 is drawn to a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule, and seed coatings comprising a growth enhancer and material fines.

Gerber teaches a combination seed capsule comprising at least one viable seed; said seed acting as a core or pseudo-core of said combination seed capsule (column 1, lines 61-63). Gerber

also teaches seed coatings comprising a growth enhancer and material fines (column 2, line 7; column 2, lines 52-56; column 3, lines 22-41).

This rejection may be obviated by inserting at the end of the claim --; said seed capsule having an inner layer comprising a soil conditioning material selected from the group consisting of municipal or sewage sludge, scrubber sludge, paper mill sludge, sawdust, fly ash, dust and animal waste;

and said seed capsule having an outer layer comprising a material selected from the group consisting of urea, an inorganic form of a plant nutrient, herbicides, fungicides, and ingredients effective to reduce susceptibility of the seed capsule to deleterious effects of animals;

wherein the inner layer is agglomerated onto the seed.--

6. New claims 76-78 and 83 are rejected under the previously applied 35 U.S.C. 102(b) as being anticipated by Roth.

Claims 76-78 and 83 are drawn to a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule, and a seed coating comprising dicalcium phosphate. Additionally, the claims are drawn to seed coatings comprising a growth enhancer and material fines such as municipal sewage.

Roth teaches a combination seed capsule comprising at least one viable seed; said seed acting as a core or pseudo-core of said combination seed capsule (column 4, lines 31-50). Roth

also teaches a coating comprising "the phosphates," which although it does not specifically state dicalcium phosphate, certainly includes dicalcium phosphate (column 3, line 10). Additionally Roth teaches coatings comprising a growth enhancer and material fines (column 3, lines 5-16). Roth also teaches material fines comprised of municipal sewage (column 4, lines 46-50).

This rejection may be obviated by inserting at the end of the claim --; said seed capsule having an inner layer comprising a soil conditioning material selected from the group consisting of municipal or sewage sludge, scrubber sludge, paper mill sludge, sawdust, fly ash, dust and animal waste;

and said seed capsule having an outer layer comprising a material selected from the group consisting of urea, an inorganic form of a plant nutrient, herbicides, fungicides, and ingredients effective to reduce susceptibility of the seed capsule to deleterious effects of animals;

wherein the inner layer is agglomerated onto the seed.--

7. New claims 77, 79-81 and 84 are rejected under the previously applied 35 U.S.C. 102(b) as being anticipated by Nilsson.

Claims 77, 79-81 and 84 are drawn to a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule, and seed coatings comprising a growth enhancer and material fines. Additionally, the claims are drawn to material fines such as industrial byproducts, byproducts of a paper making process, paper sludge, and grassy/woody substances.

Nilsson teaches a combination seed capsule comprising at least one viable seed; said seed acting as a core or pseudo-core of said combination seed capsule (column 1, lines 38-49). Nilsson also teaches seed coatings comprising a growth enhancer and material fines (column 1, lines 60-68; column 4, line 23). Additionally Nilsson teaches material fines comprised of industrial byproducts (column 1, line 65). Nilsson also teaches material fines which are byproducts of a paper making process (column 1, line 65), such as paper sludge (column 1, line 65). Nilsson also teaches material fines comprised of grassy/woody substances (column 1, lines 65).

This rejection may be obviated by inserting at the end of the claim --; said seed capsule having an inner layer comprising a soil conditioning material selected from the group consisting of municipal or sewage sludge, scrubber sludge, paper mill sludge, sawdust, fly ash, dust and animal waste;

and said seed capsule having an outer layer comprising a material selected from the group consisting of urea, an inorganic form of a plant nutrient, herbicides, fungicides, and ingredients effective to reduce susceptibility of the seed capsule to deleterious effects of animals;

wherein the inner layer is agglomerated onto the seed.--

8. Claims 77, 79-81, 84-88, and 91-94 are rejected under 35 U.S.C. 102(b) as being anticipated by Loperfido (newly applied).

Claims 77, 79-81, 84-88, and 91-94 are drawn to a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule, and seed coatings comprising a growth enhancer and material fines. Additionally, the claims are drawn to material fines such as industrial byproducts, byproducts of a paper making process, paper sludge, grassy/woody substances. The claims are also drawn to a binder which may include fertilizer and which contains lignin.

Loperfido teaches a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule (abstract, for example). The seed coatings comprise a growth enhancer (column 6, lines 37-46) and material fines (column 2, lines 63-66, for example). Loperfido teaches material fines such as cellulose derivatives, which would include byproducts of a paper making process, paper sludge, or grassy/woody substances (column 4, lines 13-14). Byproducts of a paper making process are industrial byproducts since paper making is an industry. The coating is applied by a lifting and mixing agglomeration operation (column 6, lines 65-67; column 7, lines 1-19, for example). Loperfido teaches a binder that is applied to the seed capsule (column 5, lines 26-30, for example). Additionally, Loperfido teaches a fertilizer as part of the material fines (column 6, lines 35-47). Loperfido also teaches a binder that contains lignin (column 5, line 1, for example).

This rejection may be obviated by inserting at the end of the claim --; said seed capsule having an inner layer comprising a soil conditioning material selected from the group consisting

of municipal or sewage sludge, scrubber sludge, paper mill sludge, sawdust, fly ash, dust and animal waste;

and said seed capsule having an outer layer comprising a material selected from the group consisting of urea, an inorganic form of a plant nutrient, herbicides, fungicides, and ingredients effective to reduce susceptibility of the seed capsule to deleterious effects of animals;

wherein the inner layer is agglomerated onto the seed.--

Additionally, the term "at least" in line 2 of claims 76, 77 and 85, should be deleted.

Applicant may attempt to distinguish the claimed invention by supplying a declaration which sufficiently shows that the seed capsule of the instant invention is distinct from a seed capsule made by the process of Loperfido. No commitment to patentability will be made prior to receipt and review of the declaration.

9. Claims 76-94 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loperfido in view of Roth, and further in view of Nelson (newly applied).

Claims 76-94 are drawn to a combination seed capsule comprising at least one viable seed which acts as a core or pseudo-core of the seed capsule, and a seed coating comprising dicalcium phosphate. Additionally, the claims are drawn to seed coatings comprising a growth enhancer and material fines such as municipal sewage, an industrial byproduct, paper sludge, fly ash, and grassy/woody substances. The claims are also drawn to the coating being applied by a lifting and mixing agglomeration operation.

Loperfido has been discussed previously.

Loperfido does not teach a coating or growth enhancer comprising dicalcium phosphate, nor does Loperfido teach material fines comprising fly ash or municipal sewage.

Roth teaches a coating comprising "the phosphates," which although it does not specifically state dicalcium phosphate, certainly includes dicalcium phosphate (column 3, line 10). Roth also teaches material fines comprised of municipal sewage (column 4, lines 46-50).

Nelson teaches environmentally beneficial soil amendments such as fly ash (column 3, lines 60-63.

It would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to utilize the combination seed capsules as taught by Loperfido, and to modify the capsules to include the fertilizer dicalcium phosphate, given the advantages of including fertilizers into the seed capsule as taught by Loperfido. It would also have been obvious to use material fines comprising municipal sewage or industrial byproducts such as fly ash, given the benefits of a low-cost carrier additive derived from sewage sludge as described by Roth (column 2, lines 1-45), and given the benefits of growth enhancing industrial byproducts such as fly ash as described by Nelson (column 3, lines 45-57).

This rejection may be obviated by inserting at the end of the claim --; said seed capsule having an inner layer comprising a soil conditioning material selected from the group consisting of municipal or sewage sludge, scrubber sludge, paper mill sludge, sawdust, fly ash, dust and animal waste;

and said seed capsule having an outer layer comprising a material selected from the group consisting of urea, an inorganic form of a plant nutrient, herbicides, fungicides, and ingredients effective to reduce susceptibility of the seed capsule to deleterious effects of animals;

wherein the inner layer is agglomerated onto the seed.--

Additionally, the term "at least" in line 2 of claims 76, 77 and 85, should be deleted.

Applicant may attempt to distinguish the claimed invention by supplying a declaration which sufficiently shows that the seed capsule of the instant invention is distinct from a seed capsule made by the process of Loperfido. No commitment to patentability will be made prior to receipt and review of the declaration.

Applicant's arguments filed 12/14/1999 have been fully considered but they are not persuasive.

The references supplied by the Applicant teach different coating and agglomeration techniques. The terminology in the art appears to be used interchangeably for different techniques. For example, on page 21 of Perry's Chemical Engineers' Handbook, liquid methods are characterized by spray or fluid bed agglomeration, whereas Hovmand appears to describe the same process on page 11, as a coating process. As a result, the previously applied art of Gerber, Roth and Nelson could be characterized as a coating or an agglomeration procedure.

No claim is allowed.

CLOSING REMARKS

Any inquiry concerning this or earlier communications from the examiner should be directed to Anne Marie Grünberg whose telephone number is (703) 305-0805. The examiner can normally be reached on Monday through Thursday from 7:30 to 5:00, and on alternate Fridays from 7:30 to 4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909. The fax phone number for this Group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Anne Marie Grünberg

May 8, 2000

DAVID T. FOX
PRIMARY EXAMINER
GROUP ~~180~~ 1638

Notice of References Cited

Application No.	Applicant(s)		
09/113,254	Madigan et al.		
Examiner		Group Art Unit	Page 1 of 1
Anne Marie Grunberg		1638	

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A	5,627,133	5/1997	Nelson	504	116
	B	3,905,152	9/1975	Loperfido	47	57.6
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
x	U	Hovmand, Granulation and agglomeration by fluidized bed and spray drying technology, pages 1, 10-11.	1982
x	V	Briquetting, pelletizing, extrusion &fluid bed/spray granulation, table of contents, table 8-52.	1998
x	W	Perry' Chemical Engineers' Handbook, 8-61	1978
x	X	Briquetting, Pelletizing, Extrusion and Fluid Bed/Spray Granulation, Engelleitner, tables 23-24, page 21 and 23.	1998

* A copy of this reference is not being furnished with this Office action.
(See Manual of Patent Examining Procedure, Section 707.05(a).)

Interview Summary

Application No.	Applicant(s)
09/113,254	Madigan et al.
Examiner	**Group Art Unit**
Anne Marie Grunberg	1638

All participants (applicant, applicant's representative, PTO personnel):

(1) *Anne Marie Grunberg* (3) _____

(2) *Philip Weiss* (4) _____

Date of Interview *May 4, 2000*

Type: [X] Telephonic [ ] Personal (copy is given to [ ] applicant [ ] applicant's representative).

Exhibit shown or demonstration conducted: [ ] Yes [X] No. If yes, brief description:

_____

_____

Agreement [ ] was reached. [X] was not reached.

Claim(s) discussed: *claims 70-73 and 76-100*

Identification of prior art discussed:
Discussed prior art in general terms and more thoroughly discussed Roth, Gerber, and Nilsson among others.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
Discussed the general state of the prior art and how Applicant's invention differs from the prior art. Additionally, agglomeration methods were discussed.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. [ ] It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. [ ] Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of : Madigan

Serial No.: 09/113,254

Filed: July 10, 1998

Title: SEEDING TREATMENTS

Date: 12/14/99
Group Art Unit: 1649
Examiner: Grunberg

RESPONSE TO OFFICE ACTION:

Sir:

In response to the Office Action mailed June 18, 1999 please amend the application as follows:

In the Specification

Pg. 8 Line 22 after "comprise" add --urea or--;

Line 24 delete "urea"

Line 26 change "micronutrient" to —nutrient--;

Pg. 9 Line 5 change "micronutrient" to—nutrient--;

Pg.10 Line 14 change "micronutrient" to –nutrient--;

Line 17 change "micronutrient" to –nutrient--;

Pg.14 Line 8 change "Inorganic chemical" to --chemical--;

Line 17 delete "inorganic";

Line 19 change "micronutrients" to --nutrients--;

Pg.18 Line 15 delete "inorganic";

Pg.19 Line 23 change "In" to --Referring to--;

Pg.34 Line 9 delete "inorganic";

Further, during the interview, applicant discussed that there was a difference between the agglomeration process described in the present application and the coating process described in the prior art. The Examiner requested publications which describe such a difference. These publications are attached along with this response. For example, the publications describe the difference between a tumbling and mixer agglomeration which is similar to the method described in the patent application verses spraying methods which are similar to the coating methods described in the prior art. The article, "Granulation and Agglomeration by Fluidized Bed and Spray Drying Technology" specifically describes the difference between "agglomeration technology" and "the technology of coating particles". Table 8-52 from the notes from "Briquetting, Pelletizing, Extrusion of Fluid Bed/Spray Granulation" April 1998 show the difference between tumbling and mixer agglomeration and other techniques, such as, spray methods. This same table is found in "Perry's Chemical Engineers Handbook". In "Briquetting, Pelletizing, Extrusion of Fluid Bed/Spray Granulation" 1995 describes the difference in Table 23 between "tumble agglomeration", the method described in the present patent application versus "coating techniques" which are described as a totally separate technique. The notes further described these two techniques as differing between agitation methods and liquid methods.

The present claims specifically refer to an agglomeration method which is different than a coating methods described in the prior art.

Therefore, the application is now in condition for allowance.

Philip M. Weiss
Philip M. Weiss
Reg. No. 34,751

"Express Mail" mailing label number: EL524783323US
Date of Deposit: December 14, 1999
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to BOX Response, Assistant Commissioner for Patents, Washington D.C. 20231

Nelly Bronnberg
Nelly Bronnberg, December 14, 1999

Line 15 delete "inorganic";

Pg.37 Line 23 change "micronutrients" to --nutrients--;

In the Claims:

Delete Claims 1-69, 74 and 75

Add the following claims:

76. A combination seed capsule comprising:

at least one viable seed;

said seed acting as a core or pseudo-core of said combination seed capsule;

a coating comprising dicalcium phosphate.

77. A combination seed capsule comprising:

at least one viable seed;

said seed acting as a core or pseudo-core of said combination seed capsule;

coatings comprising a growth enhancer and material fines.

78. The combination seed capsule of claim 77 wherein said growth enhancer is dicalcium phosphate.

~~79.~~ The combination seed capsule of claim ~~77~~ wherein material of said ~~fines~~ materials are comprised of ~~industrial byproduct~~ sludge or fly ash.

~~80.~~ The combination seed capsule of claim ~~79~~ wherein the material is a byproduct of a paper making process.

~~81.~~ The combination seed capsule of claim ~~79~~ wherein the byproduct is paper sludge.

82. The combination seed capsule of claim 79 wherein the byproduct is fly ash.

~~83.~~ The combination seed capsule of claim ~~77~~ wherein the material ~~fines~~ is comprised of municipal sewage.

~~84.~~ The combination seed capsule of claim ~~77~~ wherein the material ~~fines~~ are comprised of ~~grassy/woody substances~~.

85. A combination seed capsule comprising;

~~at least~~ one viable seed; said seed acting as a core or pseudo-core of said combination seed capsule;

a coating of a composition comprising material ~~fines~~;

said coating being applied to said viable seed by ~~a lifting and mixing~~ agglomeration operation.

~~86.~~ The combination seed capsule of claim ~~85~~ wherein material of said material ~~fines~~ are comprised of ~~industrial byproduct~~.

~~87.~~ The combination seed capsule of claim ~~86~~ wherein the material is a byproduct of a paper making process.

~~88.~~ The combination seed capsule of claim ~~86~~ wherein the byproduct is paper sludge.

89. The combination seed capsule of claim 86 wherein the byproduct is fly ash.

~~90.~~ The combination seed capsule of claim ~~85~~ wherein the material ~~fines~~ is comprised of municipal sewage.

~~91.~~ The combination seed capsule of claim ~~85~~ wherein the material ~~fines~~ are comprised of ~~grassy/woody substances~~.

13 7
~~92.~~ The combination seed capsule of claim ~~85~~ wherein a binder is applied to said seed capsule.

14 13 soil conditioning
~~93.~~ The combination seed capsule of claim ~~85~~ wherein a fertilizer is part of said material ~~fines~~, said binder or its own layer.

94. The combination seed capsule of claim 92 wherein said binder contains lignin.

95. A method of making seed capsules by an agglomeration operation comprising;

spraying a binder on said seed;

lifting and mixing said seeds with material fines.

96. The method of claim 95 wherein said seed capsules are coated with a growth enhancer.

97. The method of claim 96 wherein said growth enhancer is dicalcium phosphate.

98. The method of claim 95 wherein said material fines are comprised of industrial byproduct fines.

99. The method of claim 95 wherein said binder is a liquid fertilizer.

100. The method of claim 95 wherein said binder contains lignin.

Response to Office Action

Applicant has canceled the original claims in the application and has added new claims 76-100. Applicant's attorney had a telephone interview with the examiner which discussed the use of dicalcium phosphate as a seed coating, and that this was not described in the prior art presently before the Examiner.

54

Granulation and Agglomeration by Fluidized Bed and Spray Drying Technology

DR. SVEND HOVMAND
NIRO ATOMIZER INC.

INTRODUCTION

The methods to be described do not involve any mechanical agitation or compaction of the powder to be agglomerated but are agglomeration techniques derived from fluid bed dryer and spray dryer technology. With these methods, the drying and agglomeration of a product can be combined in one step in many cases. The agglomerated or granulated products from a Fluid Bed Granulator or Fluidized Spray Dryer are normally less dense and more fragile than the products agglomerated by the methods described previously in this course; however, stable and well defined agglomerates or granulates, that easily disperse in water can be produced in many applications without the addition of binder. The technology of coating particles in a fluid bed will also be described.

An overview of the techniques described here can be presented as follows:

The starting materials can influence the product characteristic. Granulation is initiated by formulation of liquid bridges. Accordingly, increasing particle surface area and absorption of water result in incomplete wetting of the surface of the particles and this will therefore result in decreasing granule size.

Granule size is directly proportional to droplet size for a given binder solution and varying the droplet size might therefore be the most suitable way of controlling the granule size.

The atomization of the liquid binder can either be performed by pressure nozzles or two fluid nozzles. Two fluid nozzles are often preferred in batch operations as they reduce the tendency to form wet agglomerates and of blockage of the nozzles. Further the position of the nozzle is an important parameter in the granulation process. Nozzles can be placed above the fluidized layer spraying downward, in the side of the fluidized layer, or at the bottom of fluidized layer near the distributor spraying upwards. Each position has advantages and disadvantages, however, no clear conclusions can be drawn from the available literature.

After granulation the granules can be dried in the fluid bed at elevated inlet gas temperatures in order to reduce the drying time.

C. Batch Fluid Bed Coating

Following the drying, the granules can be conveniently spray coated in the same equipment, as experience has shown that the fluidized bed is ideal for spray coating and is giving constant and reproducible coatings of the granules. Fluid

bed coating is an extreme example of fluid bed granulation. The layering mechanisms are made to dominate totally by applying very low liquid feed rates and keeping the fluidized layer dry; thus the drying rate rapid (16), (17), (18).

Coating is important in a number of industries such as pharmaceutical, agrochemical, seed treatment, food, and confectionery.

The reasons for coating are usually:

- appearance
- taste masking
- moisture protection or isolation from other ingredients
- enteric coating
- sustained release
- gastric release

The ideal fluid bed coater will ensure an even coating of each discrete granule/tablet's surface and thus ensure a perfect mix of the particles throughout the whole fluidized layer, by avoiding any dead zones in the fluid bed coater. It is crucial that each particle to be coated passes through the spray zone, preferably without being in contact with other particles and that the applied polymer is dried as rapidly as possible to prevent superficial sticking and picking off one surface to another.

The Wurster Process, Fig. 6, a spray coating process in a fluid bed where the granules are circulated up through the center while being coated, has specially being developed for coating of small and medium sized granules (10) (19).

Briquetting, Pelletizing, Extrusion & Fluid Bed/Spray Granulation
April 20-23, 1998
Chicago, IL

TABLE OF CONTENTS

*Optional Day Notes To Be Distributed

Optional Reading
Koerner, Robert M. and John MacDougall. *Elements II, Briquetting and Agglomeration.* Hudson: Institute for Briquetting and Agglomeration.

TABLE 8-52 Size-Enlargement Methods and Applications*

Method	Equipment	Representative applications
Pressure compaction	Piston or molding press	Plastic preforms, small machine parts from metal powders (cams, gears, gaskets), metal borings and turnings
	Tableting press	Pharmaceuticals, catalysts, industrial chemicals, ceramics, metal powders
	Roll-type press	Clay-type minerals, potassium chloride, sodium chloride, organic compounds, metal powders, ores, charcoal, lime, magnesia, titanium sponge, phosphate rock
	Pellet mill	Pharmaceuticals, plastics, clays, carbon, charcoal, industrial chemicals, fertilizers, rubber products, animal feeds
	Screw extruder	Bauxite, plastics, rare-earth fluorides, clays, catalysts
Tumbling and mixer agglomeration	Inclined pan or disk; rotary-drum agglomerator	Fertilizers, iron ores, nonferrous ores, mineral and clay products, carbon black, various finely divided solid-waste products
	Paddle mixer; horizontal pan	Fertilizers, premixing for balling, conditioning steel-plant fines
	Powder blenders; flow-jet mixing	"Instant" foods, detergent granulation
Thermal processes	Sintering and heat hardening in traveling grate, rotary kiln, grate-kiln, shaft furnace	Ferrous and nonferrous ores, minerals, cement clinker, solid-waste products
	Drying and solidification in drum dryers, flakers, endless-belt systems	Sulfur slates, urea, ammonium nitrate, caustic, various resins, hot-melt adhesives
Spray methods	Spray dryers	Instant foods, washing powders, dyestuffs, press feeds
	Prilling towers	Urea, ammonium nitrates, resins, coal-tar pitch, etc.
	Fluidized and spouted beds	Fertilizers, clays, sulfur, nuclear and other wastes
	Flash dryers	Clays, diatomaceous earths, starch, waste by-products
Liquid systems	Immiscible-liquid wetting in various high-shear and turbine mixers	Coal fines, soot and oil removal from water
	Sol-gel process in spray column	Metal dicarbide spheroids
	Pellet flocculation in drums and stirred vessels	Waste sludge, mud and clay slurries, sewage sludge

*Cf. Browning, *Chem. Eng.*, 74(25), 147 (1967).

From Ref. 7

PERRY'S CHEMICAL ENGINEERS' HANDBOOK

SIXTH EDITION

McGraw-Hill Book Company

New York
St. Louis
San Francisco
Auckland
Bogotá
Hamburg
London
Madrid
Mexico
Montreal
New Delhi
Panama
Paris
São Paulo
Singapore
Sydney
Tokyo
Toronto

Prepared by a staff of specialists under the editorial direction of

Late Editor
Robert H. Perry

Editor
Don W. Green
Conger-Gabel Professor of Chemical and Petroleum Engineering, University of Kansas

Assistant Editor
James O. Maloney
Professor of Chemical Engineering, University of Kansas

TABLE 8-52 Size-Enlargement Methods and Applications*

Method	Equipment	Representative applications
Pressure compaction	Piston or molding press	Plastic preforms, small machine parts from metal powders (cams, gears, gaskets), metal bearings and turnings
	Tableting press	Pharmaceuticals, catalysts, industrial chemicals, ceramics, metal powders
	Roll-type press	Clay-type minerals, potassium chloride, sodium chloride, organic compounds, metal powders, ores, charcoal, lime, magnesia, titanium sponge, phosphate rock
	Pellet mill	Pharmaceuticals, plastics, clays, carbon, charcoal, industrial chemicals, fertilizers, rubber products, animal feeds
	Screw extruder	Bauxite, plastics, rare-earth fluorides, clays, catalysts
Tumbling and mixer agglomeration	Inclined pan or disk; rotary-drum agglomerator	Fertilizers, iron ores, nonferrous ores, mineral and clay products, carbon black, various finely divided solid-waste products
	Paddle mixer; horizontal pan	Fertilizers, premixing for balling, conditioning feed-plant fines
	Powder blenders; flow-jet mixing	"Instant" foods, detergent granulation
Thermal processes	Sintering and heat hardening in traveling grate, rotary kiln, grate-kiln, shaft furnace	Ferrous and nonferrous ores, minerals, cement clinker, solid-waste products
	Drying and solidification in drum dryers, flakers, endless-belt systems	Sulfur slates, urea, ammonium nitrate, caustic, various resins, hot-melt adhesives
Spray methods	Spray dryers	Instant foods, washing powders, dyestuffs, press feeds
	Prilling towers	Urea, ammonium nitrates, resins, coal-tar pitch, etc.
	Fluidized and sprouted beds	Fertilizers, clays, sulfur, nuclear and other wastes
	Flash dryers	Clays, diatomaceous earths, starch, waste by-products
Liquid systems	Immiscible-liquid wetting in various high-shear and turbine mixers	Coal fines, soot and oil removal from water
	Sol-gel process in spray column	Metal dicarbide spheroids
	Pellet flocculation in drums and stirred vessels	Waste sludge, mud and clay slurries, sewage sludge

*Cf. Browning, *Chem. Eng.*, 74(25), 147 (1967).

(a) (b) (c)

FIG. 8-64 Three states of liquid content for an assembly of spherical particles. (a) Pendular state. (b) Funicular state. (c) Capillary state. [*Newitt and Conway-Jones*, Trans. Inst. Chem. Eng. (London), *36, 422 (1958).*]

Calculation of Agglomerate Strength For an agglomerate composed of equal-sized spherical particles, the tensile strength t is [Rumpf, in Knepper (ed.), *Agglomeration*, op. cit., p. 379]

$$t = \frac{9}{8}\left(\frac{1-\epsilon}{\pi X^2}\right)NF \qquad (8\text{-}38)$$

where X is the particle diameter; F is the bonding force per point of contact; N is the mean coordination number, i.e., average number of points of contact between one sphere and its neighbors; and ϵ is the volume fraction of voids in the agglomerate. Values of X and ϵ can be obtained from a size-distribution analysis of the powder and the bulk density of the packed particles. As an approximation, the coordination number N is π/ϵ (Rumpf, loc. cit.) or $N = 2 \exp 2.4(1-\epsilon)$ [Meissner, *Ind. Eng. Chem. Process Des. Dev.*, 3, 202 (1964)].

For mobile liquid binders in the pendular state

$$t = 2.8\left(\frac{1-\epsilon}{\epsilon}\right)\frac{\sigma}{Xf(\delta)} \qquad (8\text{-}39)$$

where σ is the surface tension of the binding liquid and $f(\delta)$ is a function of the angle of contact [Newitt and Conway-Jones, *Trans. Inst. Chem. Eng. (London)*, 36, 422 (1958)].

If wetting is complete, $f(\delta) = 1$. For the capillary state

$$t = 8.0\left(\frac{1-\epsilon}{\epsilon}\right)\frac{\sigma}{Xf(\delta)} \qquad (8\text{-}40)$$

The tensile strength of an agglomerate in the pendular state is about one-third of that in the capillary state, while the funicular state has intermediate strengths. A decrease in particle size and porosity yields greater strength. To improve agglomerate strength, the importance of correct particle-size distribution in attaining minimum porosity should be recognized [Ridgway and Tarbuck, *Chem. Process Eng.* (February 1968)].

For the other binding mechanisms calculated values of tensile strength shown in Fig. 8-65 indicate the strength to be expected in various size-enlargement processes.

Strength-Testing Methods Concepts of fracture mechanics (see subsection "Properties of Solids") are applicable to the methods of testing the strength of agglomerates.

Compression tests, in which agglomerates are crushed between parallel platens, are used for quick production checking. Various means of distributing the applied force uniformly over the agglomerate surface are used, including shaving off opposite sides, fitting them with hardening plastic, or covering the platen surface with compressive board.

A log-log plot of load at failure against pellet diameter for approximately spherically pellets produced under the same conditions often yields a straight line with slope approximately equal to 2. The intercept of such a plot at unit diameter yields a compressive-strength factor.

The CENTER for PROFESSIONAL ADVANCEMENT
144 Tices Lane, East Brunswick, New Jersey 08816

Academic Center, Two Tower Center, Ninth Floor,
New Jersey Turnpike (Exit 9), East Brunswick, New Jersey 08816

Administrative Offices
Telephone 908-238-1600 • FAX 908-238-9113 • Telex 139303 (CENPRO EBRW)

Briquetting, Pelletizing, Extrusion and Fluid Bed/Spray Granulation

MARCH 27-29, 1995
East Brunswick, NJ

W.H. ENGELLEITNER
Course Director

continuing education through total involvement

DALE A. LEE
RASMUSSEN

Table 23. Review of most important size enlargement processes and some of their typical applications

Technology	Method	Applications (typical)
Tumble agglomeration	Balling drum, pan, and cone	Iron ores, other ores, cement raw mix, fertilizers, dusts from dust collectors, fine particulate waste materials, ceramics, clay, finely divided minerals, etc.
	Mixer	Ceramic materials, fertilizers, minerals, chemicals, pharmaceuticals, foodstuffs, detergents, etc.
	Fluidized bed (gas)	Fertilizers, pharmaceutical materials, filter cakes, foodstuffs, chemicals
	Suspension (liquid)	Solids, e.g. coal, from suspensions
Induration	Shaft furnace Rotary kiln Straight grate Grate-Kiln Calciners	Iron ores, other ores, minerals, waste materials, e.g. metal-bearing dusts, etc.
Pressure agglomeration	Extrusion presses	Coals, ceramic materials, clays, polymers, plastics
	Pelleting machines	Animal feeds, rubber raw materials, catalysts, lubricants, stabilizers, pigments, polymers, clay, chemicals, pharmaceutical products, insecticides, herbicides, fungicides, etc.
	Piston presses (tabletting)	Pharmaceutical products, catalysts, metal powders, ceramic materials, chemicals, pigments, plastic powders
	Roller presses (briquetting, compacting)	Coals, coke, salts, minerals, ores, fertilizers, chemicals, metal powders, animal feeds, polymers, refractories, waste materials, metal-bearing fines, pharmaceutical products, sponge iron, etc.
Other agglomeration methods	Agglomeration by heat	Ores, specifically iron ores, metal-bearing wastes and dusts, mill scale, etc.
	Spray solidification	Urea, other fertilizers, pitches, asphalt, waxes, resins, sulfur, inorganic salts, etc.
	Direct capillary action	Powders, chemicals, coal (spherical agglomeration)
	Alternative sources of particle movement	Finely divided particulate solids, pharmaceuticals, chemicals, food extracts (instant characteristics)
	Coating techniques	Pharmaceuticals, food, fertilizers and agricultural chemicals (control of release), microencapsulation of pigments, etc.
	Flocculation in gases and liquids	Environmental protection, aggregation of solids in gases, flocculation of solids in liquids, selective flocculation, etc.

Table 24. Wanted and unwanted agglomeration in various process areas

Product area	Process: Wanted	Process: Unwanted
Aggregate (+3 mm)	Briquetting, compacting + crushing and screening	—
Agricultural chemicals	Tumble agglomeration (disc and drum), mixer agglomeration, briquetting, compacting + crushing and screening	Caking, bag set, build-up, flow problems, segregation due to selective agglomeration
Alumina	Granulation (tumble/pressure), calcining	Build-up, caking, flow problems
Animal feed	Pelleting, mixer agglomeration, briquetting	Caking
Carbon black	Fluid bed, mixer agglomeration, granulation by compaction	Build-up, caking, flow problems
Ceramics	Precipitation, sol-gel, spray drying and granulation, compaction + crushing and screening, tabletting	Build-up, caking
(China) clay	Tumble agglomeration, extrusion	Lumping, caking
Coal	Calcinating, briquetting, tumble agglomeration, spherical agglomeration	Build-up
Detergents	Spray drying or granulation, micropelletization (drum, disc, mixer), tabletting, pelleting	Caking, lumping, build-up
Dust (−0.5 mm)	Tumble agglomeration (disc, drum, fluid bed), briquetting, compacting, pelletizing (extrusion)	Build-up, caking
Fertilizers	Drum or disc granulation, compaction + crushing and screening, drop solidification	Caking, build-up, bag set
Filter cakes	Tumble agglomeration, briquetting, pelleting	Caking
Fumes (micrometer and submicrometer)	Fluid bed (with and without binder), disc and drum agglomeration	Build-up, caking, flow problems

(continued)

several types of agglomeration equipment can be identified. A description and list of equipment for each method follows:

Agitation methods are characterized by tumbling or particle-growth mixing, usually in the presence of a liquid. Available equipment includes disc pelletizers, drum pelletizers, cone pelletizers, paddle mixers, plow mixers, mixer-mullers, mixer-granulators, pin mixers, coating pans, vertical mixers, cone blenders, vibrating screens, and vibrating conveyor-processors.

Pressure methods are characterized by force, as with compaction techniques. Available equipment includes briquetters, compactors, extruders, pellet mills, tabletting machines, and isostatic compaction presses.

Thermal methods are characterized by applied heat, as in sintering or fusion and melt crystallization techniques. Available equipment includes heat-hardening devices, sinter strands or grates, indurating kilns, nodulizing kilns, drying and solidifying equipment, drum dryers, belt dryers, and hot-melt drum or pan granulators.

Liquid methods are characterized by spray or fluid bed agglomeration and agglomeration from liquid media. Available equipment includes spray dryers, prilling towers, spray granulators, and immiscible liquid-wetting devices.

Selection Factors for Choosing an Agglomeration Method

Selecting an agglomeration process or method depends on several factors, including the kind of raw material, the type of equipment, the intended use of the end product or agglomerate, and the use of a binder or binders. In many cases, there is a trade-off or compromise not necessarily determined by one factor alone.

Kind of Raw Material

In some instances, the selection of a method can be entirely dependent on the raw material's size or size range and uniformity of size. For example, a raw material that is 100 percent minus 325 mesh has different process requirements than a granular-fine stream ranging from 10 mesh to 325 mesh with a uniform size distribution curve.

The material's feed moisture, bulk density, angle of repose, flow characteristics, chemical composition, and toxicity can also effect the selection process. Table 1 lists the material characteristics of typical agglomerator feed streams, as well as the agglomeration methods suitable for these materials. The table shows the influence of the condition, size, handling characteristics, and moisture content of the raw material on process selection.

It should be noted, however, that there are exceptions to these guidelines. For instance, a pasty material may have to be extruded to utilize the flowability, viscosity, and moldability characteristics of the material as it flows through the auger and extruder die. In another case, a relatively coarse, but dry feed stock with the consistency of sand may not be pelletized by agitation and pellet growth alone; pressure, induced by a double-roll briquetter, may be required to compact the particles. Other feed materials, such as wood chips, are elastic and, at times, have a rather amorphous shape and size. Pelletizing and briquetting are poor agglomeration choices for these materials. A pellet mill or pellet press that applies pressure and friction and has a certain retention time in the die is a better choice.

In many cases, it is necessary to test a representative sample of a particular material in the laboratory before one or several agglomeration methods can be selected. Regardless of whether there is a previous application history, many materials are somewhat different, even within the same species, and should be tested.

Type of Equipment

The selection of an agglomeration method may not involve as wide a range of possibilities and variables as the field of equipment suggests. (See Table 2) When selecting agglomeration equipment, the processes before, during, and after the actual particle size enlargement step must also be considered. The total system, including storing feed, metering, proportioning, conveying, pretreating, binder-

Table 2
Agglomeration Capacity

Method	Maximum Capacity (tph)	Retention Time	Typical Applications
Briquetter	50	seconds	Coal, Lime, Magnesia
Compactor-Granulator	75	seconds	Fertilizer, Potash, Salt
Extruder (Auger, Screw)	30	5-10 min.	Clay, Foods, Plastics
Flexible Mixer-Agglomerator	40	seconds*	Chemicals, Flue Dust
Fluid Bed Granulator	30*	1-10 min.	Chemicals, Foods, Pharmaceuticals
Mixer-Granulator	10	> 30 min.	Ceramics, Chemicals
Nodulizing Kiln	1,000	> 30 min.	Cement, Lime, Ores
Pelletizer (Disc, Drum)	100	1-5 min.	Cement, Coal, Flue Dust
Pellet Mill	50	1-5 min.	Biomass, Plastics
Pin Mixer	25	0-5 min.	Carbon Black, Chemicals, Flue Dust
Piston Press (Ram Extruder)	5	1-10 min.	Metal chips or fines
Prill Tower	30	0-5 min.	Nitrate, Sulfur, Urea
Pugmill	300	5-10 min.	Clay, Fertilizer, Fly Ash
Sinter Strand	1,000	> 30 min.	Ferrous & Nonferrous Ores
Zig-Zag Blender	30	1-10 min.	Ceramics, Chemicals, Flue Dust

*Batch units: 0.5-1 tph
*Retention time = 1 sec.

adding, product handling, post-treating, screening, packaging, and shipping can influence the selection of an agglomeration device.

For example, almost all continuous agglomeration equipment requires a uniform and controllable feed, by either a volumetric or

ft. height, if the actual handling of the product is reasonable, not severe, and the end use is feedstock within the plant?

The physical specifications for some agglomerates are very strict, particularly if industry practice, market standards, or competitive pressures require adherence to a code. For instance, iron ore pellets, compacted potash granules, molecular sieves, catalyst supports, and metal briquettes for furnace charge require very high product strength. On the other hand, many other agglomerates have no fixed or known standards. A realistic basis for determining the desired physical specifications reduces investing and operating costs and makes the task of the equipment supplier and test engineer much easier.

Binder Use

Binderless agglomeration, using only the natural or induced bonding forces of the particulate and the optimum densification (packing) at lowest porosity, is the most desirable and economical agglomeration method. If a liquid needs to be added to induce particle flow and compaction, water is the first choice. If binderless agglomeration or water alone cannot produce a permanent bond with high tensile strength, then additional binder materials must be added to increase the final product strength.

The method of binder classification first proposed by P.I. Waters[4] and further described by K.R. Komarek,[5] distinguishes binders by type, physicals, function, and chemical composition. Binder materials are either liquid (water, alcohol, oil, silicate, acid), solid (clay, dry starch, bentonite) or semi-solid (tar, pitch). Some binders act upon the product as film between solid particles (water, starch, silicate); others act as a matrix, filling voids between the particulates and becoming part of the dense mass of the agglomerate (tar, pitch). Those classified as chemical binders rely on the chemical reaction within the binder upon curing or heating, or between the binder and the raw material. Two binders can also be added—such as cement and water, lime and water, and lime and molasses—to produce a chemical reaction and induce bonding strength in the agglomerate.

The use of a binder is often limited by the specifications of the agglomerate. Some agglomerates can or cannot use organic binders, inorganic binders, or binders containing ash-form constituents, sulphur, or toxic materials. Cost can also limit the use of a binder. The purchase cost can make the use of an otherwise excellent binder uneconomical, or the transportation cost may be higher than the binder cost at origin.

When selecting a binder, emphasis should be placed on the proper test procedures. The selection of a binder can influence the agglomerate's post-treatment process, including the type of equipment to be used for curing, drying, heating, and firing.[6] For the best results, laboratory results should be optimized and bench-tests qualified with at least one larger run in a prototype machine.

Wrapping Up the Selection Process

As this article has shown, selection of the proper agglomeration method and equipment depends on the characteristics of the raw material, the limitations of the equipment, the specifications of the desired agglomerate, and, in some instances, the choice of a binder or binders.

To help make process comparison and selection easier once this information is known, it is also useful to: study prior agglomeration applications for the same or similar raw material; review technical documentation on agglomeration methods or types of equipment made by professional societies, industry trade groups, or independent research organizations; consult industry standards on agglomerate product quality; and, compare vendor information and budget proposals. CTES

Endnotes

1. W.H. Engelleitner. *Selection of the Proper Agglomeration Process*, XVII, Institute for Briquetting & Agglomeration (1981).

2. W. Pietsch, "Pressure Agglomeration, The State of The Art," *Agglomeration*, 2, AIME (1977).

3. Ibid.

4. P.I. Waters, *Briquette Binders, A Reappraisal*, XII, Institute for Briquetting & Agglomeration, (1971).

5. K.R. Komarek, "Selecting Binders and Lubricants for Agglomeration Processes," *Chemical Engineering*, (1967).

6. J. MacDougall and V. Vellella, "Elements II: Briquetting and Agglomeration," Introduction, Institute for Briquetting and Agglomeration, (1983).

W.H. Engelleitner is a consultant specializing in agglomeration technology. He has more than twenty-five years experience in particle size enlargement by pelletizing, pressure compaction, extrusion, and other methods. Mr. Engelleitner is an executive and past president of the Institute for Briquetting and Agglomeration, a member of the Society of Mining Engineers, and a lecturer on briquetting, pelletizing, and extrusion at the Center for Professional Advancement, East Brunswick, NJ. In addition, he has authored many papers on agglomeration technology and holds several patents in this field. Mr. Engelleitner is currently manager of agglomeration for Teledyne Readco, York, PA.

For more information contact:
TELEDYNE READCO
501 South Richland Avenue
York, PA 17405
717/846-2501 Telex: 84-0439

12/15/99 GRP 1649 #17

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Madigan

SERIAL NO.: 09/113,254 ART UNIT: 1649

FILED: July 10, 1998 EXAMINER: Grunberg

FOR: SEEDING TREATMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PETITION FOR EXTENSION OF TIME

Applicants hereby petition the commissioner that the time now set for responding to the Official Action of June 18, 1999, be extended for three months to expire on December 18, 1999.

Our check for $435.00 is enclosed to cover the extension fee set in 37 CFR §1.136. A duplicate copy of this petition is enclosed.

Respectfully submitted,

Per: Philip M. Weiss
Philip M. Weiss
Reg. No.: 34,751

12/16/1999 MPRASAD 00000037 09113254
01 FC:217 435.00 OP

[redacted]

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
09/113,254			

EXAMINER	
ART UNIT	PAPER NUMBER
	6 1/2

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Anne Marie Grünberg (3) _____

(2) Philip Weiss (4) _____

Date of Interview 12/1999

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description: _____

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: _____

Identification of prior art discussed: _____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Philip Weiss, attorney for applicant, called to determine whether a declaration was appropriate. The examiner felt that a declaration at this time was not necessary, but art supporting definitions may be helpful.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary. A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has are ready been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form. Anne Marie Grünberg

FORM PTOL-413 (REV.1-96)

Manual of Patent Examining Procedure, Section 713.04 Substance of Interview must Be Made of Record

A complete written statement as to the substance of any face-to-face or telephone interview with regard to an application must be made of record in the application, whether or not an agreement with the examiner was reached at the interview.

§1.133 Interviews

* * * * *

(b) In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for response to Office action as specified in §§ 1.111,1.135. (35 U.S.C.132)

§ 1.2. Business to be transacted in writing. All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete a two-sheet carbon interleaf Interview Summary Form for each interview held after January 1, 1978 where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks in neat handwritten form using a ball point pen. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below.

The Interview Summary Form shall be given an appropriate paper number, placed in the right hand portion of the file, and listed on the "Contents" list on the file wrapper. The docket and serial register cards need not be updated to reflect interviews. In a personal interview, the duplicate copy of the Form is removed and given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephonic interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the telephonic interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Serial Number of the application
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (personal or telephonic)
- Name of participant(s)) (applicant, attorney or agent, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the claims discussed
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). (Agreements as to allowability are tentative and do not restrict further action by the examiner to the contrary.)
- The signature of the examiner who conducted the interview
- Names of other Patent and Trademark Office personnel present.

The Form also contains a statement reminding the applicant of his responsibility to record the substance of the interview.

It is desireable that the examiner orally remind the applicant of his obligation to record the substance of the interview in each case unless both applicant and examiner agree that the examiner will record same. Where the examiner agrees to record the substance of the interview, or when it is adequately recorded on the Form or in an attachment to the Form, the examiner should check a box at the bottom of the Form informing the applicant that he need not supplement the Form by submitting a separate record of the substance of the interview.

It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview:

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner. The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he feels were or might be persuasive to the examiner,
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Stimmary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete or accurate, the examiner will give the applicant one month from the date of the notifying letter or the remainder of any period for response, whichever is longer, to complete the response and thereby avoid abandonment of the application (37 CFR 1.135(c)).

Examiner to Check for Accuracy

Applicant's summary of what took place at the interview should be carefully checked to determine the accuracy of any argument or statement attributed to the examiner during the interview. If there is an inaccuracy and it bears directly on the question of patentability, it should be pointed out in the next Office letter. If the claims are allowable for other reasons of record, the examiner should send a letter setting forth his or her version of the statement attributed to him. If the record is complete and accurate, the examiner should place the indication "Interview record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

[U.S. GPO: 1997-422-310/60005]

GROUP 1600

Please type a plus sign (+) inside this box → ☐

REVOCATION OF POWER OF ATTORNEY OR AUTHORIZATION OF AGENT		
	Application Number	09/113,254
	Filing Date	July 10,1998
	First Named Inventor	Madigan
	Group Art Unit	1649
	Examiner Name	A. Grunberg
	Attorney Docket Number	29214

I hereby revoke all previous powers of attorney or authorizations of agent given in the above-identified application:

☒ A Power of Attorney or Authorization of Agent is submitted herewith.

OR

☐ Please change the correspondence address for the above-identified application to:

☐ Customer Number [ ] → Place Customer Number Bar Code Label here

OR

☒ Firm or Individual Name	Weiss & Weiss Attn: Philip M. Weiss				
Address	500 Old Country Road				
Address					
City	Garden City				
Country	U.S.A.	State	New York	Zip	11530
Telephone	516-739-1500	Fax	516-739-2189		

I am the:

☒ Applicant.

☐ Assignee of record of the entire interest.
Certificate under 37 CFR 3.73(b) is enclosed

SIGNATURE of Applicant or Assignee of Record	
Name	Feaco International by Daniel Madigan
Signature	Daniel P. Madigan
Date	10/20/99

511/07295.01
101399/2231/30864.00001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Madigan Serial No.: 09/113,254 Filed: July 10, 1998 For: SEEDING TREATMENTS	Group Art No: 1649 Attorney:

BOX RESPONSES NO FEE
Assistant Commissioner for Trademarks
2900 Crystal Drive
Arlington, VA 22202-3513

POWER OF ATTORNEY AND APPOINTMENT OF DOMESTIC REPRENSTATIVE

Sir:

Applicant hereby appoints Philip M. Weiss, Reg. No. 34,761; attorney of the firm WEISS & WEISS, located at 500 Old Country Road, Garden City, New York 11530, to prosecute this application to register, to transact all business in the Patent and Trademark Office in connection therewith and to receive the Certificate of Registration.

Philip M. Weiss, Esq., of Weiss & Weiss, whose postal address is 500 Old Country Road, Garden City, New York 11530, Telephone (516) 739-1500, is hereby designated as applicant's representative upon whom notices or process in proceedings affecting the mark may be served.

Respectfully submitted,

Feeco International

By: WEISS & WEISS

Per: Daniel P. Madigan

Daniel Paul Madigan
3913 Algoma Road
Green Bay, WI 54311

511/87286.01

TOTAL P.04

Approval for use through 09/30/2000, OMB 0651-0031
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Fesco International

Application No./Patent No.: 09/113,254 Filed/Issue Date: July 10, 1998

Entitled: SEEDING TREATMENTS

Fesco International, a Corporation
(name of Assignee) (Type of Assignee e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☐ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of an undivided part interest

in the patent application/patent identified above by virtue of either:

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the Patent and Trademark Office at Reel 9631, Frame 0624, or for which a copy thereof is attached;

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To: _____
The document was recorded in the Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.
2. From: _____ To: _____
The document was recorded in the Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.
3. From: _____ To: _____
The document was recorded in the Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] Copies of assignment or other documents in the chain of title are attached.

NOTE: A separate copy (i.e., the original assignment document or a true copy of the original document) must be submitted to Assignment Division in accordance with 37 CFR part 3, if the assignment is to be recorded in the records of the PTO. See MPEP 302-302.8]

The undersigned (whose title is supplied below) is empowered to sign this statement on behalf of assignee.

Daniel P. Madigan
Signature

Date

Daniel P. Madigan
Type or print name

President
Title

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comment on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SENT FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

TOTAL P.02

GROUP 1600

WEISS & WEISS
Attorneys At Law
500 Old Country Road, Suite 305, Garden City, NY 11530
Phone No. 516-739-1500 / Fax No. 516-739-2189

TO: Ann Marie Grunberg

FAX NO. 703-308-4242

DATE: October 28, 1999

FROM: Philip M. Weiss, Esq.

FAX NO. (516)739-2189

NUMBER OF PAGES (INCLUDING COVER SHEET): 4

RE: _____

NOTE: _____

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/113,254	07/10/98	MADIGAN D	29214

HM22/0618

THOMAS D WILHELM
WILHELM LAW SERVICE
100 W LAWRENCE STREET
THIRD FLOOR
APPLETON WI 54911

EXAMINER	
GRUNBERG, A	
ART UNIT	**PAPER NUMBER**
1649	5

DATE MAILED: 06/18/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)

Office Action Summary	Application No. 09/113,254	Applicant(s) Madigan et al.
	Examiner Anne Marie Grunberg	Group Art Unit 1649

☒ Responsive to communication(s) filed on *Jul 10, 1998* .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-75* is/are pending in the application.

Of the above, claim(s) *70-73* is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-69, 74, and 75* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☒ The drawing(s) filed on *Jul 10, 1998* is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☒ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *3*

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1649.

Election/Restriction

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

   I. Claims 1- 69, and 74-75, drawn to a seed capsule and methods of use, classified in class 47, subclass 58.1, for example.

   II. Claims 70-73, drawn to a method of making capsules, classified in class 47, subclass 57.6, for example.

2. The inventions are distinct, each from the other because of the following reasons:

Inventions II and I are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the capsules can be made by a method involving different sequences of steps than that claimed in Group II.

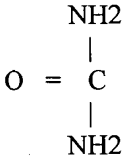
Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification or by their recognized divergent subject matter and because the search required for Invention I is not required in Inventions II, restriction for examination purposes as indicated is proper.

During a telephone conversation with Attorney Thomas Wilhelm on June 8, 1999 a provisional election was made with traverse to prosecute the invention of Group I, claims 1-69, and 74-75.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(I).

3. The disclosure is objected to because of the following informalities:

a. Throughout the specification, as on page 8, lines 22-24, for example, urea is described as inorganic. However urea has the following structure which clearly has a carbon and makes urea an organic substance;



b. Throughout the specification, as on page 9, lines 5-7, for example, sulfur, magnesium and chromium are characterized as being micronutrients. However, according to Biology of Plants (Raven et al., 1992), sulfur and magnesium are macroelements, and chromium is not listed as a micronutrient.

c. The figures are described in a confusing manner in the specification. For example, on page 19, lines 23-28, Figure 1 and 2 are said to contain a numbered "12", "14", and "16". However, Figure 1 does not seem to contain a "12" or "14", and Figure 2 does not contain a "16". The description of all the figures should be reviewed for such errors.

d. The drawings are objected to because Figure 1 has a number appearing under the labeled number "28" that is unreadable.

Appropriate correction is required. No new matter should be added.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1-2, 4-5, 7, 9, 12-13, 18, 22, 26-28, 30-31, 33, 35-36, 39, 43, 45-48, 51-52, 54-55, 58, 61-62, and 67-68, and dependent claims 3, 6, 8, 10-11, 14-17, 19-21, 23-25, 29, 32, 34, 37-38, 40, 56-57, 59-60, 63-66, and 69 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is unduly narrative in the recitation of "having an outer surface", and is vague and indefinite in the recitation of "mounted proximate, including disposed outwardly of the outer surface". It is unclear what the object of "mounted" is, nor is it clear what the second part of the phrase is referring to.

Claims 2, 28, 43, and 55 are vague and indefinite in the recitation of "enhancing", "reducing", "affects", and "assisting". This rejection may be obviated by changing the above to --enhanced--, --reduced--, --effects--, and --assistance--. Additionally, "ones of" is unclear (line 1 of iv.), and "flight" gives the impression that the seeds can fly. Since "flight" does not seem to be defined in the specification, it should be deleted.

Claim 4 is vague and indefinite in the recitation of "affect" as it is unclear what is intended. This rejection may be obviated by inserting --effects-- in its stead.

Claims 4 and 7 are vague and indefinite in the recitation of "animals, weeds, and spore-formers" for employing improper Markush terminology. See MPEP 2173.05(h). This rejection may be obviated by changing the phrase to --animals, weeds, or spore-formers.--

Claim 5 is vague and indefinite in the recitation "bitter substance". This terminology does not appear to be defined in the specification and it is subjective language that is open to interpretation.

Claims 9 and 58 are vague and indefinite in the recitation "and generally displaced from said seed" or "and generally displaced from the seeds" because it is not clear what is meant.

The recitation of "urea" in claim 12 is not in accordance with the term "inorganic" which precedes it. Urea is not an inorganic plant nutrient.

Claim 13 is vague and indefinite in grammatical composition. This rejection may be obviated by inserting --which-- before "is".

The recitation of "sulfur" and "chromium" in claim 18 is not in accordance with the term "micronutrient". According to the Biology of Plants (Raven et al., Ed, page 596), sulfur is a macronutrient, and chromium is not listed as a micronutrient.

Claim 22 is vague and indefinite in the recitation of "ones, but less than all", and in "for germination thereof' which are unduly narrative and confusing. This claim should be reworded to better reflect the intended meaning of the claim.

Claim 26 is vague and indefinite in the recitation of "having a first overall soil condition and texture", and "disposed outwardly of the outer surfaces of said seeds". The recitation "having outer surfaces," and "said coatings of said seed capsules.....in the root zone of said plant growing medium." is unduly narrative.

Claim 27 is vague and indefinite in the recitation "until respective ones of said seeds germinate." It is unclear what "ones" is referring to.

Claims 30-31 are vague and indefinite in the recitation of "affect" or "effect" and "animals, weeds, and spore-formers" for reasons stated above This rejection may be obviated by changing the above to --effects--, and --animals, weeds, or spore-formers.--

Claims 33 and 45 are vague and indefinite in the recitation "and generally displaced from said seeds" because it is not clear what is meant.

Claims 35 and 61 are vague and indefinite in the recitation of "uncoated ones of".

Claims 36, 48, and 62 are vague and indefinite in the recitation of "including" which is not U.S. recognized terminology since it is not possible to distinguish whether it is an open or closed term. This rejection may be obviated by replacing "including" with --further compromising--.

Claims 39, 51 and 67 are vague and indefinite in the recitation of "agglomerating said coatings onto said inner layers." It is unclear how the coatings can be agglomerated onto their own inner layers.

Claim 46 is vague and indefinite in the recitation of "nitrogen, phosphorus, and potassium" which employs improper Markush terminology. This rejection may be obviated by changing the above to --nitrogen, phosphorus, or potassium.--

Claim 47 is vague and indefinite in the recitation of "chromium" Chromium is not a recognized plant nutrient as taught by Biology of Plants (Raven et al., Ed, page 596).

Claim 52 is vague and indefinite in the recitation of "comprising" which is grammatically incorrect. This rejection may be overcome by deleting "comprising" and inserting --comprises--.

Claim 54 is unduly narrative in part '(a)'. It is unclear what applicant is claiming.

Claim 68 is vague and indefinite in the recitation of "the soil conditioners and plant nutrients" which lacks antecedent basis in claims 54. This rejection may be obviated by deleting "the" in line 2 of claim 68.

Clarification is required. No new matter should be added.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

7. Claims 1-5, 7-10, 12, 14-15, 17, 21, 23-35, 39-41, 43-45, 47-48, 51-59, 61, 63, 66-69, and 74-75, are rejected under 35 U.S.C. 102(b) as being anticipated by Gerber.

Claims 1-5, 7-10, 12, 14-15, 17, 21, 23-35, 39-41, 43-45, 47-48, 51-59, 61, 63, 66-69, and 74-75, are drawn to a combination seed capsule which can be easily broadcast, and which protects the seed from the weather and pests, thus increasing germination rate, and prolonging the

range during which the seed may germinate. Additionally, the seed capsule may comprise two coats and may contain nutrients, herbicides, pesticides, and a bitter substance. Moisture retaining substances and other additives, enhance the germination microenvironment of the seed and act as a soil conditioner.

Gerber teaches a seed pellet having a core containing seed, organic substrates, loess, organic fertilizers, fungicides, pesticides, and a wetting agent which promotes surface wettability (abstract, first 4 lines). At least one bitter substance may also be present to deter animals from eating the seed capsules (column 4, lines 13-28). A second, outer coat may be applied to the core and should be semipermeable to allow water to penetrate but which keeps the water-soluble constituents from leaving the core (column 4, lines 29-48). The seed capsule inhibits germination during storage (column 7, lines 62-65). The pellets allow an increase in germination to occur (column 6, lines 48-49) and allow seeds to better be dispersed from an airplane (column 7, lines 65-66).

8. Claims 1-5, 8, 19, 21-30, 32, 35, 37, 39-41, 43-44, 47, 49, 51-57, 61, 64, 66-69, and 74-75 are rejected under 35 U.S.C. 102(b) as being anticipated by Roth.

Claims 1-5, 8, 19, 21-30, 32, 35, 37, 39-41, 43-44, 47, 49, 51-57, 61, 64, 66-69, and 74-75 are drawn to a combination seed capsule which can be easily broadcast, and which protects the seed from the weather and pests, thus increasing germination rate, and prolonging the range during which the seed may germinate. Additionally, the seed capsule may comprise two coats

and may contain nutrients, herbicides, pesticides, and a bitter substance. Moisture retaining substances and other additives, enhance the germination microenvironment of the seed and act as a soil conditioner. Additionally, the soil conditioning material comprises a sludge composition.

Roth teaches a methanol treated activated sludge carrier that acts as a means for sustaining the release of agricultural chemicals and can be used as a seed pelleting composition (abstract). The sludge acts as a carrier for all types of chemicals including pesticides, fertilizers, plant growth regulators, attractants and repellants (column 2, lines 48-52). Compounds such as urea, and iron are discussed in column 3, lines 1-22. Crop seeds are coated with the pelleting composition (column 4, lines 46-48, claims 9, and 16-17) which is stable under adverse weather conditions, and although hydrating in water, does not dissolve and wash off the substrate (column 2, lines 41-44).

It is well known in the art, that seed coatings or encapsulations increase the size of the seed to make broadcasting easier and to improve flowability. Trace elements, nutrients, pesticides, and wettable substances serve to protect the seed and increase germinability , thus increasing the health and survival rate of young plants. Thus, these features are inherent properties of the coated seeds taught by Roth.

9. Claims 1-4, 7-9, 14, 20-21, 24-32, 35,38-45, 47, 50-58, 61, 65-68, and 74-75 are rejected under 35 U.S.C. 102(b) as being anticipated by Nilsson.

Claims 1-4, 7-9, 14, 20-21, 24-32, 35,38-45, 47, 50-58, 61, 65-68, and 74-75 are drawn to a combination seed capsule which can be easily broadcast, and which protects the seed from the weather and pests, thus increasing germination rate, and prolonging the range during which the seed may germinate. Additionally, the seed capsule may comprise two coats and may contain nutrients, herbicides, pesticides, and a bitter substance. Moisture retaining substances and other additives, enhance the germination microenvironment of the seed and act as a soil conditioner. Additionally, the soil conditioning material comprises a fiber-containing by-product of a paper making operation.

Nilsson teaches a seed germination improving capsule having a water absorbing ability (abstract) which may be made from paper pulp or paper fibers (column 1, lines 60-65). The capsule material may be provided with additives such as nutrients, wetting agents, and germination inhibitors, etc (column 3, lines 18-23) The capsule may also be dyed blue in order to discourage animals from eating them (column 3, lines 23-25). An additional outer material may be applied as a wetting agent (column 3, lines 36-44). Seed capsules disperse well and due to the dispersal properties and protective properties of the seed capsule, not as many seeds need to be dispersed (column 3, lines 26-35, column 6, lines 64-68, table 1 in column 7).

Claim Rejections - 35 USC § 103

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 1-69, and 74-75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schreiber in view of Aswell and Roth.

Claims 1-69, and 74-75 are drawn to a combination seed capsule which can be easily broadcast, and which protects the seed from the weather and pests, thus increasing germination rate, and prolonging the range during which the seed may germinate. Additionally, the seed capsule may comprise two coats and may contain a soil conditioning substance, nutrients, herbicides, pesticides, and a bitter substance. Moisture retaining substances and other additives,

enhance the germination microenvironment of the seed and act as a soil conditioner. Additionally, the soil conditioning material comprises a sludge composition or a fiber-containing by-product of a paper making operation.

Schreiber teaches a seed having a multiple layered coating (column 3, lines 35-39) in which the outer coating controls water imbibition of the seed to the extent necessary to delay germination until environmental factors are conducive to growth (claim 1).

Schreiber does not teach a combination seed capsule which can be easily broadcast, and contains nutrients, herbicides, pesticides, a bitter substance, and a soil conditioning substance.

Aswell teaches a waste paper soil conditioning and fertilizing pellet (column 1, lines 14-19). The densified pellets have greater water absorption and retention qualities than do most soils (column 3, lines 10-14) and may contain fertilizing ingredients (claims 5, 7).

Roth teaches an activated sludge that acts as an agricultural chemical carrier and suggests its use for seed pellets (abstract), as stated above.

It would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to use the method of coating seeds as taught by Schreiber, and to modify that method by using the waste paper soil conditioning and fertilizing pellet as taught by Aswell to contain the seeds, given that it would have been obvious to want to fertilize and condition the soil in order to realize healthy seedlings. Additionally, it would have been obvious to use the activated sludge as taught by Roth to plug the hollow pellets taught by Aswell in order to minimize any contact to herbicides included within the capsules, and to increase fertilizing

(Roth, column 2, lines 25-27) and water retention values. Choice of fungicides, pesticides and animal repellents would have been the optimization of process parameters.

No claim is allowed.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne Marie Grunberg whose telephone number is (703) 305-0805. The examiner can normally be reached from Monday through Thursday from 7:30 until 5:00, and every other Friday from 7:30 until 4:00.

If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached at (703) 308-3909. The fax number for the unit is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

AMG

June 16, 1999

DAVID T. FOX
PRIMARY EXAMINER
GROUP ~~180~~ 1649

Notice of References Cited

Application No.	Applicant(s)		
09/113,254	Madigan et al.		
Examiner		Group Art Unit	Page 1 of 1
Anne Marie Grunberg		1649	

U.S. PATENT DOCUMENTS

		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A	4,759,151	7/26/1988	Gerber	47	57.6
	B	4,628,633	12/16/86	Nilsson	47	57.6
	C	3,269,824	~~10/25/63~~ 8/1966	Aswell	47	57.6
	D	3,698,133	10/17/72	Schreiber	47	57.6
	E	4,065,287	12/27/77	Roth	71	13
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U	Biology of Plants, Raven et al., Worth Publishers, page 596	1992
	V		
	W		
	X		

Form PTO 948 (Rev. 8-98) U.S. DEPARTMENT OF COMMERCE - Patent and Trademark Office Application No. 9/113,654

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

The drawing(s) filed (insert date) 7-10-98 are:

A. ☐ approved by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 for the reasons indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawing must be submitted according to the instructions on the back of this notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:
   - Black ink. Color.
   - Color drawings are not acceptable until petition is granted.
   - Fig(s) _____
   - Pencil and non-black ink not permitted. Fig(s) _____

2. PHOTOGRAPHS. 37 CFR 1.84 (b)
   - ___ 1 full-tone set is required. Fig(s) _____
   - ___ Photographs not properly mounted (must use brystol board or photographic double-weight paper). Fig(s) _____
   - ___ Poor quality (half-tone). Fig(s) _____

3. TYPE OF PAPER. 37 CFR 1.84(e)
   - ___ Paper not flexible, strong, white, and durable. Fig(s) _____
   - ___ Erasures, alterations, overwritings, interlineations, folds, copy machine marks not accepted. Fig(s) _____
   - ___ Mylar, velum paper is not acceptable (too thin). Fig(s) _____

4. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:
   - ___ 21.0 cm by 29.7 cm (DIN size A4)
   - ___ 21.6 cm by 27.9 cm (8 1/2 x 11 inches)
   - ___ All drawing sheets not the same size. Sheet(s) _____
   - ___ Drawings sheets not an acceptable size. Fig(s) _____

5. MARGINS. 37 CFR 1.84(g): Acceptable margins:
   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm
   SIZE: A4 Size
   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm
   SIZE: 8 1/2 x 11
   Margins not acceptable. Fig(s) 1-3, 7-10
   - ✓ Top (T) ✓ Left (L)
   - ___ Right (R) ___ Bottom (B)

6. VIEWS. 37 CFR 1.84(h)
   REMINDER: Specification may require revision to correspond to drawing changes.
   Partial views. 37 CFR 1.84(h)(2)
   - ___ Brackets needed to show figure as one entity. Fig(s) _____
   - ___ Views not labeled separately or properly. Fig(s) _____
   - ___ Enlarged view not labeled separetely or properly. Fig(s) _____

7. SECTIONAL VIEWS. 37 CFR 1.84 (h)(3)
   - ___ Hatching not indicated for sectional portions of an object. Fig(s) _____
   - ___ Sectional designation should be noted with Arabic or Roman numbers. Fig(s) _____

8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(f)
   - ___ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____

9. SCALE. 37 CFR 1.84(k)
   - ___ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) _____

10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(i)
    - ✓ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (poor line quality). Fig(s) 1-8

11. SHADING. 37 CFR 1.84(m)
    - ___ Solid black areas pale. Fig(s) _____
    - ___ Solid black shading not permitted. Fig(s) _____
    - ___ Shade lines, pale, rough and blurred. Fig(s) _____

12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)
    - ✓ Numbers and reference characters not plain and legible. Fig(s) 1-8
    - ✓ Figure legends are poor. Fig(s) 1-8
    - ___ Numbers and reference characters not oriented in the same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____
    - ___ English alphabet not used. 37 CFR 1.84(p)(2)
    - ___ Figs _____
    - ___ Numbers, letters and reference characters must be at least .32 cm (1/8 inch) in height. 37 CFR 1.84(p)(3) Fig(s) _____

13. LEAD LINES. 37 CFR 1.84(q)
    - ___ Lead lines cross each other. Fig(s) _____
    - ___ Lead lines missing. Fig(s) _____

14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)
    - ___ Sheets not numbered consecutively, and in Arabic numerals beginning with number 1. Sheet(s) _____

15. NUMBERING OF VIEWS. 37 CFR 1.84(u)
    - ___ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____

16. CORRECTIONS. 37 CFR 1.84(w)
    - ___ Corrections not made from prior PTO-948 dated _____

17. DESIGN DRAWINGS. 37 CFR 1.152
    - ___ Surface shading shown not appropriate. Fig(s) _____
    - ___ Solid black shading not used for color contrast. Fig(s) _____

COMMENTS

REVIEWER [illegible] DATE 6-17-99 TELEPHONE NO. 703 305 8430

ATTACHMENT TO PAPER NO. 5

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

OIPE OCT 21 1998 PATENT AND TRADEMARK OFFICE

ATTY DOCKET NO.	SERIAL NO.
29214	09/113,254
APPLICANT (S) Daniel Paul Madigan et al	
FILING DATE 07/10/98	GROUP ~~Unassigned~~ 1649

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
AMG		56,140	07/1866	Blessing	—	—	
AMG		2,664,350	12/1953	Hale et al	71	1	
AMG		3,545,129	12/1970	Schreiber et al	47	57.6	
AMG		3,621,612	11/1971	Porter	47	58	
AMG		3,698,133	10/1972	Schreiber	47	57.6	
AMG		3,936,976	02/1976	Porter et al	47	57.6	
AMG		3,947,996	04/1976	Watts	47	57.6	
AMG		3,950,891	04/1976	Hinkes	47	57.6	
AMG		4,116,666	09/1978	Willard, Sr.	71	77	
AMG		4,192,095	03/1980	Haslam et al	47	58	
AMG		4,272,417	06/1981	Barke et al	260	22 R	

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
AMG		2354101	05/1974	Germany	A 01 c	07/00		✓

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

AMG			Pietsch, Wolfgang, "Part 2. Agglomerate Bonding and Strength," Date unknown. (Reprinted from W. Pietsch (98)).
AMG			Staub-Reinhalt, Luft, "Part 3, THE AGGLOMERATIVE BEHAVIOR OF FINE PARTICLES," (Reprinted from W. Pietsch (7), English edition). Vol. 27, No. 1, January 1967.

EXAMINER	DATE CONSIDERED
Anne Marie Grimberg	13 June 99

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

OCT 2 1 1998 JC58 PATENT & TRADEMARK OFFICE

ATTY DOCKET NO.	SERIAL NO.
29214	09/113,254
APPLICANT (S) Daniel Paul Madigan et al	
FILING DATE 07/10/98	GROUP ~~Unassigned~~ 1649

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
AMG		4,344,979	08/1982	Gago et al	427	4	
AMG		4,438,593	03/1984	McNew et al	47	57.6	
AMG		4,452,008	06/1984	Sandhu et al	47	57.6	
AMG		4,493,162	01/1985	Langan et al	47	57.6	
AMG		4,752,319	06/1988	DelliColli	71	77	
AMG		4,759,151	07/1988	Gerber	47	57.6	
AMG		5,044,116	09/1991	Gago et al	47	57.6	
AMG		5,087,475	02/1992	Bazin et al	427	4	
AMG		5,106,648	04/1992	Williams	427	3	
AMG		5,127,185	07/1992	Kojimoto et al	47	57.6	
AMG		5,300,127	04/1994	Williams	47	57.6	

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

EXAMINER	DATE CONSIDERED
Anne Marie Grimberg	13 June 1999

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY DOCKET NO.	SERIAL NO.
29214	09/113,254
APPLICANT(S) Daniel Paul Madigan et al	
FILING DATE 07/10/98	GROUP ~~Unassigned~~ 1649

OIPE JC58 OCT 21 1998 PATENT & TRADEMARK OFFICE

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
AMG		5,368,626	11/1994	Schnuda	71	23	
AMG		5,525,131	06/1996	Asano	47	57.6	
AMG		5,623,781	04/1997	Legro	47	576	

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

EXAMINER	DATE CONSIDERED
Anne Marie Grunberg	13 June 1999

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Receipt #4

29214

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Daniel Paul Madigan et al

Group Art Unit: 3616

Serial Number: 09/113,254

Examiner: Unknown

Filed: 07/10/98

For: SEEDING TREATMENTS

REQUEST FOR CORRECTION TO FILING RECEIPT

Application Processing Division
Customer Correction Branch
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants note that the subject application has an incorrect city name for RONALD DEAN EICHHORN on the Filing Receipt (PTO-103X).

The correct city for RONALD D. EICHHORN should be "GREEN BAY".

Applicants respectfully request that the city of the inventor be corrected. These corrections have been noted on the copy of the **Filing Receipt** enclosed herewith. A copy of the title page as originally submitted is also enclosed.

Respectfully submitted,
Daniel Paul Madigan et al

By: Thomas Wilhelm
Thomas D. Wilhelm
Attorney for Applicants
(Reg. No. 28,794)

January 22, 1999
Appleton, Wisconsin 54911
(920) 831-0100
(920) 831-0101 FAX

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO APPLICATION PROCESSING DIVISION, CUSTOMER CORRECTION BRANCH, ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231

ON January 22, 1999.

Kerri Bruchs
(Typed name of person mailing paper or fee)

Kerri Bruchs
(Signature)

1/22/99
(Date Of Signature)

PTO-103X
(Rev. 8-95)

RECEIVED JAN 1 1999

OIPE JC23 JAN 25 1999 PATENT & TRADEMARK OFFICE

FILING RECEIPT

CORRECTED

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS	TOT CL	IND CL
09/113,254	07/10/98	3616	$1,147.00	29214	6	75	5

THOMAS D WILHELM
WILHELM LAW SERVICE
100 W LAWRENCE STREET
THIRD FLOOR
APPLETON WI 54911

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to the Application Processing Division's Customer Correction Branch within 10 days of receipt. Please provide a copy of the Filing Receipt with the changes noted thereon.

Applicant(s)

DANIEL PAUL MADIGAN, GREEN BAY, WI; MICHAEL DENNIS KRYSIAK, GREEN BAY, WI; RONALD DEAN EICHHORN, ~~EICHHORN,~~ GREEN BAY WI; GLEN H. WESENBERG, GREEN BAY, WI.

FOREIGN FILING LICENSE GRANTED 07/28/98
TITLE
SEEDING TREATMENTS

PRELIMINARY CLASS: 047

RECEIVED
FEB 0 8 1999
MATRIX CUSTOMER
SERVICE CENTER

DATA ENTRY BY: WHITE, JACKIE P. 134 TEAM: 03 DATE: 01/07/99

(see reverse)

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "FOREIGN FILING LICENSE GRANTED" followed by a date appears on the reverse side of this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.11. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related application(s) filed under 37 CFR 1.62 which meets the provisions of 37 CFR 5.15(a). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations, especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR Parts 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "FOREIGN FILING LICENSE GRANTED" DOES NOT appear on the reverse side of this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the fiing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PATENT APPLICATION

TITLE: SEEDING TREATMENTS

By: Daniel Paul Madigan
804 S. Madison
Green Bay, WI 54301
Citizenship: USA

Michael Dennis Krysiak
3554 Highland Center Drive
Green Bay, WI 54311
Citizenship: USA

Ronald Dean Eichhorn
1524 ½ Cedar Street
Green Bay, WI 54302
Citizenship: USA

Glen H. Wesenberg
920 Laverne Drive
Green Bay, WI 54311
Citizenship: USA

"Express Mail" mailing number EM 469 259 847 US
Date of Deposit June 10, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Jerry F. Janssen
(Typed or printed name of person mailing paper or fee)

Jerry F. Janssen
(Signature of person mailing paper or fee)

TDW, JSK

OIPE JC54 DEC 14 1998 PATENT & TRADEMARK OFFICE

Sector $

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO./TITLE
09/113,254	07/10/98	MADIGAN	D 29214

0232/0813

THOMAS D WILHELM
WILHELM LAW SERVICE
100 W LAWRENCE STREET
THIRD FLOOR
APPLETON WI 54911

NOT ASSIGNED

3616

DATE MAILED: 08/13/98

NOTICE TO FILE MISSING PARTS OF APPLICATION
Filing Date Granted

An Application Number and Filing Date have been assigned to this application. The items indicated below, however, are missing. Applicant is given TWO MONTHS FROM THE DATE OF THIS NOTICE within which to file all required items and pay fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a). **If any of items 1 or 3 through 5 are indicated as missing, the SURCHARGE set forth in 37 CFR 1.16(e) of ☐ $65.00 for a small entity in compliance with 37 CFR 1.27, or ☑ $130.00 for a non-small entity, must also be timely submitted in reply to this NOTICE to avoid abandonment.**

If all required items on this form are filed within the period set above, the total amount owed by applicant as a ☐ small entity (statement filed) ☑ non-small entity is $ 1294

☑ 1. The statutory basic filing fee is:
- ☑ missing.
- ☐ insufficient.

Applicant must submit $ 790 to complete the basic filing fee and/or file a small entity statement claiming such status (37 CFR 1.27).

☑ 2. Additional claim fees of $ 1374, including any multiple dependent claim fees, are required.

$ 164 for 2 independent claims over 3.

$ 126 for 55 dependent claims over 20.

$ _____ for multiple dependent claim surcharge.

Applicant must either submit the additional claim fees or cancel additional claims for which fees are due.

☐ 3. The oath or declaration:
- ☐ is missing or unexecuted.
- ☐ does not cover the newly submitted items.
- ☐ does not identify the application to which it applies.
- ☐ does not include the city and state or foreign country of applicant's residence.

An oath or declaration in compliance with 37 CFR 1. 63, including residence information and identifying the application by the above Application Number and Filing Date is required.

☐ 4. The signature(s) to the oath or declaration is/are by a person other than inventor or person qualified under 37 CFR 1.42, 1.43 or 1.47.
A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.

☐ 5. The signature of the following joint inventor(s) is missing from the oath or declaration:

_____

An oath or declaration in compliance with 37 CFR 1.63 listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.

☐ 6. A $50.00 processing fee is required since your check was returned without payment (37 CFR 1.21(m)).

☐ 7. Your filing receipt was mailed in error because your check was returned without payment.

☐ 8. The application does not comply with the Sequence Rules.
See attached "Notice to Comply with Sequence Rules 37 CFR 1.821-1.825."

☐ 9. OTHER: _____

Direct the reply and any questions about this notice to "Attention: Box Missing Parts."

A copy of this notice MUST be returned with the reply.

Customer Service Center
Initial Patent Examination Division (703) 308-1202

PART 3 - OFFICE COPY

12/21/1998 CHENG 00000010 232/0813 09113254
01 FC:205 53.00 CH
02 FC:202 82.00 CH
03 FC:203 605.00 CH
04 FC:201 256.00 CH
139.00 CH

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SEEDING TREATMENTS,
the specification of which
(check one) ____ is filed herewith.
__X__ was filed on July 10, 1998
__X__ Application Serial No. 09/113,254
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56 (a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	(Yes)	(No)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

60/052,287	07/11/97	Provisional
(Application Serial No.)	(Filing Date)	(Status-Patented, Pending, Abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:
THOMAS D. WILHELM (REG. NO. 28794), JERRY F. JANSSEN (REG. NO. 29175), JASBIR S. KINDRA (REG. NO. 41115)
Address all telephone calls to THOMAS D. WILHELM at telephone no. 920-831-0100
Address all correspondence to THOMAS D. WILHELM at the following address:
100 W. LAWRENCE ST. FLOOR 3
APPLETON, WI 54911 USA

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Daniel Paul Madigan
Inventor's signature x Daniel Paul Madigan Date x 10/13/98
Residence 804 S. Madison, Green Bay, Wisconsin 54301 Citizen USA
Post Office Address 804 S. Madison, Green Bay, Wisconsin 54301 USA

Full name of second joint inventor, if any Michael Dennis Krysiak
Second Inventor's signature x Michael Dennis Krysiak Date x 10/19/98
Residence 3554 Highland Center Drive, Green Bay, Wisconsin 54311 Citizen USA
Post Office Address 3554 Highland Center Drive, Green Bay, Wisconsin 54311 USA

Additional inventors are named on sheet 2 of 2 sheets.

Full name of third joint inventor, if any Ronald Dean Eichhorn
Third Inventor's signature x [signature] Date x 10/19/98
Residence 1524 ½ Cedar Street, Green Bay, Wisconsin 54302 Citizen USA
Post Office Address 1524 ½ Cedar Street, Green Bay, Wisconsin 54302 USA

Full name of fourth joint inventor, if any Glen H. Wesenberg
Inventor's signature x [signature] Date x 20 Oct. 98
Residence 920 Laverne Drive, Green Bay, Wisconsin 54311 Citizen USA
Post Office Address 920 Laverne Drive, Green Bay, Wisconsin 54311 USA

Applicant or Patentee: DANIEL PAUL MADIGAN ET AL Attorney's
Serial No. or Patent No.: 09/113,254 Docket No. 29214
Date Filed or Issued: July 10, 1998
For: SEEDING TREATMENTS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled SEEDING TREATMENTS and described in

[ ] the specification filed herewith
[X] application serial no. 09/113,254, filed 07/10/98
[ ] patent no. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[X] persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME FEECO International Inc.
ADDRESS 3913 Algoma Road, Green Bay, Wisconsin 54311
[ ] INDIVIDUAL [X] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Daniel Paul Madigan	Michael Dennis Krysiak	Ronald Dean Eichhorn
TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR
X Daniel Paul Madigan	X Michael Dennis Krysiak	X Ronald Dean Eichhorn
Signature of Inventor	Signature of Inventor	Signature of Inventor
X 10/13/98	X 10-15-98	X 10-19-98
Date	Date	Date

Applicant or Patentee: DANIEL PAUL MADIGAN ET AL Attorney's
Serial No. or Patent No.: 09/113,254 Docket No. 29214
Date Filed or Issued: July 10, 1998
For: SEEDING TREATMENTS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled SEEDING TREATMENTS and described in

[ ] the specification filed herewith
[X] application serial no. 09/113,254, filed 07/10/98
[ ] patent no. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[X] persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME FEECO International Inc.
ADDRESS 3913 Algoma Road, Green Bay, Wisconsin 54311
[ ] INDIVIDUAL [X] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

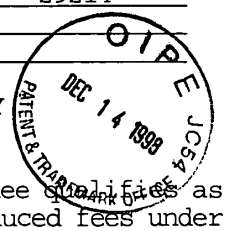
FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Glen H. Wesenberg		
TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR
X Glen H. Wesenberg		
Signature of Inventor	Signature of Inventor	Signature of Inventor
X 20 Oct. 98		
Date	Date	Date

Applicant or Patentee: [redacted] PAUL MADIGAN ET AL Attorney's
Serial or Patent No.: [redacted] 254 Docket No. 29214
Date Filed or Issued: July 10, 1998
For: SEEDING TREATMENTS



VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27(b)) - SMALL ENTITY

As a representative of the assignee, I hereby declare that the assignee qualifies as a small entity as defined in 37 CFR 1.9(d) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35 United States Code, to the Patent and Trademark Office with regard to the invention entitled SEEDING TREATMENTS and described in

[ ] the Provisional Patent Application filed herewith
[X] application serial no. 09/113,254, filed 07/10/98
[ ] patent no. _____, issued _____.

The assignee has not signed, granted, conveyed, or licensed and is under no obligation under contract of law to assign, grant, convey, or license any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which the assignee has assigned, granted, conveyed, or licensed or is under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[x] no such person, concern, or organization
[ ] person, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dan Madigan,	President
TYPED NAME OF PERSON SIGNING	TITLE
FEECO International	Green Bay, Wisconsin
IDENTITY OF ASSIGNEE BEING REPRESENTED	ASSIGNEE'S CITY AND STATE
x Dan Madigan	x 10/13/98
SIGNATURE	DATE SIGNED

29214
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Daniel Paul Madigan et al

Group Art Unit: 3616

Serial Number: 09/113,254

Examiner: Unassigned

Filed: 07/10/98

For: SEEDING TREATMENTS

RESPONSE TO NOTICE TO FILE MISSING PARTS

Attention: Box Missing Parts
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This paper is submitted in response to the Notice To File Missing Parts dated 08/13/98 in the above-identified application. The Missing Parts required are the Surcharge and Filing Fees.

Applicants enclose herewith the following documents:

Petition For Extension of Time - two months;
copy of Notice to File Missing Parts of Application;
Declaration, signed 10/13/98, 10/19/98 and 10/20/98(2 sheets);
Inventors' Small Entity Statement, signed 10/13/98, 10/15/98, 10/19/98, and 10/20/98 respectively (2 sheets);
Assignee Small Entity Statement, signed 10/13/98 (1 sheet);
Check #5283 for $65.00, for the Missing Parts Fee;
Check #5284 for $953.00, for the Filing Fee; and
Check #5285 for $190.00, for the Petition for Extension Fee

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO BOX MISSING PARTS, ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231

ON December 11, 1998.

Kerri Bruchs
(Typed name of person mailing paper or fee)

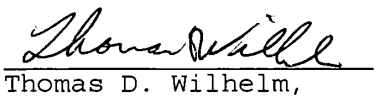
Kerri Bruchs
(SIGNATURE)

12/11/98
(DATE OF SIGNATURE)

Applicants submit that all parts of the application are now present in the PTO, and request that the Official Receipt be issued forthwith.

Should any additional fee be properly due, kindly charge same to Deposit Account 23-2130.

Respectfully submitted,
Daniel Paul Madigan et al

By: 
Thomas D. Wilhelm,
Attorney for Applicants
(Reg. No. 28,794)

December 9, 1998
Appleton, Wisconsin
920-831-0100
920-831-0101 FAX

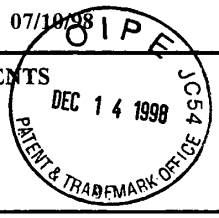
G:\DATA\WPDATA\PATENT\29214\missingpart.pto

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) (Small Entity)	Docket No. 29214

In Re Application Of: **Daniel Paul Madigan et al**

Serial No.	Filing Date	Examiner	Group Art Unit
09/113,254	07/10/98	Unassigned	3616

Invention: **SEEDING TREATMENTS**



TO THE ASSISTANT COMMISSIONER FOR PATENTS:

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a response to the Office Action of 08/13/98 (Date) in the above-identified application.

The requested extension is as follows (check time period desired):

☐ One month ☒ Two months ☐ Three months ☐ Four months ☐ Five months

from: 10/13/98 (Date) until: 12/13/98 (Date)

A verified statement of small entity status as a small entity under 37 CFR 1.27:

☒ is enclosed.
☐ has already been filed in this application.

The fee for the extension of time is $190 and is to be paid as follows:

☒ A check in the amount of the fee is enclosed.
☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account No. 23-2130
A duplicate copy of this sheet is enclosed.
☐ If an additional extension of time is required, please consider this a petition therefor and charge any additional fees which may be required to Deposit Account No. 23-2130 A duplicate copy of this sheet is enclosed.

Thomas D Wilhelm
Signature

Dated: December 11, 1998

Thomas D. Wilhelm (Reg. No. 28,794)
Wilhelm Law Service
100 W. Lawrence St., Third Floor
Appleton, WI 54911

I certify that this document and fee is being deposited on **December11,1998** with the U.S. Postal Service as first class mail under 37 C.F.R. 1.8 and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Kerri Bruchs
Signature of Person Mailing Correspondence

Kerri Bruchs
Typed or Printed Name of Person Mailing Correspondence

12/21/1998 CHOANG 00000010 232130 09113254
01 FC:216 200.00 OP

cc:

0360

29214
Patent

#31 4.R.
01/22/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Daniel Paul Madigan et al

Group Art Unit: Unassigned

Serial Number: 09/113,254

Examiner: Unassigned

Filed: 07/10/98

For: SEEDING TREATMENTS

INFORMATION DISCLOSURE STATEMENT

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to Applicants' duty of disclosure set forth in 37 C.F.R. § 1.56, the Applicants wish to bring to the Examiner's attention the references listed here and on the attached PTO Form 1449.

No representation is made, and no representation is intended, that more relevant material does not exist or that the order of presentation of these materials in any way reflects their relative pertinence. The references cited on the attached PTO Form 1449 are not intended to constitute an admission of any kind. Specifically, this presentation is not an admission that any of the items listed on the attached PTO Form 1449 are properly citable against the above-identified application.

In accordance with the provisions of 37 C.F.R. § 1.98, the references are listed on the attached PTO Form 1449 and copies

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231

ON October 19, 1998.

Kerri Bruchs
(TYPED NAME OF PERSON MAILING PAPER OR FEE)

Kerri Bruchs
(SIGNATURE)

October 19, 1998
(DATE OF SIGNATURE)

are submitted herewith. The attached copies have been pulled from the Applicants' or assignee's file. Accordingly, some of the references may have written indicia thereon. It is requested that the Examiner ignore all such written indicia as such indicia may not be relevant to the instant case or may not be an accurate characterization of the reference.

This Information Disclosure Statement is being filed before issuance of a first Office Action or within three months of the filing date of the referenced patent application. Accordingly, no fee is due. Nevertheless, the Commissioner is hereby authorized to charge payment of any additional fees due under 37 C.F.R. § 1.17 or credit any overpayment to Deposit Account No. 23-2130. It is Applicants' desire to have these references available in the record for both the Examiner and the public to review. Applicants, therefore, request that the Examiner review the entire disclosure of each reference and make all references of record.

U.S. Patent Documents

56,140	Blessing
2,664,350	Hale et al
3,545,129	Schreiber et al
3,621,612	Porter
3,698,133	Schreiber
3,936,976	Porter et al
3,947,996	Watts
3,950,891	Hinkes
4,116,666	Willard, Sr.
4,192,095	Haslam et al
4,272,417	Barke et al
4,344,979	Gago et al
4,438,593	McNew et al
4,452,008	Sandhu et al
4,493,162	Langan et al
4,752,319	DelliColli
4,759,151	Gerber
5,044,116	Gago et al
5,087,475	Bazin et al
5,106,648	Williams
5,127,185	Kojimoto et al
5,300,127	Williams
5,368,626	Schnuda

5,525,131 Asano
5,623,781 Legro

Foreign Patent Document

2354101 Germany

Other Documents

Pietsch, Wolfgang, "Part 2. Agglomerate Bonding and Strength," Date unknown. (Reprinted from W. Pietsch (98)).

Staub-Reinhalt, Luft, "Part 3, THE AGGLOMERATIVE BEHAVIOR OF FINE PARTICLES," (Reprinted from W. Pietsch (7), English edition). Vol. 27, No. 1, January 1967.

Respectfully submitted,
Daniel Paul Madigan et al

By: Thomas D. Wilhelm
Thomas D. Wilhelm,
Attorney for Applicants
(Reg. No. 28,794)

October 19, 1998
Appleton, Wisconsin
920-831-0100
920-831-0101 FAX

G:\DATA\WPDATA\PATENT\29214\156ids

Best Available Copy

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO./TITLE
09/113,254	07/10/98	MADIGAN	D 29214

0232/0813

THOMAS D WILHELM
WILHELM LAW SERVICE
100 W LAWRENCE STREET
THIRD FLOOR
APPLETON WI 54911

NOT ASSIGNED

3616

DATE MAILED: 08/13/98

NOTICE TO FILE MISSING PARTS OF APPLICATION
Filing Date Granted

An Application Number and Filing Date have been assigned to this application. The items indicated below, however, are missing. Applicant is given TWO MONTHS FROM THE DATE OF THIS NOTICE within which to file all required items and pay fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a). **If any of items 1 or 3 through 5 are indicated as missing, the SURCHARGE set forth in 37 CFR 1.16(e) of ☐ $65.00 for a small entity in compliance with 37 CFR 1.27, or ☑ $130.00 for a non-small entity, must also be timely submitted in reply to this NOTICE to avoid abandonment.**

If all required items on this form are filed within the period set above, the total amount owed by applicant as a ☐ small entity (statement filed) ☑ non-small entity is $ 1852.00

☐ 1. The statutory basic filing fee is:
- ☐ missing.
- ☐ insufficient.

Applicant must submit $ 790 to complete the basic filing fee and/or file a small entity statement claiming such status (37 CFR 1.27).

☑ 2. Additional claim fees of $ 1374, including any multiple dependent claim fees, are required.

$ 164 for 8 independent claims over 3

$ 126 for 35 dependent claims over 20.

$ _____ for multiple dependent claim surcharge.

Applicant must either submit the additional claim fees or cancel additional claims for which fees are due.

☐ 3. The oath or declaration:
- ☐ is missing or unexecuted.
- ☐ does not cover the newly submitted items.
- ☐ does not identify the application to which it applies.
- ☐ does not include the city and state or foreign country of applicant's residence.

An oath or declaration in compliance with 37 CFR 1. 63, including residence information and identifying the application by the above Application Number and Filing Date is required.

☐ 4. The signature(s) to the oath or declaration is/are by a person other than inventor or person qualified under 37 CFR 1.42, 1.43 or 1.47.
A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.

☐ 5. The signature of the following joint inventor(s) is missing from the oath or declaration:

_____
An oath or declaration in compliance with 37 CFR 1.63 listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.

☐ 6. $50.00 processing fee is required since your check was returned without payment (37 CFR 1.21(m)).

☐ A corrected filing receipt was mailed in error because your check was returned without payment.

☑ 8. The application does not comply with the Sequence Rules.
See attached "Notice to Comply with Sequence Rules 37 CFR 1.821-1.825."

☐ 9. OTHER: _____

Direct the reply and any questions about this notice to "Attention: Box Missing Parts."

A copy of this notice MUST be returned with the reply.

Customer Service Center
Initial Patent Examination Division (703) 308-1202

Express Mail Label No. EM 469 259 847 US

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 29214

Total Pages in this Submission 82

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

SEEDING TREATMENTS

and invented by:

DANIEL PAUL MADIGAN
MICHAEL DENNIS KRYSIAK
RONALD DEAN EICHHORN
GLEN H. WESENBERG

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

1. ☐ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 70 pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
29214
Total Pages in this Submission
82

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
   a. ☐ Formal b. ☒ Informal Number of Sheets 6

4. ☐ Oath or Declaration
   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
   c. ☐ With Power of Attorney ☐ Without Power of Attorney
   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission *(if applicable, all must be included)*
   a. ☐ Paper Copy
   b. ☐ Computer Readable Copy
   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & documents)*

9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing
   ☐ First Class ☒ Express Mail *(Specify Label No.)*: EM 469 259 847 US

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
29214
Total Pages in this Submission
82

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Small Entity Statement(s) - Specify Number of Statements Submitted: _____

17. ☒ Additional Enclosures *(please identify below):*

Correspondence Address

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	75	- 20 =	55	x	$11.00	$605.00
Indep. Claims	5	- 3 =	2	x	$41.00	$82.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$395.00
OTHER FEE *(specify purpose)* _____						$0.00
					TOTAL FILING FEE	$1,082.00

☐ A check in the amount of to cover the filing fee is enclosed.

☐ The Commissioner is hereby authorized to charge and credit Deposit Account No. as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☐ Credit any overpayment.
- ☐ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: July 10, 1998

Thomas D. Wilhelm
Signature

Thomas D. Wilhelm (Reg. No. 28,794)

cc:

29214
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Daniel Paul Madigan et al

Group Art Unit: Unassigned

Serial Number: Unassigned

Examiner: Unassigned

Filed: July 10, 1998

For: SEEDING TREATMENTS

CORRESPONDENCE ADDRESS

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Kindly address all correspondence regarding the above-referenced Application to the following address:

Thomas D. Wilhelm
Wilhelm Law Service, S.C.
100 W. Lawrence Street
Third Floor
Appleton, WI 54911

Phone 920-831-0100
FAX 920-831-0101

Respectfully submitted,
Daniel Paul Madigan et al

By _____
Thomas D. Wilhelm
Attorney for Applicants
(Reg. No. 28,794)

July 10, 1998
Appleton, Wisconsin

PATENT APPLICATION

TITLE: SEEDING TREATMENTS

By: Daniel Paul Madigan
804 S. Madison
Green Bay, WI 54301
Citizenship: USA

Michael Dennis Krysiak
3554 Highland Center Drive
Green Bay, WI 54311
Citizenship: USA

Ronald Dean Eichhorn
1524 ½ Cedar Street
Green Bay, WI 54302
Citizenship: USA

Glen H. Wesenberg
920 Laverne Drive
Green Bay, WI 54311
Citizenship: USA

"Express Mail" mailing number EM 469 259 847 US

Date of Deposit June 10, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Jerry F. Janssen
(Typed or printed name of person mailing paper or fee)

Jerry F. Janssen
(Signature of person mailing paper or fee)

TDW, JSK

SEEDING TREATMENTS

Field of the Invention

This present invention relates to improvements in seed and seed-related products, processes for making such products and processes for establishing and improving seed beds and seed bed germination. As additional benefits, this invention is directed at improving soil productivity through enhancements in soil fertility, soil condition/tilth, and control of soil moisture. Further, the invention relates to productive use of certain types of abundantly available manufacturing waste, which waste is currently being disposed of in landfills.

Background of the Invention

Agricultural growers, gardeners, landscape operators, flower growers, and the like produce a wide variety of cultivated crops. Many such crops are grown from seed. The sizes, shapes, and physical characteristics of the various kinds of seeds are as varied as the number of crops produced therefrom.

Producers of such cultivated crops encounter a variety of challenges in handling and distributing such seed, as well as with sowing of such seed in suitable growing media. Certain seed may desirably be sowed by a broadcast method if the seed were compatible with broadcast application. For example, grass seed for lawns is desirably broadcast, but the low density and generally non-aerodynamic shape of some grass seed can limit the range of such broadcast, and make such seed susceptible to being blown about

by wind, or washed away by surface water, even if initially well placed in a good seeding application.

Another difficulty encountered in sowing seed is that the seed may be so small as to be difficult to handle, thereby to place properly-spaced seeds at a desired spacing as to make cost-effective use of the seed, thereby to produce a crop of the related plants without using any more seed than necessary, thus to gain maximum benefit from the amount of seed used.

While small seed may be efficiently handled by industrial equipment especially designed for handling such seed, typically the user of such seed also handles various other types of seed; and may be unable to justify the cost of such specialty seed-handling equipment. Rather, the seed user typically has a limited range of seed handling equipment which must be capable of being used and/or adapted to handle and apply all the types of seeds being used by that user. Where the seed itself can be adapted to the equipment, specialty seed can be handled without need for any specialized equipment.

Even where the seed may be sown by hand, such as in seedling or bedding trays or pots, some seeds are so small as to be difficult for the sower/user to effectively manipulate and control by hand. Typical of such difficult-to-handle seeds are seeds of lettuce, carrots, the cabbage family, ground cherries, and alfalfa. Many flower seeds are equally small and/or difficult to handle and/or manipulate, for example poppy seed.

When seed is planted, the seed has immediate use for moisture to aid in germination of the seed, and subsequent early development of the resulting young plant. Where moisture is not readily available to the seed when planted, the seed may lie in a dormant state for some period of time before germinating. While the seed is thus dormant, awaiting suitable moisture, the seed is subject to a variety of hazards which may destroy its viability. The seed may be attacked by worms, parasites, and other pests. The seed may be

eaten by foraging animals including insects and larvae. The seed may be overheated by a hot sun. The seed may lie dormant without germinating for so long that any plant emerging therefrom will have insufficient time to mature before the end of the growing season.

If and when the seed does germinate, the seedling plant has a continuing need for a proper balance of moisture and oxygen, as well as for such plant nutrients as nitrogen, phosphorous, and potash, as well as the micronutrients, in relatively predictable quantities. To the extent the proper balance of such materials is available to the young plant, a healthy young plant may be produced, with optimum potential for maximum crop production, assuming germination occurs at a seasonably-desirable time.

To the extent one or more such materials is not available to the seed and/or the young plant, plant growth, plant health, and ultimately maturity, may be adversely affected. For example, the soil may be too dry to support germination, or optimum germination. Or while the soil may in general have a desired moisture content, moisture content at a macro level can vary widely. Thus, while the soil in general may have a desirable moisture content, the microcosm of the soil adjacent an individual seed may be too dry, or too wet, to support any germination, or optimum germination.

Similarly, the soil may be generally depleted of one or more plant nutrients needed by the germinated seedling. Or while the soil may in general have desired nutrient levels, the nutrient levels at a macro level can vary widely. Thus, the microcosm of the soil adjacent an individual seed may be too low in one or more nutrients to support a desired level of plant growth, or so high as to be toxic to a desired level of plant growth.

Further, plant nutrient chemicals may be present in the soil, but so tied up chemically in the soil as to be unavailable, or poorly available, relative to the quantities and use rates needed for desired plant growth. Or the soil may become so hard, dry, and/or caked shortly after the seed germinates that the seedling

plant has difficulty penetrating such soil, difficulty becoming associated suitable nutrients, and/or difficulty taking up such nutrients because of insufficient moisture availability.

After the plant has further developed such that the plant roots extend deeper into the soil, conditions of the soil near the surface are less critical. However, until such time as the roots so penetrate, conditions of the soil at and near the top surface of the soil may be critical.

Soil fertility generally relates to uptake of plant nutrients from the soil by plants. Uptake is generally the result of two factors, the presence of plant nutrients in the soil, and the availability of the plant nutrients for plant uptake. Presence of plant nutrients in the soil is generally a function of the combination of (a) the basic level of soil fertility, (b) depletion by previous crop production and (c) replenishment with fertilizer. Availability of a plant nutrient physically present in the soil for plant uptake is in general related to solubility of the respective nutrient or nutrient combination in a solvent for the nutrient, which solvent is present in the soil, such solvent as water, along with any other material affecting solvation of the plant nutrient into the water or other solvent.

Plant nutrients are routinely depleted from the soil by crop production, and are routinely added back, or otherwise replenished, to the soil by conventional inorganic fertilizers.

In order for plant nutrients in the soil to be available for uptake by plants, the nutrients must be held in the soil without excessive leaching, but must not be held so tightly that the nutrients cannot be released for plant uptake. Thus, nutrient availability requires a balance between holding tightly enough to retain the nutrient in the root zone, without leaching, but not so tight as to make the nutrient unavailable for plant uptake. Thus, the general "condition" or "tilth" of the soil is instrumental in

determining the efficiency with which plant nutrients are utilized for plant nutrition.

A properly conditioned soil has advantageous soil chemistry in combination with advantageous soil texture. Thus, in addition to providing specific plant nutrients, soil users also use products that modify basic soil chemistry, and soil texture.

Basic soil chemistry is modified by adding to the soil, for example, calcium products to provide pH control, and flyash or like products to provide pH control as well as micronutrients.

Soil texture is generally modified by adding to the soil organic matter such as manures, sludges, wood and other plant products and by-products, and the like. While such materials have good soil conditioning properties, plant nutrient value of such materials is fixed and is generally so low that other "fertilizer"-type products must in general be used in addition to the organic matter in order to preserve plant nutrient values in the soil.

The primary object of this invention is to provide solid plant seed capsule products that supply both soil conditioning properties and the seed, which can benefit from such conditioned soil, in a given seed capsule particle.

It is a further object to provide a plant nutrient material, in the seed capsule particle, in amount beneficial to the seedling emerging from the seed, and higher than a naturally-occurring amount of such nutrient in such soil conditioning material, so as to have enhanced chemical nutrient qualities over use of the soil conditioning material alone.

In another aspect, a further object is to provide soil conditioning and optionally nutrient qualities to seed products that reach the soil as the result of fulfilling objectives separate from providing soil fertility or soil conditioning.

Still another object is to provide seed capsules containing fertility-enhancing elements having a high level of plant food

nutrients in combination with a high level of soil conditioning properties.

Still another object is to encapsulate a seed in a soil conditioning material using materials rich in plant nutrients as part of the encapsulating agent.

Yet another object is to provide a seed product which reduces the tendency for light weight seeds to be washed away by surface water runoff.

Still another object is to provide a seed product which obviates the typical practice of adding straw as a mulch over e.g. grass seed, to protect the seed from being washed away by surface water, from heat of the sun, and to hold moisture in the soil.

A further object is to provide products wherein a single seed capsule product particle provides enhanced soil texture and enhanced soil nutrient value at nutrient levels traditionally needed by newly-germinated seedlings, optionally with higher levels of plant nutrient suitably spaced from the seed itself so as to not be toxic to seedling growth, optionally in combination with time-release technology.

Yet another object is to provide fertility-enhancing seed capsule products having a suitable level of plant food nutrients in combination with a high level of organic matter as soil conditioning material.

Summary of the Invention

The invention generally addresses a combination seed capsule, comprising at least one viable seed, having an outer surface and acting as a core or pseudo-core of said combination seed capsule; and a coating of a composition comprising a soil conditioning material mounted proximate, including disposed outwardly of the outer surface of said seed.

In general, the coating provides at least one of (i) enhancing broadcast flight properties of the combination seed capsule; (ii) reducing susceptibility to deleterious affects of weather on the combination seed capsule; (ii) enhancing resistance of the combination seed capsule to attack by animals, weeds, or spore-formers; (iv) staged germination of ones of the seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in the seeds; (v) enhancing control of moisture about the seed thereby to assist in seed germination; (vi) release of plant nutrients into soil onto which the combination seed capsule is placed; (vii) soil conditioning effect to soil onto which the combination seed capsule is placed; (viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released; (ix) higher embryo emergence and survival rate in a population of the seed capsules, thereby reducing required seed planting density for a desired plant population density; and (x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

While a wide variety of seeds may be used, in general such seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

Preferably, the coating comprises the soil conditioning material in combination with at least one ingredient effective to

reduce susceptibility of the seed capsule to deleterious affect of at least one of animals, weeds, and spore-formers. In some embodiments, the ingredient for reducing such susceptibility of the seed capsule is selected from the group consisting of herbicides, fungicides, for example metalaxyl, and a bitter substance.

In some embodiments, the combination seed capsule further comprises a second coating, separate from the first coating, and comprising at least one ingredient effective to reduce susceptibility of the seed capsule to deleterious effect of at least one of animals, weeds, and spore-formers.

Some embodiments are effective to provide a plant nutrient at a desirable controlled distance from a plant seedling emerging from the seed, in an amount beneficial to the plant seedling.

In other embodiments, the second coating material is intermingled with the first coating material in an outer portion of the first coating, and generally displaced from the seed.

The second coating material can comprise a plant nutrient, beneficial in location and in amount of availability, to a plant seedling emerging from the seed. The second coating composition can comprise an inorganic form of a plant nutrient and can be selected from the group consisting of nitrogen, phosphorus, and potassium. The second coating composition can comprise an inorganic form of a plant nutrient and can be selected from the group consisting of e.g. urea, monammonium phosphate, diammonium phosphate, superphosphate, triple superphosphate, dicalcium phosphate, and potash or a micronutrient such as sulfur, manganese, copper, boron, iron, magnesium, or chromium.

A population of the seed capsules can comprise coatings having a range of properties affecting germination rate of the seeds, thereby to stage germination of the seeds in the population over a period of time longer than the range of germination times inherent in uncoated ones of the seeds. Such properties can be, for

example, a range of hardnesses, or a range of thicknesses, of the coatings.

The coating can comprise a first layer of the soil conditioning material, and a second layer comprising an inorganic, and/or organic, fertilizer, and/or at least one micronutrient, such as, for example, sulfur, manganese, copper, boron, iron, magnesium, or chromium.

A preferred soil conditioning material is a sludge composition, such as a fiber-containing by-product of a paper making operation, or sewage sludge.

The seed capsule can comprise a water-leachable plant nutrient, and/or a leach-retardant composition, such as wax, effective to retard leaching of the leachable plant nutrient out of the combination seed capsule.

In some embodiments, in a population of the combination seed capsules, the coatings in ones, but less than all, of the population, comprise ingredients effective to retard effective penetration of a seed-germinating environment to the seed for germination thereof.

In embodiments preferred for some applications, the seed capsule comprises an inner layer on the outer surface of the seed, and an outer layer, the inner layer enhancing properties of the seed for acting as nucleus in an agglomeration operation agglomerating the coating onto the inner layer.

In some embodiments, the coating comprises an admixture of the soil conditioner and a plant nutrient.

In preferred embodiments, the coating remains generally disposed about the seed, and preferably but not necessarily remains generally intact about the seed, until the seed germinates.

The invention further comprises a plant growing medium extending over an area, the plant growing medium having a root zone, and a top surface of the root zone generally corresponding with a top surface of the plant growing medium, the plant growing

medium having a first overall soil condition and texture; and a population of seed capsules disposed over the top surface of the plant growing medium, the seed capsules comprising individual seeds, having outer surfaces, and coatings of soil conditioning material disposed outwardly of the outer surfaces of the seeds, the coatings of the seed capsules providing localized germination and growth environments, at and adjacent the seeds, having texture, and nutrient and water holding properties for supporting seedling health, superior to respective properties as provided overall in the root zone of the plant growing medium.

The invention yet further comprises a method of providing plant micronutrients to soil, the method comprising placing onto the soil a population of combination seed capsules, each comprising at least one seed, and a coating comprising a plant micronutrient material.

The coating can comprise a first coating comprising the plant micronutrient, and a second coating, separate and distinct from the first coating, and comprising a soil conditioning material.

The invention yet further comprehends a method of providing a seed bed having enhanced growing conditions for growing seed, the method comprising coating a population of the seeds with a coating material, and thereby providing coatings thereon of such material, the material tending to stabilize, in the seed capsules, or in soil on which the seed capsules are disposed coating compositions which tend to hold, moisture adjacent the seeds in the seed capsules or in soil adjacent the seed capsules, in such quantities and for such times as to enhance growing conditions for the seeds; and placing the population of seeds on soil effective to support germination of the seeds which are in the seed capsules.

In some embodiments, the seed capsules comprise inner layers on the outer surfaces of the seeds, and outer layers, the inner layers enhancing properties of the seeds for acting as nuclei in

agglomeration operations agglomerating the coatings onto the inner layers.

The invention yet further comprehends a method of making a population of combination seed capsules, each comprising a seed, and a coating of a soil conditioning material, the method comprising pre-coating the seed with a material which enhances the ability of the seed to act as a nucleus in an agglomeration operation, to form a pre-coated substrate; and subsequently coating the pre-coated substrate with a soil conditioning material. A preferred pre-coating material comprises dicalcium phosphate.

In general, the pre-coating step typically results in an overall increase in the density of pre-coated seed combination. The pre-coating step can be accomplished by, for example, spraying the pre-coating material onto the seed, and subsequently driving off such as by drying, as necessary, any solvent or other liquid carrier used for application of the coating material to the seed.

In yet other expressions, the invention comprehends a method of providing an enhanced seed germination environment in combination with placement of a controlled amount of plant nutrients in controlled proximity to each seed, the method comprising providing a population of seeds, coated with a soil conditioning material which tends to enhance germination of the seeds, and with plant nutrient composition effective to enhance growth of plant embryos emerging from the seeds; and placing the population of seeds on soil effective to support germination of the seeds. In such method, the coating material can include a second ingredient comprising plant nutrient moieties.

Brief Description of the Drawings

FIGURE 1 is a transverse cross-sectional view of a coating drum suitable for spray-coating substrate seed according to the present invention.

FIGURE 2 is a partially cut away view showing a length of the drum of FIGURE 1.

FIGURE 3 is a schematic representative flow diagram illustrating a first manufacturing process for producing combination seed capsule product of the invention.

FIGURE 4 is a block diagram illustrating a second manufacturing process for producing combination seed capsule product of the invention.

FIGURE 5 is a schematic representative flow diagram illustrating a third manufacturing process for producing combination seed capsule product of the invention.

FIGURES 6A, 6B, 6C, and 6D show cross sections of seed capsules of the invention.

FIGURE 7 illustrates a cross-section of the soil root zone, and a representative population of seed capsules at the top surface of the soil.

FIGURE 8 illustrates a single seed capsule on the soil surface, and the micro-environment developing about the seed capsule.

DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

The following is a detailed description of the illustrated embodiments of the present invention which provides combination seed capsule products that provide for a combination of efficient and proper seed placement in the soil, soil conditioning properties at the specific site of the seed, plant nutrients at or near the specific site of the seed, ingredients effective to reduce deleterious effects of spore-formers and animals, and/or other various physical benefits/properties of the combination seed capsule not previously available in a single product.

In general, at least one seed substrate and at least one soil conditioning material are selected as raw materials, and are combined to make a combination soil conditioning seed capsule product of the invention.

The invention can operate with any of a wide variety of soil conditioning materials such as municipal or other sewage sludge, scrubber sludge, paper mill sludge, fly ash, dust, animal waste, other organic materials, and mineral soil conditioning materials.

The soil conditioning material can be a solid material having a melting temperature so high that handling such material in the melt state is impractical and/or undesirable in view of the limited temperatures at which the seed will remain viable. For example, the soil conditioning material may be combustible at a temperature lower than its melt temperature, or will melt only above temperatures which can be tolerated by the seed, such that viability of the seed would be destroyed if melting were attempted in an environment which exposed the seed to such temperatures. Thus, handling such material in the melt state is impractical, whereby other methods of handling the soil conditioning material may be desired.

Solid sewage sludge, sawdust, and solid animal waste are representative of soil conditioning materials which cannot be

readily melted. In the alternative, some soil conditioning materials such as sewage sludge, paper mill sludge, sawdust, and solid animal waste can be suitably comminuted and then dissolved or suspended in water or other solvent composition for processing purposes, optionally along with other soil conditioning materials and/or inorganic chemical fertilizer materials, and the solvent subsequently driven off to make a resulting solid product.

Inorganic chemical fertilizers generally are distributed in commerce as solid state materials. Such material is generally produced in manufacturing steps either in solution or in the melt state to meet a specified narrow range of size, hardness, and plant nutritional characteristics, distinct to the application of each such product. Examples of such fertilizers include nitrogen, phosphorus, and potassium containing products such as urea, monoammonium phosphate, diammonium phosphate, superphosphate, triple super phosphate, dicalcium phosphate, potash, and the like. The inorganic chemical fertilizer can be a mixture or other physical combination of known inorganic fertilizer chemicals, and may include desired amounts of micronutrients such sulfur, manganese, copper, boron, iron, zinc, and the like.

In preferred embodiments of this invention, a precursor seed capsule, having one or more coatings of the soil conditioning and/or other material thereon may first be prepared as a solid or semi-solid particle or agglomerate. The soil conditioning raw material may be a particulate powder, or may be fibrous, or may be a suspension of a powder or fibrous material in a liquid carrier, and is preferably coated onto the substrate seed to form a seed capsule or other agglomeration of particles, fibers, or the like. Where the soil conditioning material is, for example, sewage sludge, the sewage sludge raw material can be obtained as a slurry that may be bound together as with a binder, preferably an organic binder, when dried. The slurry may be spray-applied to the substrate seeds, for example to a rolling bed of such seeds, in

combination with a flow of air to evaporate water from the thus-applied coating. Such sewage sludge, or paper mail sludge, need not be reacted or otherwise treated with any acid, caustic, or any other chemical before being applied and/or dried, or partially dried, either in preparation for, or after, the slurry application of the sludge to the seed substrate.

Specifically, the sewage sludge or paper mill sludge used herein as soil conditioning raw material need not be treated to transform such sludge into colloidal form. Thus, the sludge preferred for use herein is generally non-colloidal in nature, and is distinguished by its non-colloidal nature from conventional sludges which are specifically treated to provide the colloidal characteristics thereto.

Natural lignin, lignosulfonates, and the like, may serve as suitable binders where the soil conditioning material is, for example, paper mill sludge, raw wood, sewage sludge, or other organic or inorganic material. In the case of, for example, calcium chloride or other inorganic additives, such materials may be added to the primary coating, e.g. onto or into the sludge coating, by well-known processes.

Soil conditioning material used herein may be devoid of such conventional plant nutrients as nitrogen, potassium, and phosphorous, or may have such limited plant nutrient value, or may be so unbalanced in nitrogen, phosphorous, and potassium content, that the soil conditioning material may not, by itself, be a desirably complete material for use as the only ingredient in the seed coating. Thus, such soil conditioning material may have limited application herein where basic level of soil fertility is seriously degraded. However, all soil conditioning materials contemplated herein beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients. By use of soil conditioner in intimate association with the seed, this

invention not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated, and in intimate association with the seed, irrespective of the general tilth condition of the growth medium into or onto which the seed capsule is applied.

Further to preferred embodiments, typically a first coating material (e.g. soil conditioning material) is readily converted into liquid state such as liquid suspension, and is provided to the process as a liquid. As a general statement, the first coating material may be sprayed onto the substrate seed, then is converted back to solid state on the thus-created seed capsules or seed capsule precursors. In the alternative, the coating material may be mixed with the seed in an (e.g. ribbon) blender, or may be otherwise coated onto the substrate seed in an agglomeration process according to well-known conventional agglomeration principles.

Regarding the coating process, the coating material can accumulate as a single or multiple layer coating on the outside of the seeds to form a population of combination seed capsules. The layer or layers of coating material can be a homogeneous or heterogeneous mixture of the desired elements. Further, such population of combination seed capsules can have a range of hardnesses and thicknesses for improved seeding treatments.

Cooperating inner and/or outer layers may be used e.g. to control direct contact between the seed and moisture. Suitable materials and processes therefore are taught in USA Patent 3,698,133 Schreiber and 4,759,151 Gerber, and are thus well known in the art.

In some embodiments, a second coating material may penetrate into the layer of soil conditioning coating material. Such penetration may comprise a generally uniform distribution of the second coating material throughout the first coating material, or

may represent a more stratified or otherwise heterogeneous distribution of second coating material in or on the first coating material.

In other embodiments, the coating materials may be mixed into a heterogenous layer. Such layer or layers of heterogenous material can then be coated upon the outside surface of the seed.

Where the liquid state of a coating material was obtained by slurrying or otherwise combining the coating material with water, the liquid fraction is reduced after application of the liquid-state material to the substrate seed, or to the growing seed capsule, to effect solidifying of the coating material after application of the coating material to the substrate seed. The liquid fraction is reduced by driving off the liquid carrier, as by medium or low temperature air, or vacuum or other flash drying, after or during application of the coating material to the substrate seed. The resulting solid seed capsule, comprising the seed coated with the e.g. sludge coating material, is then recovered as a combined soil conditioning seed capsule product of the invention.

Spraying of the liquid coating material can be accomplished by a variety of known processes such as, but not limited to, pneumatic, hydraulic, or electrostatic spraying processes. The temperature and pressure of the material being sprayed depends on the material selected, and the viscosity and other parameters of the respective material in the respective liquid state. While high atomization is desired, such is not critical. The liquid coating material need only be atomized sufficiently to provide a generally uniform coating on the substrate seeds, as determined after the coating and solidification steps in fabricating the seed capsule product are completed.

Indeed, the uniformity of coating or coating thickness about the seed is typically not critical so long as the seed is not on or immediately adjacent an outside surface of the capsule such that

the seed may fall out, or be easily broken out, of the capsule, or easily removed by dissolution of materials at and near the surface of the seed capsule. In addition, the seed should not be so near the outside surface of the capsule as to be in a nutrient layer having such high concentration of nutrient as to be toxic or otherwise detrimental to viability or growth of a plant emergent from the seed.

Spray application of the coating is suitably controlled to achieve the required addition of the spray material, liquid and/or powder, coating to the substrate seed or precursor seed capsule. An illustrated method of applying the liquid material to the substrate seed or precursor seed capsule is by using a rotating drum spray-coating apparatus. Other apparatus and methods, for example a tilted pan coating process, can be used to apply the soil conditioning material and optionally an inorganic chemical fertilizer material onto the substrate seed. The coating operations can be batch operations or continuous operations.

As illustrated in FIGURES 1, 2, and 4, spray apparatus can operate within a rotating drum disposed in a generally horizontal orientation. The drum may incorporate internal lifting flights which lift free-flowing (e.g. seed and growing seed capsule) particles in the drum and then let the particles fall to the bottom of the drum as a continuously falling curtain or cascade. In some embodiments, the interior of the drum is either clean and free from any flighting, or has only mixing fingers or flights that expand the area covered by the bed, that keep the bed rolling as the drum rotates, and that generally improve mixing, rather than lifting particles to the top of the drum and then releasing them in a falling cascade. However, such lifting of particles to the top of the drum, and corresponding falling cascade or falling curtain, are not excluded from processes of the invention. Rather, both such finger mixing, and such lifting coupled with falling cascade or curtain, are included within the scope of the invention.

Stationary spray nozzles are positioned within the drum to project the sprayed material onto the rolling bed, and optionally onto any curtain or cascade of falling particles. For a continuous process, the drum is preferably inclined at a small angle from horizontal, such as, without limitation, about 0.25 inch to about 0.38 inch from the horizontal for each foot of length of the drum, so that rotation of the drum causes the particles to move from the inlet end of the drum to the discharge end, while maintaining a relatively uniform bed thickness. The optimum degree of incline varies with each set-up and may thus be outside the above range. The important parameter is that the incline contribute to maintaining a bed of seed and seed capsule particles having sufficient uniformity that the spray material can be effectively applied to the particles passing through the drum. The particles are then discharged at the discharge end of the drum.

FIGURES 1 and 2 show schematically a first embodiment of processing equipment which may be used to produce seed capsules of the invention. Such processing equipment includes a drum and sprayer combination suitable for continuously producing coated seed capsules in accord with the invention. Use of the illustrated drum and sprayer combination is not critical, however, as other drum and sprayer combinations, or other coating methods such as pan coating methods, are also suitable. In FIGURES 1 and 2, drum **10** has an inlet end **12** for receiving the substrate seed material or materials, or partially formed or pre-coated seed capsule precursors. Drum **10** has a discharge end **14** through which agglomerated or otherwise coated seed capsule product particles are discharged over discharge retaining ring **16**. A variable speed rotary drive (not shown) is provided for supporting and rotating the drum **10** in a counterclockwise direction as viewed in FIGURE 1 at controlled, and changeable drive speeds. Conventional slope adjustment apparatus (not shown) is provided for routine and ongoing adjustment the slope of the drum from horizontal.

Air is preferably supplied from discharge end **14** as shown in FIGURE 2, and flows countercurrent to the direction of travel of the seed substrate material. Since the contemplated coating materials are generally applied to the seed in liquid, or semi-liquid, or other moist form, and since some coating materials may thus tend to form clumps or otherwise self-agglomerate when exposed to ambient moisture conditions, air supplied at discharge end **14**, and elsewhere in the process for contact with the coated seed and seed capsules, is preferably dried in order to cost-effectively remove an optimum amount of the moisture from the coating material and to assist in maintaining suitably low moisture content in the thus coated and dried seed capsules.

A first stationary spray assembly **28** extends longitudinally within drum **10** above and adjacent the bed **20** of seed and/or seed capsules. First spray assembly **28** includes pipe **29** and nozzles **30**. A second spray assembly **32** extends longitudinally within drum **10** generally adjacent first spray assembly **28**. Second stationary spray assembly **32** includes pipe **33** and nozzles **34**, which transport the material to be sprayed. Nozzles **30** and **34** are connected to pipes **29** and **33** respectively, and project sprays of liquid or otherwise particulate coating material toward the bed of seeds and/or seed capsule precursors. The description of spray assemblies **28**, **32** as stationary means that the spray assemblies do not rotate with drum **10**. However, the positions of either nozzles **30**, **34** or pipes **29**, **33**, or both, can be adjusted within the drum for proper direction of the respective spray or sprays onto the bed of seeds and/or seed capsules or seed capsule precursors.

A stationary protective cover **24** is mounted over the spray assemblies. Seeds and/or seed capsules falling from the inner surface of the drum and the flights, above the spray assemblies, fall onto the cover, and are deflected away from the spray assemblies, as shown in FIGURE 1. Thus, cover **24** protects the

pipes and nozzles from the falling seeds and seed capsules falling onto and fouling the pipes and spray nozzles.

As drum **10** rotates, flights **22** lift and mix the seeds, seed capsule precursors, and seed capsules, but do not generally carry the bulk of the seeds and seed capsules up to the top of the drum. Some small amount of seeds, seed capsule precursors, and seed capsules will be carried upwardly to the top of the drum by even a drum devoid of any flights. Thus, all drums experience some amount of seeds and seed capsules falling from the upper part of the rotating drum whereby cover **24** is beneficial for protecting spray assemblies **28** and **32**.

Preferred flights **22** are primarily directed toward enhancing mixing of the bed **20** of seeds and seed capsules, continually refreshing the surface of the bed with a newly-emergent supply of seeds and seed capsules, rather than lifting and subsequently dropping the seeds and seed capsules which may be fragile when initially coated. To that end, each flight **22** preferably, but without limitation, has a leading surface **23A** extending at an obtuse angle "A1" of at least 90 degrees with respect to the inner surface of the drum. A more preferred angle "A1" is about 100 degrees to about 150 degrees. Trailing surface **23B** of flight **22** can be virtually any angle, with the inner surface of the drum, which angle does not interfere with the operation of adjacent leading surfaces **23A**.

Additional retaining rings can be added to the assemblage shown in the drawings, in order to provide that height "H" to the retaining ring which will provide and maintain the optimum configuration of bed **20** inside drum **10**.

As noted above, inlet end **12** of the drum may be raised above discharge end **14**. When in use, the drum rotates continuously. Seeds or previously thinly-coated or partially-coated seed capsules are continuously fed into inlet end **12** and thus added to rolling bed **20**. Flights **22** continuously mix the bed as the drum rotates,

refreshing the bed surface with newly fed seeds, or seeds and seed capsules newly brought to the surface by the continuous rotation of the drum in combination with the mixing action of the flights. Spray assembly **28** sprays the desired coating material (e.g. sewage sludge, paper mill sludge, or other coating composition, onto the continuously moving and mixing surface of bed **20** from a plurality of nozzles **30** distributed along the length of pipe **29**, and similarly along the length of drum **10**, adding the sprayed material to the seeds and seed capsules in bed **20**. After receiving the spray coating from spray assembly **28**, the seed capsules are discharged through discharge end **14**. In some embodiments, the seed capsules pass through a cooling chamber, not shown, integral in drum **10**, before being discharged through discharge end **14**.

In general, as the seeds traverse the drum, from inlet to discharge, nozzles **30** atomize the liquid or other coating material and spray such atomized coating material as e.g. droplets of the coating material onto the seeds in the bed. The result is that the seeds become generally uniformly coated with one or more layers of the coating material such that the coating material becomes an integral part of the respective seed capsules fabricated in the drum. As the coating material solidifies on the seeds, the coating material tightly bonds to the respective portions of the seeds.

As the seeds and seed capsules roll and mix with rotation of the drum, the incline of the drum causes the seeds and seed capsules to travel from inlet end **12** toward discharge end **14**.

In the alternative, or where a coating material is not readily self-bonding to the seed material, a binder material can be provided toward the inlet end of the drum at spray assembly **32**, through pipe **33** and nozzles **34**. In such embodiment, the binder is preferably sprayed onto the seeds closer to inlet end **12** rather than along the entire length "L" of the drum. The coating material is then preferably sprayed onto the seeds downstream from the inlet end, and preferably relatively downstream of nozzles **34**. Thus, the

seeds receive a first coating of the binder, and a subsequent second coating of e.g. liquid soil conditioning coating material overlying the binder.

Binder material applied as e.g. through spray assembly **32** may contain additional coating components such as e.g. flyash, lime, gypsum, or the like, as one or more components for assisting in adding bulk and thickness to an inner binder layer prior to any, or the majority of, the application of the organic coating material (e.g. sewage sludge or paper mill sludge).

In some embodiments, binder and liquid soil conditioning coating material are applied at similar locations along length "L" of the drum whereby binder and soil conditioning coating material may become intermingled/mixed before reaching the seeds, or on the seeds. For example, liquid soil conditioning coating material may be sprayed onto the seeds along the full length of the coating chamber in drum **10** while spraying of the binder material onto the substrate seeds is done relatively closer to or adjacent the inlet end of the coating chamber of the drum. Thus, a first binder layer may underlie or be mixed with the soil conditioning coating material, and may be overlain by a second layer of the soil conditioning coating material. Thus, in this embodiment, the binder layer may typically be a combination of binder material and coating material.

Further, it is contemplated that the soil conditioning coating may be applied first, followed by application of binder or inorganic fertilizer or sealer coating, in which case the binder or inorganic fertilizer or sealer may serve as an outer shell, temporarily trapping the inwardly-disposed materials inside the seed capsule. In the alternative, the soil conditioning coating may be applied first, followed by application of the binder, and wherein the binder penetrates through the soil conditioning coating, either physically or chemically, to the underlying substrate seed and there provides the binding property.

Additional spray assemblies can be provided, spraying additional materials (e.g. inorganic fertilizer materials) onto the substrate seed. Thus, e.g. 6 spray assemblies can spray 6 different coating materials onto the substrate seed. For example, a first spray material can be a binder or primer material intended primarily to enhance bonding of subsequent sprays to the substrate seed. Continuing the example, a second spray can be a combination of binder and finely comminuted particulate material such as lime and/or flyash. A third spray may be a soil conditioning material such as a paper mill sludge or a municipal sewage sludge. Fourth, fifth, and/or sixth sprays can add nitrogen, phosphorous, and/or potassium plant nutrient ingredients, alone or in combination, or as combinations. In this manner, the soil conditioning properties of the seed capsule can be established, and the plant nutrient level of the seed capsule can be enhanced to provide substantially any level of major and/or minor plant nutrients desired in the seed capsule, at substantially any relative ratios of the respective plant nutrients, and wherein the preferably primarily soil conditioning coating provides desired soil conditioning properties in the resulting product, initially for use by the specific seed contained therein, and ultimately as additive to the overall tilth of the growth medium such as soil into or onto which the seed capsule is eventually planted.

A preferred, and rather simplistic, embodiment of the invention is provided by spraying a soil conditioning liquid suspension of sewage sludge or paper mill sludge onto seeds to be encapsulated to make seed capsules. By controlling the amount of the soil conditioning sludge, or by controlling the residence time of the seeds in the drum, a desired thickness of soil conditioning coating can be provided in the resulting coated product.

Typical dried sewage sludge, as a raw material, contains about 2-6% nitrogen, up to about 2% phosphorous, and generally no potassium, and thus has little or no market value as a fertilizer

(plant food) product per se. However, by adding e.g. urea, the nitrogen content can be raised if desired, especially as a coating on or adjacent the outside surface of the seed capsule, whereby the combination fertility-enhanced, soil conditioning, seed capsule product has real market value as a comprehensive, self-contained, value-added, seed capsule product. Such product thus contains the seed, a soil conditioning composition which operates somewhat as a seed incubator providing a beneficial germination environment, and a starter quantity of fertilizer selected in quantity and placed in location so as to provide improved, ideally optimum, amounts of plant nutrients at optimum location for use by the newly-emerged embryonic plant at the germination stage of seed development.

Starting with a sludge coating having 2% by weight nitrogen, sufficient urea may be added to bring the nitrogen content to, for example, 5%, 7%, 8% or 10% nitrogen, or more, depending what analysis is desired. Starting with a sludge coating having 6% nitrogen, sufficient urea may be added to bring nitrogen content to, for example, 10%, or whatever other analysis is desired. Phosphorous and/or potassium components and/or materials having combinations of plant nutrient elements (e.g. NPK) can, similarly, be added to the sludge, either before, after, or during addition of the urea. In addition, nitrogen, potassium, and/or phosphorous-containing materials can be combined with the sludge prior to the sludge being applied to the seed.

It should be understood that the more porous the established soil conditioning coating, or e.g. the outer surface of such coating, the more any subsequent spray material penetrates the established coating. All such penetration is contemplated in use of the term "coating" herein.

In some preferred embodiments, the overall coated combination seed capsule product comprises seed capsules wherein substantially the entirety of the soil conditioning material is confined to a contiguously-defined portion of the seed capsule. In such

embodiments, the structures of the finished product seed capsules comprise coatings of contiguously arranged elements of the soil conditioning material, generally arrayed entirely or substantially entirely about the seed, which coatings may be overlain by an additional layer, optionally discontinuous, of organic or inorganic chemical fertilizer. Further coating layers of either soil conditioning material or organic or inorganic chemical fertilizer can be applied over the additional layer.

In addition, or in the alternative, other layers of other materials whether soil conditioning materials, organic or inorganic fertilizers, or other materials, can be applied to the substrate seed before applying the above mentioned layer of soil conditioning sludge. Thus, the substrate seed can be coated with a layer of a calcium compound e.g. calcium chloride, calcium carbonate, or dicalcium phosphate, or with a sulfur moiety, and/or a further layer of urea, all with optional use of binder materials.

Further to the structure of the seed capsules of the invention, the coatings on the seed capsules need not generally represent a uniform mixture of the inorganic chemical fertilizer and the soil conditioner. Rather, in a typical seed capsule a core substrate seed is overlain or encapsulated by a soil conditioning material, and is generally free from a second overlying soil conditioning coating material, and wherein the inorganic fertilizer content at the seed/coating interface is relatively higher so as to represent a second coating material such as an inorganic fertilizer coating, as compared to the inorganic fertilizer content at locations at and adjacent the seed.

The second coating can, and preferably does, in some embodiments, penetrate into voids or other interstices in an underlying e.g. soil conditioning coating. However, preferably most if not all elements of the underlying e.g. soil conditioning coating material are generally interconnected with each other without intervening coating material of the second layer, except

for an optional binder used to hold the first coating material together as a unitary structure, separate from any structure and bonding provided by the second coating material.

While the combination seed capsule can comprise discontinuities in the soil conditioning sludge coating layer, in combination with an inorganic fertilizer material in such seed capsules, such compositions are less preferred.

Regarding the coating process, FIGURE 4 illustrates in flow sheet form a manufacturing process for producing seed capsules of the invention, using the coating drum **10** as described above. It should be understood, however, that other equipment such as a pan pelletizer, a paddle mixer, or the like can be used in place of the rotary drum to obtain combination seed capsules of the invention.

The coating process operates according to conventional and generally well known agglomeration principles, as described by Wolfgang B. Pietsch in an article entitled "The Agglomerative Behavior Of Fine Particles." Such coating process uses water and heat, along with physical and/or chemical adhesives and like properties, to bind or agglomerate a plurality of types of particles and/or materials into coated seed capsules, each typically containing an individual seed.

To obtain agglomerates from relatively smaller particles of raw materials, binding forces must act within the individual developing agglomerate particles. According to known agglomeration principles, five different binding mechanisms are known to be useful for building agglomerate particles including solid bridges, interfacial attractions and capillary pressure, adhesion and cohesion, attraction between solid particles, and form-closed bonds.

At elevated temperatures, solid bridges can form by diffusion of molecules from one particle to another at the points of contact. Heat can be introduced from an external, secondary source or created during agglomeration by friction and/or energy conversion.

Solid bridges can also be built up by chemical reaction, crystallization of dissolved substances, hardening binders, and solidification of melted components.

Capillary pressure and interfacial attraction forces in liquid bridges can create strong bonds that disappear if the liquid evaporates and no other binding mechanisms take over.

Highly viscous bonding media such as tar and other high molecular weight organic liquids can form adhesive and/or cohesive bonds very similar to those of solid bridges. Thin adsorption layers are immobile and can contribute to such bonding together of fine particles under certain circumstances.

Typical short-range forces of the van der Waals electrostatic or magnetic type can cause attraction between solid particles whereby the particles stick together if such particles are sufficiently close to each other. Decreasing particle size clearly favors such attraction between solid particles.

Fibers, little platelets or bulky particles can interlock or fold about each other resulting in "form-closed" bonds.

Now referring to FIGURE 3, in some embodiments of the coating/agglomeration process, it is desirable to pre-coat the seeds prior to implementing agglomeration principles to produce the above described coating of soil conditioning material. Such embodiments comprise light-weight and/or elongate shaped seeds (i.e. grass seeds), or other similar type of seed which may not readily or inherently serve as a nucleating agent in a conventional agglomeration process with the respective soil conditioning material which is desired to be coated on the seed. Pre-coating the grass seed, for example, enhances the agglomeration of paper sludge as a coating material, of binder and/or of other coating substances, by increasing the weight of the pre-coated grass seed and by providing a more filled in, more rounded shape to such long and narrow seeds. The increased weight and more filled in shape of the grass seed enables more effective, more efficient, processing

of the seed in coating apparatus such as that illustrated in FIGURES 3 and 4.

Referring to FIGURE 3, the form and composition of such pre-coating, when needed, can vary according to the weight, shape, composition, and surface properties of the seeds, and according to the binder, if any, the soil conditioning coating or coating materials to be applied, and any other inorganic or organic coating material to be applied.

The seeds, whether pre-coated or not, are received within the rotary drum where the soil conditioning material is spray coated onto the substrate seeds to obtain combination seed capsules.

Before coating the seeds with a soil conditioner, the organic soil conditioner material (e.g. paper sludge) is preferably processed through a dryer such as a rotary drum dryer, as needed, to reduce the amount of moisture in the organic soil conditioner material to less than about 8% water by weight. Such drying is an essential step where the material is otherwise above the nominal 8% effective water content, to enable grinding the sludge to a size less than US Standard 20 mesh screen, and to prevent the particles from agglomerating with each other. Certain of the coating materials, e.g. fly ash, because of their physical properties, need not be dried before being ground to a suitable size for participating in the agglomeration operation.

The seeds, whether pre-coated or not pre-coated, and the one or more soil conditioners, are received within a mixer where growth enhancers such as time release agents and/or other environmental conditioners may be added to form a combination seed capsule. The thus pre-coated seeds are then received into a pan pelletizer, a rotary drum, or the like, where binders such as lignin, lignosulphonates, molasses, sodium silicate, wax, monammonium phosphate, or urea can be added and thereby coated onto the pre-coated seeds. Other materials which can be added to the seed capsule at the e.g. rotary drum include anti-fungal coatings such

as with metalaxyl fungicide, for example, Apron® and/or Subdue®, available from Novartis, Inc. of Greensboro, North Carolina.

The such-coated seeds are then passed into a rotary or other dryer in order to obtain a seed capsule containing 5% or less water. The maximum water fraction in the coating can vary according to the composition of the coating material, so long as the resultant seed capsules remain suitably structurally strong and so long as a population of such coated seed capsules remains free flowing in solid condition. The process for fabricating the seed capsules must maintain a temperature sufficiently low that the seeds are not heated so hot that viability of the seeds, for germination purposes, is not dramatically compromised. It is generally preferred that the temperature of the seeds be suitably controlled such that any binder and/or coating material, or other materials applied to the seeds, cool at a controlled rate while bonds form between the seeds, or seed capsule precursors and the one or more soil conditioning and/or other coating materials. Such temperatures of all materials are suitably controlled to avoid decomposition of the respective materials, loss of viability of the seeds, or breakage of seed capsules or seed capsule precursors, or coatings or coating or other materials during such processing. The temperature at the rolling seed bed inside drum **10** generally can range from about 130 degrees F to up to at least 230 degrees F for seed residence times up to at least 1 hour. At drum operating temperatures of less than 130 degrees F, drying time can become excessive. At temperatures above 230 F, the viability of the seed may be at risk, depending on the sensitivity of the seed, residence time, and other influential parameters.

The above stated temperature range is illustrative and not limiting, and will vary depending on the seed, the coating materials, and the specific process parameters of a particular coating system and coating operation. Thus, maximum e.g. drum coating temperatures can be less than 130 degrees F or more than

230 degrees F. However, the stated range is preferred, including all temperatures within such range such as, for example, 150 degrees F, 180 degrees F, 210 degrees F, and the like.

Referring to the drum of FIGURES 1 and 2, and to the pan pelletizer block in FIGURE 3, the seeds are fed continuously to an inlet as at inlet end **12** of drum **10**. Combination seed capsules, produced as described above, are released from a discharge locus such as discharge end **14** of the drum to a sizing apparatus **36** in which the seed capsules are sized through conventional sizing elements. Suitably-sized seed capsules are discharged from the sizing apparatus as product for distribution. Undersize seed capsules are fed back into mixer as shown in FIGURE 3. Oversized seed capsules are fractured and screened for reprocessing.

The recovered seed product can be further coated with any of the coating materials described above, such as urea or other inorganic or organic fertilizer, and/or with growth enhancers or other desirable materials. Further, other types of coating materials such as water repellants can be coated onto the discharged seed capsules for the purpose of importing additional desirable properties to the seed capsules.

In the process of coating porous organic materials such as sewage sludge or paper mill sludge as is optional in the invention, with a second material which is applied for other than imparting soil conditioning properties, for example an inorganic fertilizer, the general size of the coated seed capsule may be the same after applying the second material (e.g. inorganic fertilizer) as the size of the previously-coated seed capsule, or may be similar in size. Namely, the quantity of coating material added to the seed capsule can be so small as to not materially affect seed capsule size, or the coating material can be received into an e.g. porous interior of the soil conditioning coating of the seed capsule, or both.

It is contemplated that the operation and functions of the invention have become fully apparent from the foregoing description of elements, but for completeness of disclosure, the usage of the invention will be briefly described.

EXAMPLE 1

A coating drum as illustrated in FIGURES 1, 2 and 4 is used to place a coating of paper mill sludge on grass seed. Raw material grass seed about 4-6 millimeters long and about 0.5-1.0 millimeter thick, is continuously fed to pre-treater **11**, where the seed is blended with powdered lime, powdered flyash, and a lignosulfonate binder, to form partially-developed seed capsules comprising seeds coated with relatively thinner coatings of the recited mixture of coating materials. The partially-developed seed capsules are continuously fed to inlet end **12** of drum **10**, to form a bed **20** of the partially-developed seed capsules. The drum rotates continuously. The rolling of the drum, and the associated mixing affect of the flights, provide a constantly changing top surface of the bed. A paper mill sludge slurry is supplied in pipe **28** at pressure sufficient to atomize the liquid sludge slurry. A liquid sludge slurry is thus sprayed from nozzles **30** onto the top surface of the bed of partially-developed seed capsules, applying a sludge coating on those partially-developed seed capsules which are at the upper surface of the bed at any given point in time.

The resulting seed capsules, of paper mill sludge coated seeds, have a coating of soil conditioning sludge thick enough to make the material a product marketable for its soil conditioning content as well as for the seeds contained therein. Increased levels of nitrogen and/or other plant nutrients can be added by, without limitation, providing sprays of the other desired materials, preferably subsequent to at least the initial sludge slurry spray. Other materials can be included in one or more of

the sprays e.g. to retard or enhance moisture permeation into or out of the combination product in accord with the anticipated storage and/or use environment of the product.

EXAMPLE 2

FIGURE 5 illustrates the equipment used in this EXAMPLE 2. As seen therein, grass seed, lime, flyash, and calcium lignosulfonate binder are fed to ribbon blender **111** by respective screw feeders **112A**, **112B**, **112C**, **112D** respectively. Ribbon blender **111** encapsulates the seed with a thin layer of the mixture of lime, flyash, and lignosulfonate to thereby make partially-formed seed capsules. The partially-formed seed capsules are discharged from the ribbon blender and conveyed by conveyor **114** and belt feeder **116** to a tilted-pan pelletizer **118**, which rotates about a fixed axis.

Paper mill sludge is received into a weigh hopper **120** at about 60% by weight water, and is fed by screw feeder **122** and belt **124** to pin mixer **126**. The pin mixer breaks down the fiber and fiber clusters of the sludge into loose separate fibers, and discharges the resultant material onto conveyor **128** which transports the material to screw feeder **130**, and thence into the tilted pan pelletizer.

In the tilted pan pelletizer, the partially-formed seed capsules, (seeds being coated with lime, flyash, and lignosulfonate) are mixed with the comminuted paper mill sludge and thereby coated with the sludge. By operation of the tilted rotating pan pelletizer, the larger seed capsules generally rise to the top of the bed of seed capsules in the pan, and as additional material (sludge and partially-formed seed capsules) are added to the pan, the larger seed capsules overflow the lower edge of the rotating pan, onto vibrating feeder conveyor **132**.

The vibrating feeder conveyor feeds the seed capsules into granulator **134** (e.g. rotating drum) where the seed capsules may be

(e.g. spray) coated with inorganic fertilizer or other desired material.

From the granulator, the seed capsules flow into dryer **136** and are dried to a final product moisture of about 2-3% by weight water. The resultant product is then screened and sized as before, with undersized and oversized product seed capsules being recycled for further processing.

Urea and other liquid inorganic chemical fertilizers can, as indicated, be used as binders to bind together soil conditioning coatings which are not readily self-bonded together. In such embodiments, the urea or other liquid fertilizer composition serves as the binder or glue which holds together the soil conditioning material which is used as the coating. Other binding materials may be used either alone or in combination with the inorganic chemical fertilizer. Any plant nutrient components of the binder/glue composition contribute to the plant nutrient value, e.g. nitrogen, phosphorous, and/or potassium, provided by the so-made seed capsules. Thus, a binder/glue, or a multiplicity of binders/glues, properly selected as to nutrient value can provide, in the finished product, significant contribution to any desired fertility analysis.

A primary purpose of soil conditioning products is to condition the soil in terms of properties other than direct provision of plant nutrients.

The primary purpose of conventional inorganic chemical fertilizer products is to directly provide plant nutrients. It is well known that highly purified forms of inorganic chemical materials are more concentrated than desired in close or intimate proximity with seed, in the growing medium. Thus, inorganic chemical fertilizers can be diluted in concentration and still have sufficient nutrient content to be highly useful additives in soil conditioning seed capsules of the invention. It is common practice

to modify and thus dilute inorganic chemical fertilizer products with filler materials that do not provide plant nutrients, in order to provide less concentrated fertilizer products. To the inventor's knowledge, such diluents, however, do not include soil conditioning products, especially not organic soil conditioning products.

It is conventionally known to apply commercially available soil conditioning materials and inorganic fertilizers, in separate applications, to a given common plot of soil to assist the soil in growing a crop. For example, it is known to make a first broadcast or other placement of lime to control pH of the soil, followed by a second broadcast and/or row-applied placement of granular inorganic chemical fertilizer. It is also known to make sequential applications of a soil conditioning material such as fresh or aged manure followed by inorganic fertilizer, all of which may be separate from the step of applying seed. And where seed is indeed applied in the same step, the seed and soil conditioner are not intimately bound in controlled positioning with respect to each other in common in individual particles of the product so applied, as in the invention.

To the inventor's knowledge, it is not known to apply soil conditioning material and inorganic chemical fertilizer in a common carrier/particle. Nor is it known to apply seed in a seed capsule wherein the seed is intimately combined with a soil conditioning material in a common particle, optionally with an inorganic fertilizer component in controlled positioning with respect to the seed in the same capsule as a seed-soil conditioning particle.

In those embodiments of the invention comprehending both soil conditioning and inorganic fertilizer in the same seed capsule/particle, the ratio of soil conditioning material to inorganic chemical fertilizer material can vary, from, for example, about 80% by weight up to less than 100% by weight soil conditioning material, with corresponding greater than 0% up to

about 20% by weight inorganic chemical fertilizer. Generally, the invention as practically applied, however, is somewhat more narrowly defined, because the practical benefits of the invention are achieved at more balanced combinations of the soil conditioning material and the inorganic chemical fertilizer.

Thus, a preferred amount of soil conditioning material is about 90% by weight to about 98% by weight soil conditioning material, in combination with about 2% by weight to about 10% by weight inorganic chemical fertilizer. To the extent the soil conditioning material is present in amount less than about 80% by weight, the corresponding 20% by weight organic fertilizer in such close and intimate proximity to the seed may be toxic to the seed. To the extent the inorganic fertilizer is present in an amount of less than 2% by weight, the beneficial fertility affects of the fertilizer may not be perceived.

To the extent the inorganic fertilizer can be confined in a layer displaced from the seed, a higher level of inorganic fertilizer may be used while limiting risk of a toxic response from the seed. Referring now to FIGURES 6A-6D, in the embodiment of FIGURE 6A, seed capsule **38A** comprises a seed **40A** coated with a single generally homogeneous coating **42A**. Coating **42A**, as illustrated in FIGURE 6A, may comprise only the soil conditioning material (e.g. paper mill sludge or sewage sludge), or may comprise both the soil conditioning material and an inorganic fertilizer or other inorganic material generally dispersed in coating **42A**.

In FIGURE 6B, seed capsule **38B** comprises a seed **40B** coated with a first layer **42B** of soil conditioning material. A second coating material is shown penetrated part-way through the first layer **42B**, thus to make a combination outer layer **44B** comprising the combination of the material of layer **42A** and the material of the second material, such as inorganic fertilizer.

In FIGURE 6C, seed capsule **38C** comprises a seed **40C** coated with a first layer **42C** of soil conditioning material. A second

generally separate and distinct layer **46C** of a second coating material (e.g. inorganic fertilizer) is disposed outwardly on the underlying first layer **42C**. Layer **46C** generally does not penetrate layer **42C**, whereby higher levels of inorganic fertilizer may be used because of the effective displacement distance between the seed and the second layer **46C**. The second layer may be prevented from penetrating the first layer by applying e.g. an intervening layer which repels the second layer, for example wax, lignin, or the like.

In FIGURE 6D, seed capsule **38D** comprises a seed **40D** coated with a pre-coating layer **48D** of dicalcium phosphate to densify and configure the seed capsule precursor for the primary coating steps in drum **10** or pan pellitizer **118**. Layer **42D** of soil conditioning material is disposed outwardly of pre-coating layer **48D**. Other materials such as at layers **44B** or **46C** can be added to any of the embodiments, including that of FIGURE 6D to provide the properties associated therewith.

In alternative embodiments, seed capsules can comprise a seed coated with at least one heterogenous layer. The heterogenous layer comprises at least two different materials substantially commingled, uniformly or non-uniformly, within a single layer. Such materials can include, for example, soil conditioning material and inorganic fertilizer, micronutrients, herbicides, fungicides, binders and/or any other layer material contemplated by the present invention.

While the soil conditioning material/sewage sludge or paper mill sludge may contain a nominal amount of nitrogen and lesser quantities of phosphorous, potassium, and micronutrients, these small levels of plant nutrient content are generally not high enough for the plant nutrients to be considered a primary commercial asset. Yet only small nutrient amounts are desired so close to the seed. Thus, in some uses, the nutrient content of the sludge may be fully acceptable as the sole coating material on the

seed in making suitable and acceptable seed capsules of the invention.

Products of the invention offer a new combination of properties, namely readily available excellent soil conditioning properties in combination with the seed in a seed capsule wherein size and density of the seed capsule are controlled to the desired size and weight.

One of the properties offered by soil texture conditioners such as sewage sludge and paper mill sludge is that of maintaining soil condition by retaining moisture in the soil, retarding leaching of soil nutrients from the root zone, and attenuating hardening, clumping, or other hard agglomeration characteristics of the soil, which harder soils are more difficult for plant roots to penetrate than are softer soils. Thus, improving the soil texture condition, soil tilth, increases the efficiency with which plant nutrients are retained and used for plant nutrition, as well as generally improving the environment of the soil to accommodate, and readily receive, root growth.

When soil conditioning materials and plant nutrients are applied separately to the soil, as in the prior art, the ratio of applied plant nutrients to applied soil conditioning material typically varies widely according to variations in the uniformity of the two applications of the two materials. Further, the soil conditioning material is generally not closely associated with the plant nutrient-containing fertilizer in the soil, and certainly neither soil conditioner nor the fertilizer are controllably-closely associated with the seed, such that nutrient absorption benefits provided by the soil conditioning material are not assuredly associated with respective particles of inorganic chemical fertilizer materials, and neither the soil conditioning material nor the inorganic fertilizer is controllably and intimately associated with the seed as in a common capsule or other particle as in the invention.

Rather, where soil conditioning and fertilizer materials are applied in separate applications and/or in applications separate from the application of the seed, the bulk of the soil conditioning material and the bulk of the inorganic chemical fertilizer are generally at least somewhat separated from each other in space, and physically separated from the seeds, such that potential cooperative benefit of the soil conditioning material as relates to solvation and up-take of soil moisture and/or of the inorganic chemical fertilizer by the seed are not obtained, and/or are not obtained in controlled close association with the seed.

When the soil conditioning material, the inorganic chemical fertilizer materials, and the seed are separately applied to soil with different sets of equipment, the respective rates of application vary such that the desired ratios between the quantities of the several materials are applied somewhat non-uniformly. The variances from uniformity will be different for each of the applications, thus adversely skewing the relative ratios of the materials with respect to each other at different locations in the e.g. field. Further, when applied separately to the soil, the seed and the soil conditioner are not necessarily in intimate contact with each other as they are when both materials are combined into a single combined seed capsule product as in the invention. Nor is the seed in closely controlled proximity (e.g. within the same capsule) with the inorganic fertilizer. In reality, then, any fertilizer added to the soil but not in close proximity to the seed applied to the same soil during e.g. the same growing season, is of reduced value or no value to that application of seed, whereby little or no value is realized, during that growing season, from the application of such material to the soil.

The amounts of soil conditioning material and inorganic fertilizer added to the soil at any given time represent a small fraction of the "soil" in the plant growing zone (root zone). Thus, in the conventional practice of providing separate

applications of plant nutrients and soil conditioning material, in addition to the seed, only small fractions of the newly applied soil conditioning material and plant nutrient come into proximate cooperating relationship with each other and with the seed. Thus, the seed and any plant newly emergent from the seed are benefitted only to the extent the overall average root zone of the soil is benefitted by the applied soil conditioning material

Even were combinations of soil conditioner, inorganic chemical fertilizer, and seed are to be applied as separate and distinct physical product particles, using a single application apparatus and a single application process, the individual particles of soil conditioner, individual particles of inorganic chemical fertilizer, and individual particles of seed would be separated from each other to a significant degree, during the application process, such that the benefits of intimate association with each other in the soil would be lost. Indeed, the seed benefits from intimate contact with a substantial quantity of soil conditioner, but can tolerate intimate contact with only limited concentrations of fertilizer chemicals. Rather, fertilizer chemicals should in general be displaced from, but controllably located close to the seed.

In an uncontrolled application of fertilizer by an application separate from application of the seed, as in the prior art, some of the seed might be expected to be placed so close to some of the inorganic fertilizer as to be damaged by the toxic affect of such close association. Thus, the benefit of intimate contact between organic soil conditioning material, inorganic chemical fertilizer, and seed, is reduced and largely lost because of low levels of intimate association between the soil conditioning material and the seed, and unpredictable, uncontrolled levels of association between the seed and the inorganic chemical fertilizer, outside the combination of the invention, of soil conditioning coating of the seed, and optional addition of inorganic fertilizer at controlled

location with respect to the seed, all in the same seed capsule, as taught herein.

By combining an organic soil conditioning material in the same seed capsule with the seed, highly effective levels of soil conditioner are assuredly associated with the seed as the seed germinates and begins to grow. Where suitable levels of plant nutrient fertilizer are incorporated into the same seed capsule, growth of the newly-germinated plant is further enhanced. In either case, the soil conditioning materials can and do tend to retain moisture and nutrients in the soil in the defined area of the seed capsule by a variety of mechanisms, providing an extended time period during which nutrients can be taken up by the plants. For example, organic soil conditioning material may retain moisture, reducing moisture drainage from the soil, such that the rate of leaching of the nutrients is, in general, reduced. Further, the soil conditioning material may absorb or otherwise physically or chemically attach to plant nutrient materials in the chemical fertilizer material, thus further retarding leaching of the plant nutrient away from the seed.

While applicant cannot place an exact time period on the increase in the extent to which the soil conditioning materials retard leaching of the plant nutrients from proximity with the seed, thereby holding the plant nutrients available for up-take by the plant, any increase in time during which the nutrients are held in the soil proximate the newly-emerging plant is beneficial to meeting the nutritional needs of the plant being so fed.

By incorporating soil conditioning materials and optionally plant nutrient fertilizers, in the seed capsules, the invention offers an efficiency of application of soil conditioning materials in proximity to the seeds most beneficially affected thereby, in a beneficial association never before available. Optional addition of plant nutrients to the same seed capsule provides a largely self-contained microcosm of seed, soil conditioner, and inorganic

fertilizer in intimate yet controlled spatial relationship with each other, whereby the controlled spacings provide enhanced plant growth benefit. Namely, soil conditioning materials and plant nutrients are somewhat beneficial to each other for the overall cooperative achievement of soil fertility in the presence of the newly emerging plant which is dependent on such plant nutrients, and on moisture retained by the soil conditioner for uptake of such plant nutrients.

While soil conditioning materials do perform a number of highly interdependent tasks, one such task is in assisting in maintaining the plant nutrients in the root zone where they can be effectively used by the plants when needed. Another such task is in assisting in making the soil soft and friable in the root zone whereby the newly-emerged and very tender plant roots more readily penetrate the soil as they grow.

Where both soil conditioner and fertilizer are incorporated with the seed into the seed capsule, the soil conditioner assists in strategically maintaining the combination of soil conditioner and plant nutrients in close and controlled proximity to each other and to the seed in the soil. Such strategic placement virtually assures that the soil conditioning material and inorganic chemical fertilizer are bound to each other, in proximate relationship with the seed, for a time, such that wherever the seed capsule may land when the seed is sown, the seed will have the initial benefit of both soil conditioner and plant nutrients in intimate proximity with itself, irrespective of any condition of the surrounding growth medium. Thus, in the invention, soil conditioning material and optionally inorganic chemical fertilizer, are inherently bound to each other, and to the seed, as by the coating process, and inherently assist the seed in achieving desired germination and strong early growth.

By incorporating the soil conditioning material in the same seed capsule with the seed, the invention ensures that the seed has

benefit of intimate relationship with a beneficial amount of soil conditioner material. The seed thus receives the advantage of the beneficial amount of soil conditioner material irrespective of the overall tilth of the soil and irrespective of the overall level soil conditioner, e.g. soil texture conditioner, in the root zone of the soil with which the seed capsule becomes associated for seed and plant growth purposes.

Referring to FIGURE 7, a population of seed capsules **38** are disposed at the top surface of a cross section of soil. Root zone **150** of the soil is generally defined to that depth of the soil which typically receives roots of growing plants, and is generally defined within 20-30 inches of the top surface of the soil. Generally, and preferably, the root zone should have a soft texture, rich in organic and/or other soil conditioning material in order to provide good tilth, and desirable moisture and nutrient holding properties. Underlying root zone **150** is subsoil **152** which typically contains little organic matter.

It is a well known agricultural phenomenon that, in soil used for intensive crop production, the root zone tends, over time, to become relatively depleted of organic soil conditioning material, illustrated at **154** in FIGURE 7, negatively affecting soil tilth and texture. While wholesale addition of organic soil conditioning material can improve the overall tilth of the soil, FIGURE 7 illustrates application of the invention wherein the texture of the material immediately adjacent the seed, namely coating **42**, provides beneficial properties attributable to soil having desirable texture.

FIGURE 8 illustrates that coating **42** draws moisture **154** from the soil, into the capsule, where the moisture is available to assist in germination of seed **40**. In the process, traverse of the moisture through second coating **46C** releases plant nutrient material into the moisture, as well as downwardly into the soil adjacent the seed capsule, as illustrated at **156**. Thus, the root

158 emerging from the seed emerges into an initial growth medium, coating **42**, having texture, moisture, and plant nutrient highly advantageous to early plant growth. As root **158** advances further downward, the upper portion of the underlying soil under the capsule where the seed first enters the soil, has also been beneficially affected to the good of the plant by plant nutrients **156**, and by moisture attracted or held in the vicinity of the capsule, as a result of the presence of the soil conditioning material in the capsule.

The relative amounts of the soil conditioning material and the inorganic chemical fertilizer material in the seed capsule vary significantly in accord with the specific application, and any specific interactivity desired of the soil conditioning material and inorganic chemical fertilizer. For example, in a particular combination of soil conditioning material and inorganic fertilizer a particular plant crop to be nourished by the product may require a higher amount of plant nutrient, or a specific analysis of plant nutrients, in order to be properly fed at and shortly after the stage of germination.

Thus, for a given specific application of combination seed capsule (with fertilizer) product of the invention, the relative amount of inorganic chemical fertilizer, and the fertilizer analysis, may be increased or decreased from some "standard" in the interest of achieving a functionally adequate feeding of the newly germinated seedlings. Namely, the NPK etc. nutrient levels provided in a given seed capsule product of the invention can be set and controlled at the fertilizer manufacturing plant in accord with the respective NPK etc. nutrient needs of the seed to be supported, or of the soil or other growth medium to which the combination fertilizer of the invention is to be applied.

In any embodiments, whether or not specifically discussed here, the fabricated seed capsules are kept sufficiently cool, and are kept sufficiently dry, to avoid the seed capsules sticking to

each other, caking, and the like, and to prevent premature germination of the seed. Where liquid is used to obtain the coating material in liquid state, sufficient liquid is removed during or shortly after the coating step to avoid the seed capsules sticking to each other, or caking, or the like. Where the seed capsules are made by process other than the process described here, the details of the process will determine proper cooling, drying, or other steps to provide a finished, dry, solid seed capsule or like product. A dry such product generally has moisture content less than 10% by weight, preferably less than 5% by weight, most preferably less than 3% by weight.

As suggested by the description hereinabove, the processes of the invention are generally carried out to make combination seed improvement products solely by using physical processes such as coating and drying. While some minor chemical reactions may inadvertently accompany such physical processes, the invention does not rely on any chemical reaction for achievement of the objectives thereof. Rather the invention is focused on a physical combination of starting materials, which physical combination results in mutual benefits of the two starting materials (seed and soil conditioner, and optional inorganic chemical fertilizer) functioning intimately together, in primarily physical and physico-chemical relationship, to produce an overall increase in benefits of plant germination and early plant growth with such combination seed improvement products.

The relative amounts of seed and coating material depend on the overall benefits desired to be achieved from the coating operations. In general, the seed will comprise from about 0.1% to about 75% of the overall weight of the seed capsule. the coating material thus represents about 25% to about 99.9% by weight of the seed capsule. Where the seed content is low, the general benefit of the product is that of soil conditioning, with some seed application. Such product is well suited for application to e.g.

a healthy lawn for general improvement of soil condition, and modest fill-in of bare spots with seed.

Another benefit of low seed content by weight, especially with quite small seeds, is in creating a larger size seed capsule, and thereby facilitating the handling of such seed in commonly-used seed handling machines such as grain drills or seed broadcast machines.

Typically, however, a higher seed content is preferred so as to have major impact on the number of plants which are caused to germinate by application of such product. Thus, for a seed about 0.5-1.0 mm thick and about 4-7 mm long, a preferred fraction of seed is about 1% to about 50%, preferably about 1.5% to about 20%, more preferably about 2% to about 10% by weight seed, with respective amount of soil conditioner and optionally fertilizer. For example, in a preferred product of the invention, an above mentioned grass seed about 0.5-1.0 mm thick and about 4-7 mm long, when coated produces a seed capsule about 4 mm across and about 6-9 mm long. Smaller, or larger, seed capsules may be made and used as desired.

The size and density of the seed capsules can be readily controlled using conventional sizing equipment and processing parameters of the coating process, so as to provide a uniform product of a wide range of sizes and densities. With the size and density of any seed thus controllable, the size and density may be selected and specified for enhancing control and efficiency of seed handling and/or distribution. For example, tiny seeds such as lettuce, carrots, cabbage, and alfalfa, may be sized and weighted for easy and assured handling and distribution, whether by hand or by machine. Seeds which are non-aerodynamic, or which are so light as to be blown around, such as grass seed, can be made heavy and compact enough as to assuredly remain on location where sown after being planted. For example, non-aerodynamic seeds, after treatment according to the invention, can be broadcast-applied using

conventional equipment such as is used to broadcast apply granular fertilizer over e.g. 40 foot wide application paths.

Where time controlled germination is desirable, a population of combination seed capsules, having at least one soil conditioner and one or more nutrients, can be planted in conjunction with non-coated seeds. As a result, non-coated seeds will germinate at an earlier stage than the population of combination seed capsules. Such staggering of germination times allows, for example, the non-coated seeds to use the available soil nutrients with less competition (i.e. less seeds using limited nutrient supply). At a later time, when the coated seeds germinate, such seeds can use the nutrients leached from their combination seed capsules to germinate.

Where e.g. small such seeds are desirably planted in close proximity with each other, and wherein a relatively larger size seed capsule is desired for ease of handling such that the large size seed capsule would potentially interfere with such close placement of the seeds with respect to each other, then and in such situation, multiple seeds may be employed in individual seed capsules, e.g. generally uniformly distributed throughout the seed capsule, so as to provide for sufficiently close spacing of the seeds from each other.

Paper mill sludge, as is suggested as a coating material herein, is a resultant by-product of papermaking, typically from e.g. a de-inking process in the paper mill.

By utilizing paper mill sludge and/or sewage sludge as taught herein, one contemplates beneficially and suitably disposing of significant quantities of industrial waste which otherwise is disposed of by landfilling.

Where the product of the invention is applied as to a residential or like lawn, as in an agricultural field, the seed is applied to the soil in intimate combination (seed capsule) with the soil conditioner, such that the soil conditioner serves as moisture

retainer and sun shield. In addition, the seed capsule is much heavier and dense than the seed itself, whereby the seed capsule provides substantial protection against the seed being washed away in surface water run-off. Thus, the coating about the seed serves many of the functions typically performed by the conventionally-used straw mulch. Accordingly, product of the invention can be used to seed new lawns without any need for use of straw or any other mulch material.

Where seed is desirably used to fill in bare spots in the lawn, such seed, especially fertility-enhanced seed capsules, may be applied desirably in one of two ways. First, the coated seed capsule product may be applied only to perceived bare spots, without use of straw. The soil conditioner in the seed capsules serve the functions of the straw as described above, but perform better than straw because of the close association between the seed and the soil conditioner.

In the alternative, the coated seed capsule product may be broadcast generally over the entire lawn. Where the lawn is already healthy with thick grass growth, the soil conditioner and fertilizer will benefit the existing grasses, with minimal germination and growth of new seed from the seed capsules. Where the existing grass is thinner, the seeds in the seed capsules will have room and light to grow, whereby the combined properties of seed, soil conditioner, and fertilizer, in intimate relationship with one another, will be efficaciously used.

Where seed capsules of the invention are used to establish a new lawn, the soil conditioner in the seed capsules serve the functions of the straw as described above, obviating the need for straw in establishing the lawn seeding.

Those skilled in the art will now see that certain modifications can be made to the apparatus and methods herein disclosed with respect to the illustrated embodiments, without departing from the spirit of the instant invention. And while the

invention has been described above with respect to the preferred embodiments, it will be understood that the invention is adapted to numerous rearrangements, modifications, and alterations

To the extent the following claims use means plus function language, it is not meant to include there, or in the instant specification, anything not structurally equivalent to what is shown in the embodiments disclosed in the specification.

CLAIMS

Having thus described the invention, what is claimed is:

1. A combination seed capsule, comprising:

(a) at least one viable seed, having an outer surface and acting as a core or psuedo-core of said combination seed capsule; and

(b) a coating of a composition comprising a soil conditioning material mounted proximate, including disposed outwardly of the outer surface of said seed.

2. A combination seed capsule as in Claim 1, said coating providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

3. A combination seed capsule as in Claim 1 wherein said seed is selected from the group consisting of grass, vegetables, grains, and flowers.

4. A combination seed capsule as in Claim 1, said coating further comprising said soil conditioning material in combination with at least one ingredient effective to reduce susceptibility of

said seed capsule to deleterious affect of at least one of animals, weeds, and spore-formers.

5. A combination seed capsule as in Claim 4 wherein said at least one ingredient to reduce susceptibility of the seed capsule is selected from the group consisting of herbicides, fungicides, and a bitter substance.

6. A combination seed capsule as in Claim 5 wherein said fungicide comprises metalaxyl.

7. A combination seed capsule as in Claim 1, said coating comprising a first coating, said combination seed capsule further comprising a second coating, separate from said first coating, and comprising at least one ingredient effective to reduce susceptibility of said seed capsule to deleterious effect of at least one of animals, weeds, and spore-formers.

8. A combination seed capsule as in Claim 1, effective to provide a plant nutrient at a desirable controlled distance from a plant seedling emerging from said seed, in an amount beneficial to said plant seedling.

9. A combination seed capsule as in Claim 1, said coating comprising a first coating, said combination seed capsule further comprising a second coating of a second coating material intermingled with said first coating material in an outer portion of said first coating, and generally displaced from said seed.

10. A combination seed capsule as in Claim 9 wherein said second coating material comprises a plant nutrient, beneficial in location and in amount of availability, to plant seedling emerging from said seed.

11. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient and is selected from the group consisting of nitrogen, phosphorus, and potassium.

12. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient and is selected from the group consisting of urea, monammonium phosphate, diammonium phosphate, superphosphate, triple superphosphate, dicalcium phosphate, and potash.

13. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient is selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

14. A population of combination seed capsules of Claim 1, said population of seed capsules comprising coatings having a range of properties affecting germination rate of said seeds, thereby to stage germination of said seeds in said population over a period of time longer than the range of germination times inherent in uncoated ones of said seeds.

15. A population of combination seed capsules as in Claim 14 wherein said range of properties comprises at least one of (i) a range of hardnesses and (ii) a range of thicknesses, of said coatings.

16. A combination seed capsule as in Claim 1, said coating comprising a first layer of said soil conditioning material, and including a second layer comprising an inorganic fertilizer.

17. A combination seed capsule as in Claim 1, said coating comprising a first layer of said soil conditioning material, and including a second layer comprising at least one micronutrient.

18. A combination seed capsule as in Claim 17 wherein said micronutrient is selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

19. A combination seed capsule as in Claim 1, said soil conditioning material comprising a sludge composition.

20. A combination seed capsule as in Claim 1, said soil conditioning material comprising a fiber-containing by-product of a paper making operation.

21. A combination seed capsule as in Claim 1, said seed capsule comprising a water-leachable plant nutrient, and a leach-

retardant composition effective to retard leaching of said leachable plant nutrient out of said combination seed capsule.

22. A population of combination seed capsules of Claim 1, said coating in ones, but less than all, of said population, comprising an ingredient effective to retard effective penetration of a seed-germinating environment to said seed for germination thereof.

23. A combination seed capsule as in Claim 1, said seed capsule comprising an inner layer on the outer surface of said seed, and an outer layer, said inner layer enhancing properties of said seed for acting as nucleus in an agglomeration operation agglomerating said coating onto said inner layer.

24. A combination seed capsule as in Claim 1 wherein said coating comprises an admixture of said soil conditioner and a plant nutrient.

25. A combination seed capsule as in Claim 1 wherein said coating remains generally disposed about said seed until said seed germinates.

26. A plant growing system, comprising:

(a) a plant growing medium extending over an area, said plant growing medium having a root zone, and a top surface of said root zone generally corresponding with a top surface

of said plant growing medium, said plant growing medium having a first overall soil condition and texture; and

(b) a population of seed capsules disposed over the top surface of said plant growing medium, said seed capsules comprising individual seeds, having outer surfaces, and coatings of soil conditioning material disposed outwardly of the outer surfaces of said seeds,

said coatings of said seed capsules providing localized germination and growth environments, at and adjacent said seeds, having texture, and nutrient and water holding properties for supporting seedling health, superior to respective said properties as provided overall in the root zone of said plant growing medium.

27. A growing system as in Claim 26, said coatings remaining generally disposed about said seeds until respective ones of said seeds germinate.

28. A growing system as in Claim 26, said coatings providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

29. A growing system as in Claim 26 wherein said seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

30. A growing system as in Claim 26, said coatings further comprising said soil conditioning material in combination with at least one ingredient effective to reduce susceptibility of said seed capsules to deleterious affect of at least one of animals, weeds, and spore-formers.

31. A growing system as in Claim 26, said coating comprising a first coating, said combination seed capsules further comprising a second coating, separate from said first coating, and comprising at least one ingredient effective to reduce susceptibility of said seed capsules to deleterious effect of at least one of animals, weeds, and spore-formers.

32. A growing system as in Claim 26, effective to provide plant nutrients at desirable controlled distances from plant seedlings emerging from said seeds, in amounts beneficial to said plant seedlings.

33. A growing system as in Claim 26, said coatings comprising first coatings, said combination seed capsules further comprising second coatings of second coating materials intermingled with said first coating materials in outer portions of said first coatings, and generally displaced from said seeds.

34. A growing system as in Claim 33 wherein said second coating materials comprise plant nutrients, beneficial in location and in amount of availability, to plant seedlings emerging from said seeds.

35. A growing system as in Claim 26, said population of seed capsules comprising coatings having a range of properties affecting germination rates of said seeds, thereby to stage germination of said seeds in said population over a period of time longer than the range of germination times inherent in uncoated ones of said seeds.

36. A growing system as in Claim 26, said coatings comprising first layers of said soil conditioning material, and including second layers comprising inorganic fertilizer.

37. A growing system as in Claim 26, said soil conditioning material comprising a sludge composition.

38. A growing system as in Claim 26, said soil conditioning material comprising a fiber-containing by-product of a paper making operation.

39. A growing system as in Claim 26, said seed capsules comprising inner layers on the outer surfaces of said seeds, said inner layers enhancing properties of said seeds for acting as nucleus in an agglomeration operation agglomerating said coatings onto said inner layers.

40. A growing system as in Claim 26 wherein said coatings comprise admixtures of said soil conditioner and plant nutrient.

41. A method of providing plant micronutrients to soil, the method comprising placing onto the soil a population of combination seed capsules, each comprising at least one seed, and a coating comprising a plant micronutrient material.

42. A method as in Claim 41, the coating comprising a first coating comprising the plant micronutrient, and a second coating, separate and distinct from the first coating, and comprising a soil conditioning material.

43. A method as in Claim 41, the coating providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

44. A method as in Claim 41, the coating providing a plant nutrient at a desirable controlled distance from a plant seedling emerging from the seed, in an amount beneficial to the plant seedling.

45. A method as in Claim 41, the coating comprising a first coating, the combination seed capsule further comprising a second coating of a second coating material intermingled with the first coating material in an outer portion of the first coating, and generally displaced from the seed.

46. A method as in Claim 45 wherein the first coating comprises plant micronutrient material and the second coating

comprises plant nutrient material comprising at least one of nitrogen, phosphorus, and potassium.

47. A method as in Claim 41 wherein the micronutrient composition comprises a plant nutrient selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

48. A method as in Claim 41, the coating comprising a first layer of the soil conditioning material, and including a second layer comprising an inorganic fertilizer.

49. A method as in Claim 41, the coating comprising a sludge composition.

50. A method as in Claim 41, the coating comprising a fiber-containing by-product of a paper making operation.

51. A method as in Claim 41, the seed capsule comprising an inner layer on an outer surface of the seed, and an outer layer, the inner layer enhancing properties of the seed for acting as nucleus in an agglomeration operation agglomerating the coating onto the inner layer.

52. A method as in Claim 41 wherein the coating comprising an admixture of soil conditioner and a plant nutrient.

53. A method as in Claim 41 wherein the coating remains generally disposed about the seed until the seed germinates.

54. A method of providing a seed bed having enhanced growing conditions for growing seed, the method comprising:

(a) coating a population of the seeds with material, and thereby providing coatings thereon of such material, tending to stabilize, in the seed capsules, or in soil on which the seed capsules are disposed coating compositions which tend to hold, moisture adjacent the seeds in the seed capsules or in soil adjacent the seed capsules, in such quantities and for such times as to enhance growing conditions for the seeds; and

(b) placing the population of seeds on soil effective to support germination of the seeds which are in the seed capsules.

55. A method as in Claim 54, the coatings providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vi) soil conditioning effect to soil onto which said combination seed capsule is placed;

(vii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released; and

(viii) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density.

56. A method as in Claim 54 wherein the seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

57. A method as in Claim 54, effective to provide a plant nutrient at desirable controlled distances from plant seedlings emerging from the seeds, in amounts beneficial to the plant seedlings.

58. A method as in Claim 54, the coatings comprising first coatings, the combination seed capsules further comprising second coatings of second coating materials intermingled with the first coating materials in outer portions of the first coatings, and generally displaced from the seeds.

59. A method as in Claim 58 wherein the second coating materials comprise plant nutrients, beneficial in location and in amount of availability, to plant seedlings emerging from the seeds.

60. A method as in Claim 58 wherein the second coating compositions comprise inorganic forms of plant nutrients and are selected from the group consisting of nitrogen, phosphorus, and potassium.

61. A method as in Claim 54, the population of seed capsules comprising coatings having a range of properties affecting germination rate of the seeds, thereby to stage germination of the seeds in the population over a period of time longer than the range of germination times inherent in uncoated ones of the seeds.

62. A method as in Claim 54, the coatings comprising first layers of the soil conditioning material, and including second layers comprising inorganic fertilizer.

63. A method as in Claim 54, the coatings comprising first layers of the soil conditioning materials, and including second layers comprising micronutrients.

64. A method as in Claim 54, the soil conditioning materials comprising sludge compositions.

65. A method as in Claim 54, the soil conditioning materials comprising fiber-containing by-products of paper making.

66. A method as in Claim 54, the seed capsules comprising water-leachable plant nutrients, and leach-retardant compositions effective to retard leaching of the leachable plant nutrients out of the combination seed capsules.

67. A method as in Claim 54, the seed capsules comprising inner layers on the outer surfaces of the seeds, and outer layers, the inner layers enhancing properties of the seeds for acting as nuclei in agglomeration operations agglomerating the coatings onto the inner layers.

68. A method as in Claim 54 wherein the coatings comprise admixtures of the soil conditioners and plant nutrients.

69. A method as in Claim 54 wherein the coatings remain generally disposed about the seeds until the seeds germinate.

70. A method of making a population of combination seed capsules, each comprising a seed, and a coating of a soil conditioning material, the method comprising:

(a) pre-coating the seed with a material which enhances the ability of the seed to act as a nucleus in an agglomeration operation, to form a pre-coated substrate; and

(b) subsequently coating the pre-coated substrate with a soil conditioning material.

71. A method as in Claim 70 wherein the pre-coating material comprises dicalcium phosphate.

72. A method as in Claim 70 wherein the pre-coating step results in an overall increase in the density of pre-coated seed combination.

73. A method as in Claim 70 wherein the pre-coating is accomplished by spraying the pre-coating material onto the seed.

74. A method of providing an enhanced seed germination environment in combination with placement of a controlled amount of plant nutrients in controlled proximity to each seed, the method comprising:

(a) providing a population of seeds, coated with a soil conditioning material which tends to enhance germination of the seeds, and with plant nutrient composition effective to enhance growth of plant embryos emerging from the seeds; and

(b) placing the population of seeds on soil effective to support germination of the seeds.

75. A method as in Claim 74 wherein the coating material includes therein a second ingredient comprising plant nutrient moieties.

ABSTRACT OF THE DISCLOSURE

This invention pertains to combination seed capsules wherein each seed capsule includes both moieties of at least one soil conditioner and at least one seed, and optionally, one or more inorganic chemical fertilizer, growth enhancer, binder, and/or anti-fungal agent. The combination seed capsules are made by physically combining the respective soil conditioner and seed with one other, in the absence of any requirement for chemical reactions in the process of so combining the respective materials. The combination seed capsules provide cooperative and beneficial effects of the soil conditioner and the optional inorganic fertilizer, working together in controlled intimate relation with the seed, to enhance the germination and growth processes of the seed, and the plant emergent therefrom, greater than when the soil conditioner and seed, and optionally inorganic chemical fertilizer, are applied to the soil separately; the improvement being a result of the intimate relationship of the respective materials in the combination seed capsule, whereby the respective materials cooperate with each other in support of germination and plant growth.

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Daniel Paul Madigan et al**			Docket No. **29214**
Serial No. **Unassigned**	Filing Date **07/10/98**	Examiner **Unassigned**	Group Art Unit **Unassigned**

Invention: **SEEDING TREATMENTS**

I hereby certify that this **Informal Drawings (6)**
(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231 on

July 10, 1998
(Date)

Jerry F. Janssen
(Typed or Printed Name of Person Mailing Correspondence)

(Signature of Person Mailing Correspondence)

EM 469 259 847 US
("Express Mail" Mailing Label Number)

Note: Each paper must have its own certificate of mailing.

Fig. 2

Fig. 1

Seed/Paper Sludge
Agglomeration Process

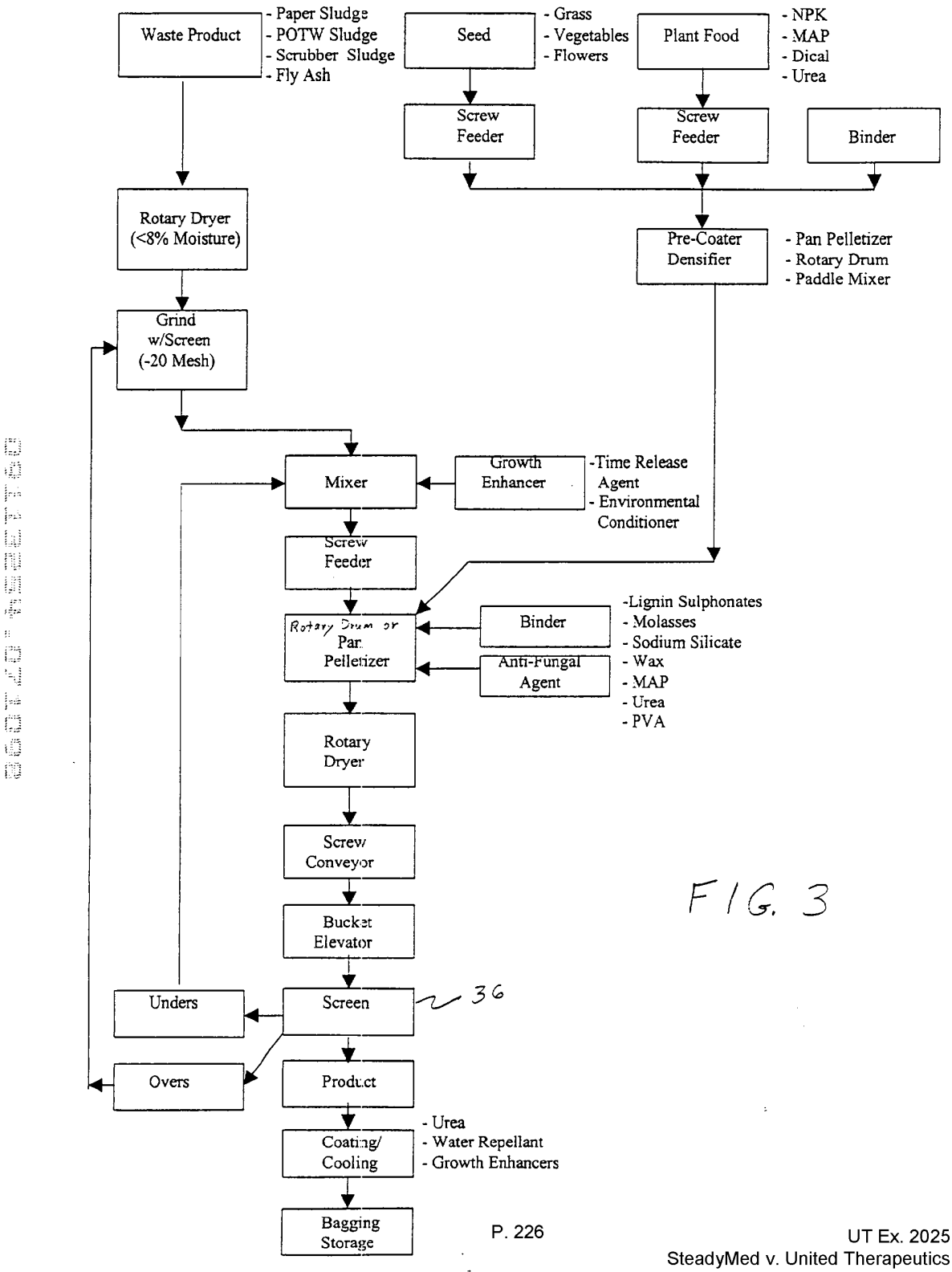


FIG. 3

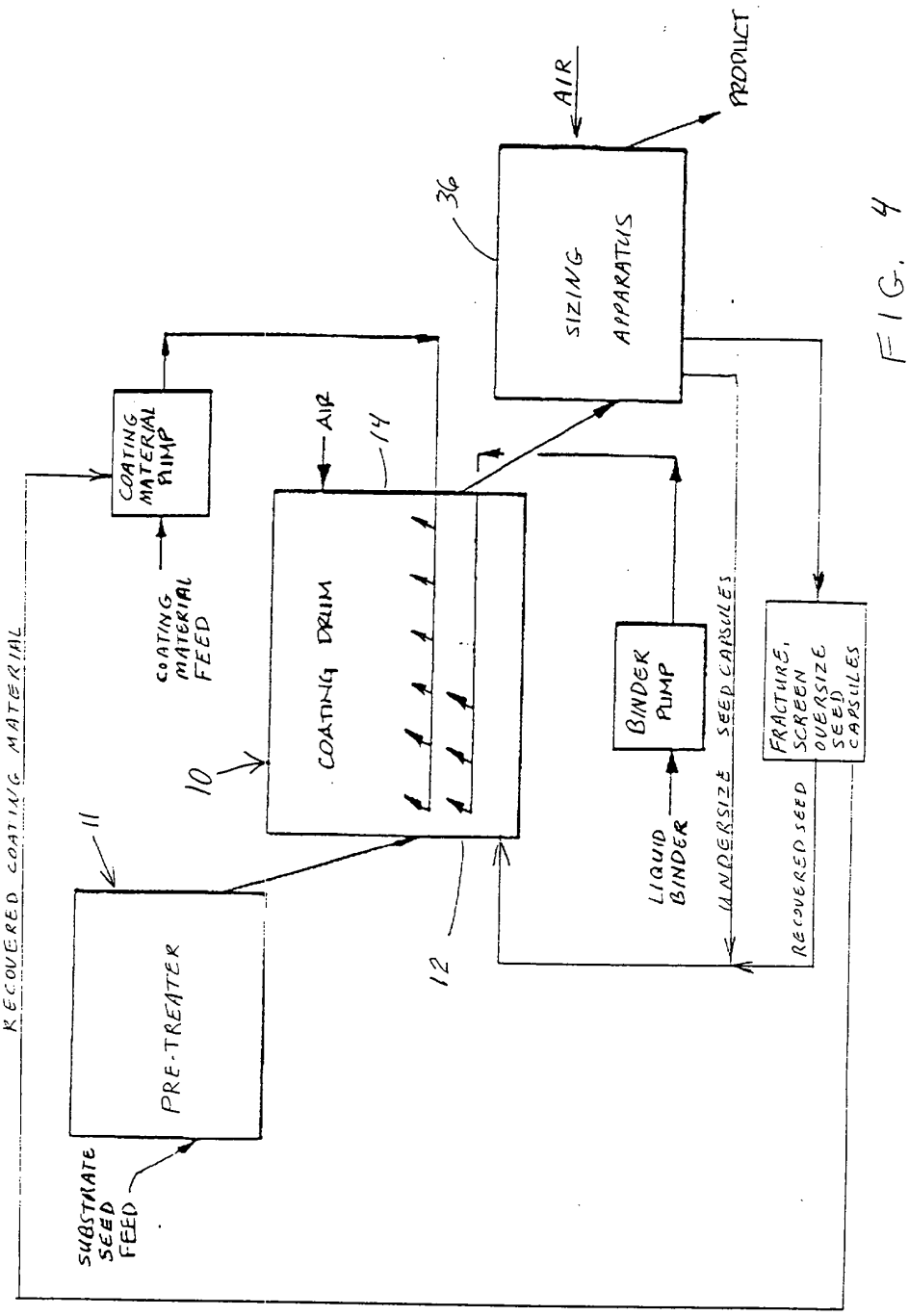


FIG. 4

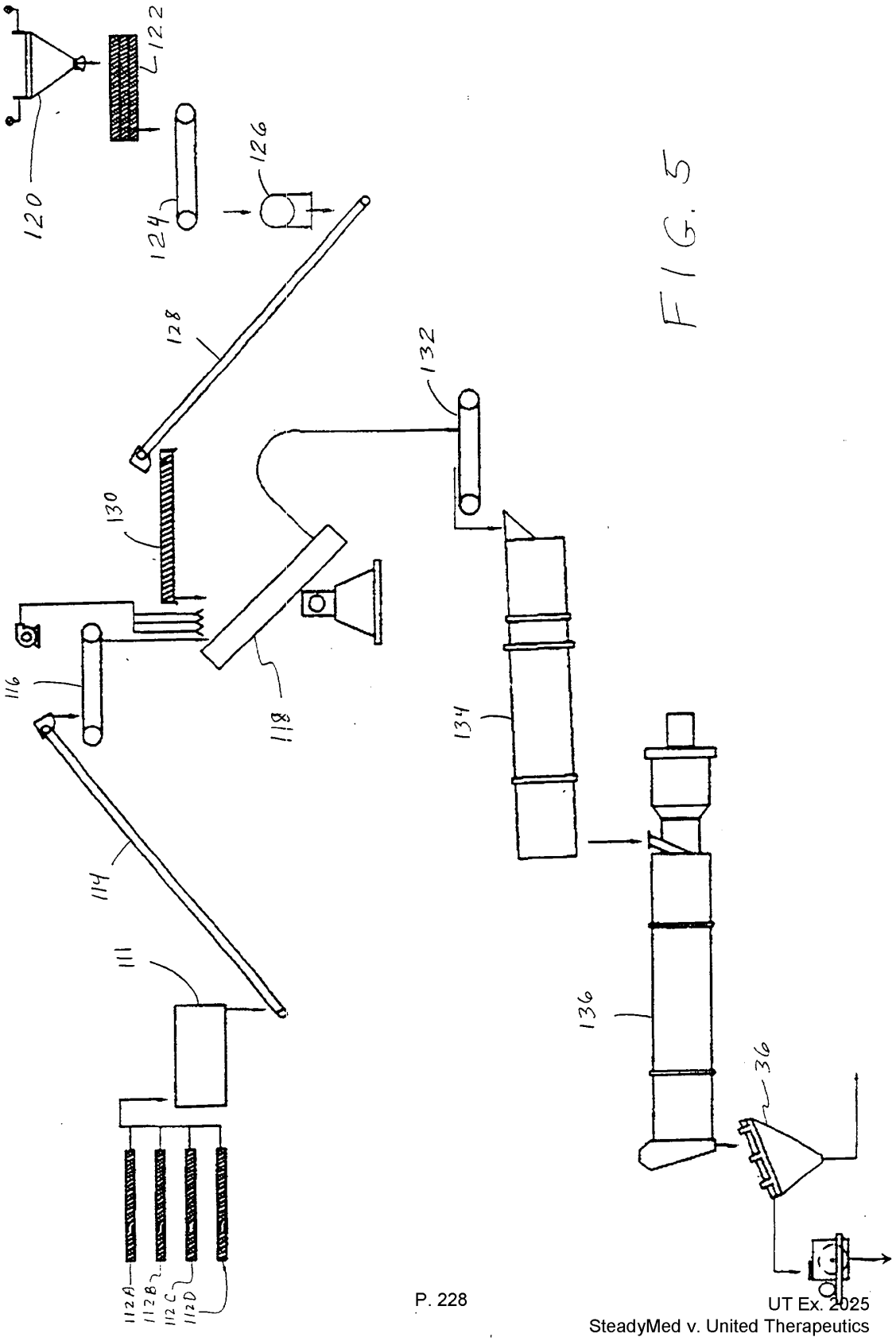


FIG. 5

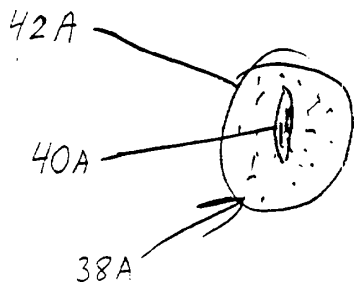


FIG. 6A

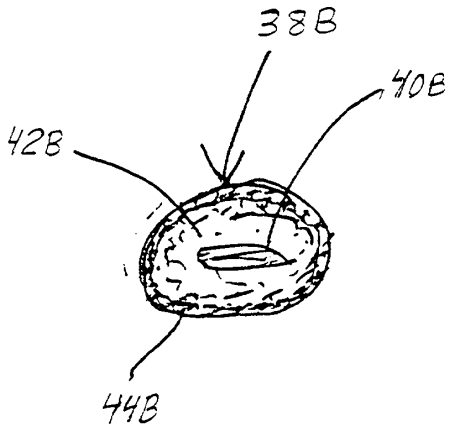
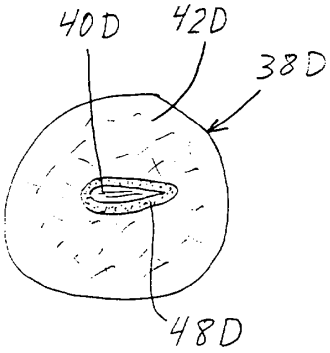


FIG. 6B



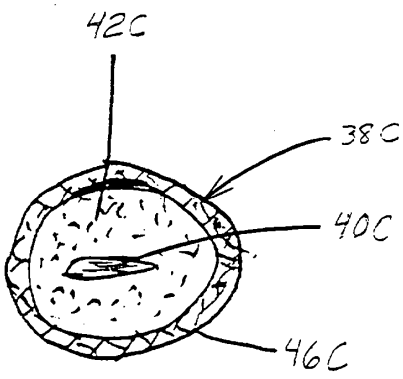
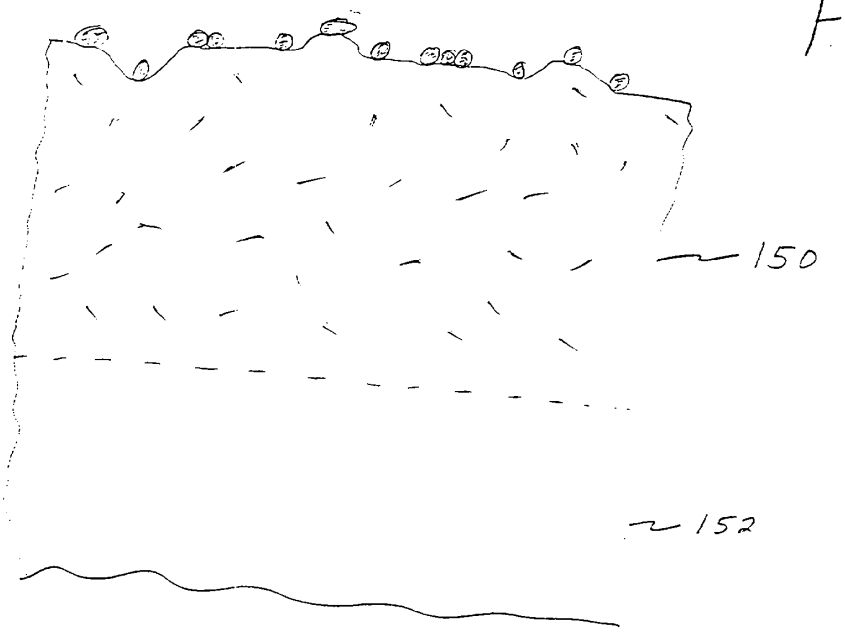


FIG. 6C

FIG. 6D

FIG. 7



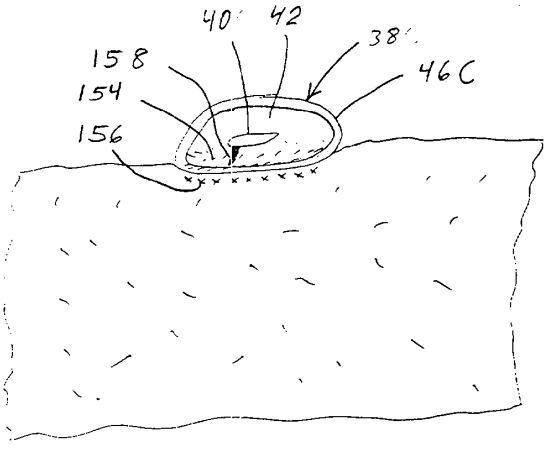
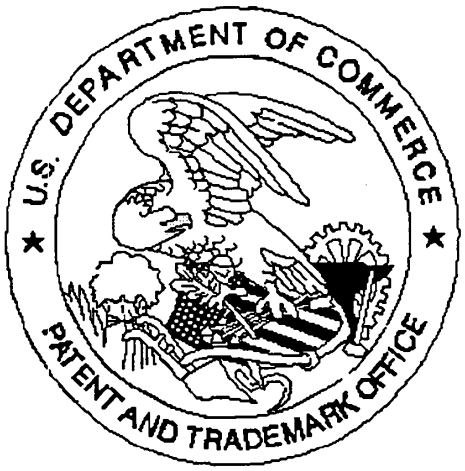


FIG. 8

PRINT OF DRAWINGS
AS ORIGINALLY FILED

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

1. Application papers are not suitable for scanning and are not in compliance with 37 CFR 1.52 because:
   - ☐ All sheets must be the same size and either A4 (21 cm x 29.7 cm) or 8-1/2"x 11". Pages _____ do not meet these requirements.
   - ☐ Papers are not flexible, strong, smooth, non-shiny, durable, and white.
   - ☐ Papers are not typewritten or mechanically printed in permanent ink on one side.
   - ☐ Papers contain improper margins. Each sheet must have a left margin of at least 2.5 cm (1") and top, bottom and right margins of at least 2.0 cm (3/4").
   - ☐ Papers contain hand lettering.

2. Drawings are not in compliance and were not scanned because:
   - ☐ The drawings or copy of drawings are not suitable for electronic reproduction.
   - ☐ All drawings sheets are not the same size. Pages must be either A4 (21 cm x 29.7 cm) or 8-1/2" x 11".
   - ☐ Each sheet must include a top and left margin of at least 2.5 cm (1"), a right margin of at least 1.5 cm (9/16") and a bottom margin of at least 1.0 cm (3/8").

3. Page(s)_____ are not of sufficient clarity, contrast and quality for electronic reproduction.

4. Page(s)_____ are missing.

5. OTHER: No Declarations Enclosed

Express Mail Label No. EM 469 25[illegible]US

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 29214

Total Pages in this Submission 82

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

SEEDING TREATMENTS

and invented by:

DANIEL PAUL MADIGAN
MICHAEL DENNIS KRYSIAK
RONALD DEAN EICHHORN
GLEN H. WESENBERG

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

1. ☐ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 70 pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
29214
Total Pages in this Submission
82

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
   a. ☐ Formal b. ☒ Informal Number of Sheets 6

4. ☐ Oath or Declaration
   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
   c. ☐ With Power of Attorney ☐ Without Power of Attorney
   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission *(if applicable, all must be included)*
   a. ☐ Paper Copy
   b. ☐ Computer Readable Copy
   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & documents)*

9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing
   ☐ First Class ☒ Express Mail *(Specify Label No.):* EM 469 259 847 US

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
29214
Total Pages in this Submission
82

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Small Entity Statement(s) - Specify Number of Statements Submitted: _____

17. ☒ Additional Enclosures *(please identify below):*

Correspondence Address

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	75	- 20 =	55	x	$11.00	$605.00
Indep. Claims	5	- 3 =	2	x	$41.00	$82.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$395.00
OTHER FEE *(specify purpose)* _____						$0.00
					TOTAL FILING FEE	$1,082.00

☐ A check in the amount of to cover the filing fee is enclosed.

☐ The Commissioner is hereby authorized to charge and credit Deposit Account No. as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☐ Credit any overpayment.
- ☐ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: July 10, 1998

Thomas D. Wilhelm
Signature

Thomas D. Wilhelm (Reg. No. 28,794)

cc:

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Daniel Paul Madigan et al**			Docket No. **29214**
Serial No. **Unassigned**	Filing Date **07/10/98**	Examiner **Unassigned**	Group Art Unit **Unassigned**

Invention: **SEEDING TREATMENTS**

I hereby certify that this **Correspondence Address**
(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231 on

July 10, 1998
(Date)

Jerry F. Janssen
(Typed or Printed Name of Person Mailing Correspondence)

Jerry F. Janssen
(Signature of Person Mailing Correspondence)

EM 469 259 847 US
("Express Mail" Mailing Label Number)

Note: Each paper must have its own certificate of mailing.

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Daniel Paul Madigan et al**			Docket No. **29214**
Serial No. **Unassigned**	Filing Date **07/10/98**	Examiner **Unassigned**	Group Art Unit **Unassigned**

Invention: **SEEDING TREATMENTS**

I hereby certify that this **Informal Drawings (6)**
(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231 on

July 10, 1998
(Date)

Jerry F. Janssen
(Typed or Printed Name of Person Mailing Correspondence)

Jerry F. Janssen
(Signature of Person Mailing Correspondence)

EM 469 259 847 US
("Express Mail" Mailing Label Number)

Note: Each paper must have its own certificate of mailing.

Fig. 2

Fig. 1

Seed/Paper Sludge Agglomeration Process

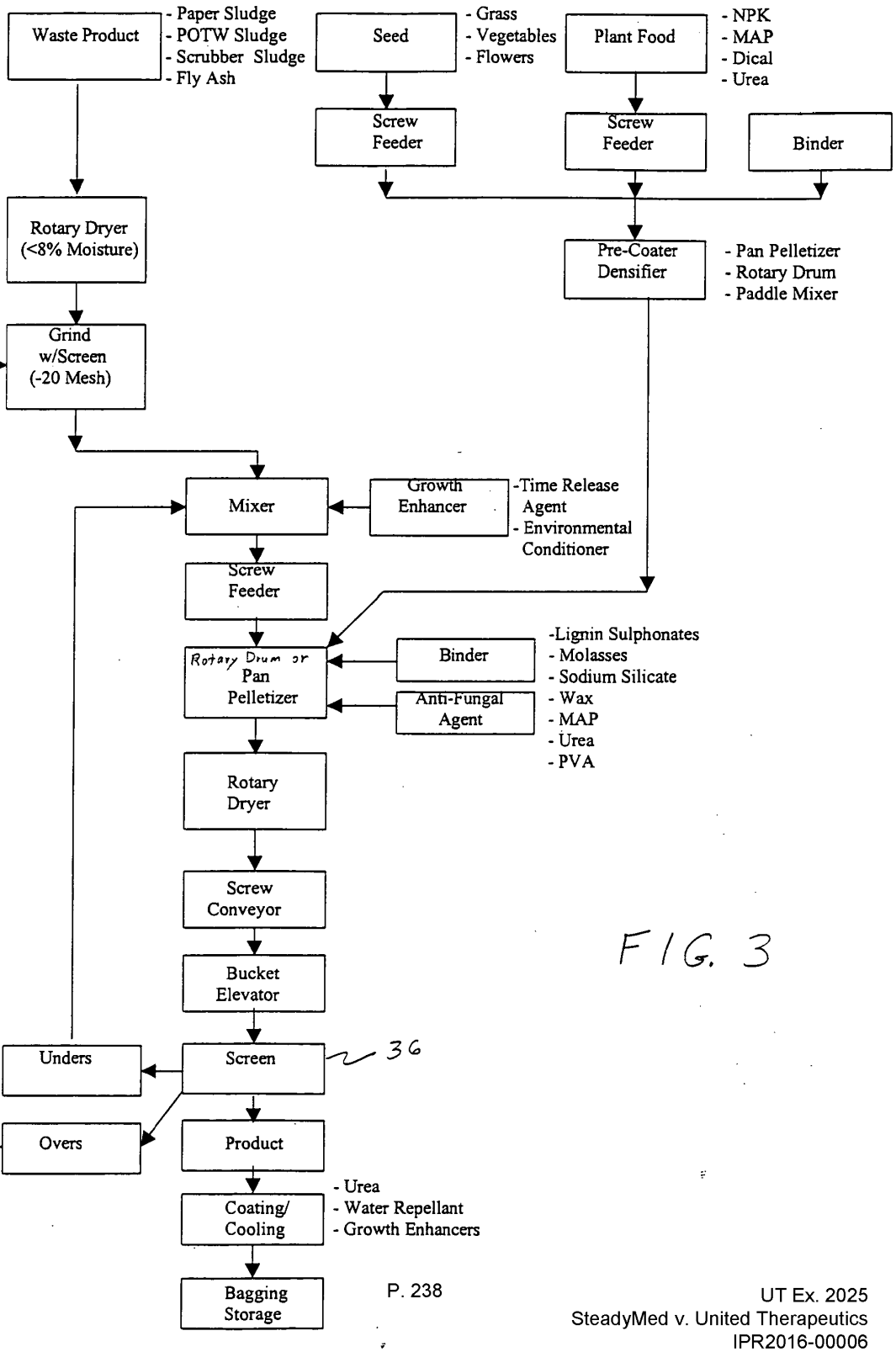


FIG. 3

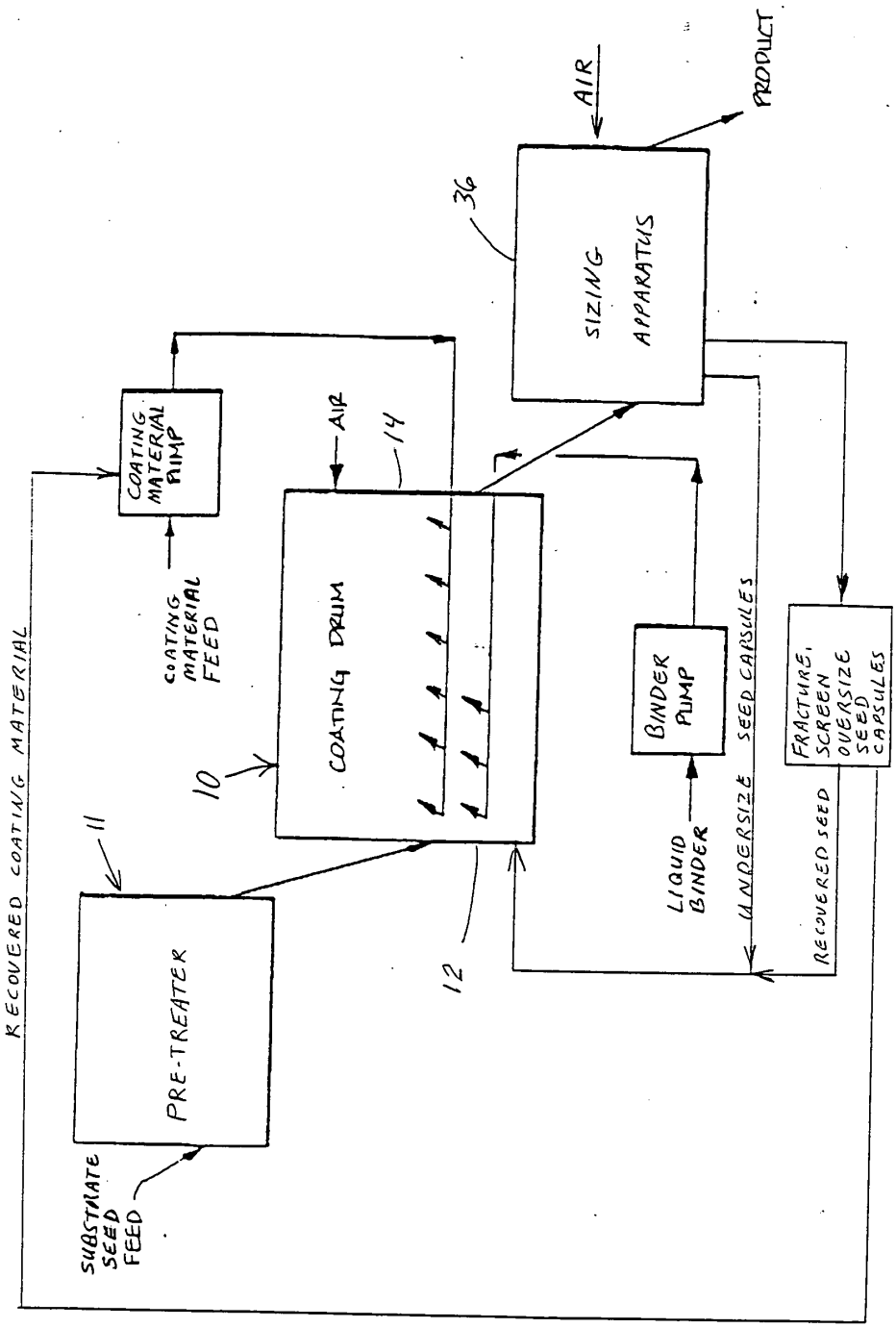


FIG. 4

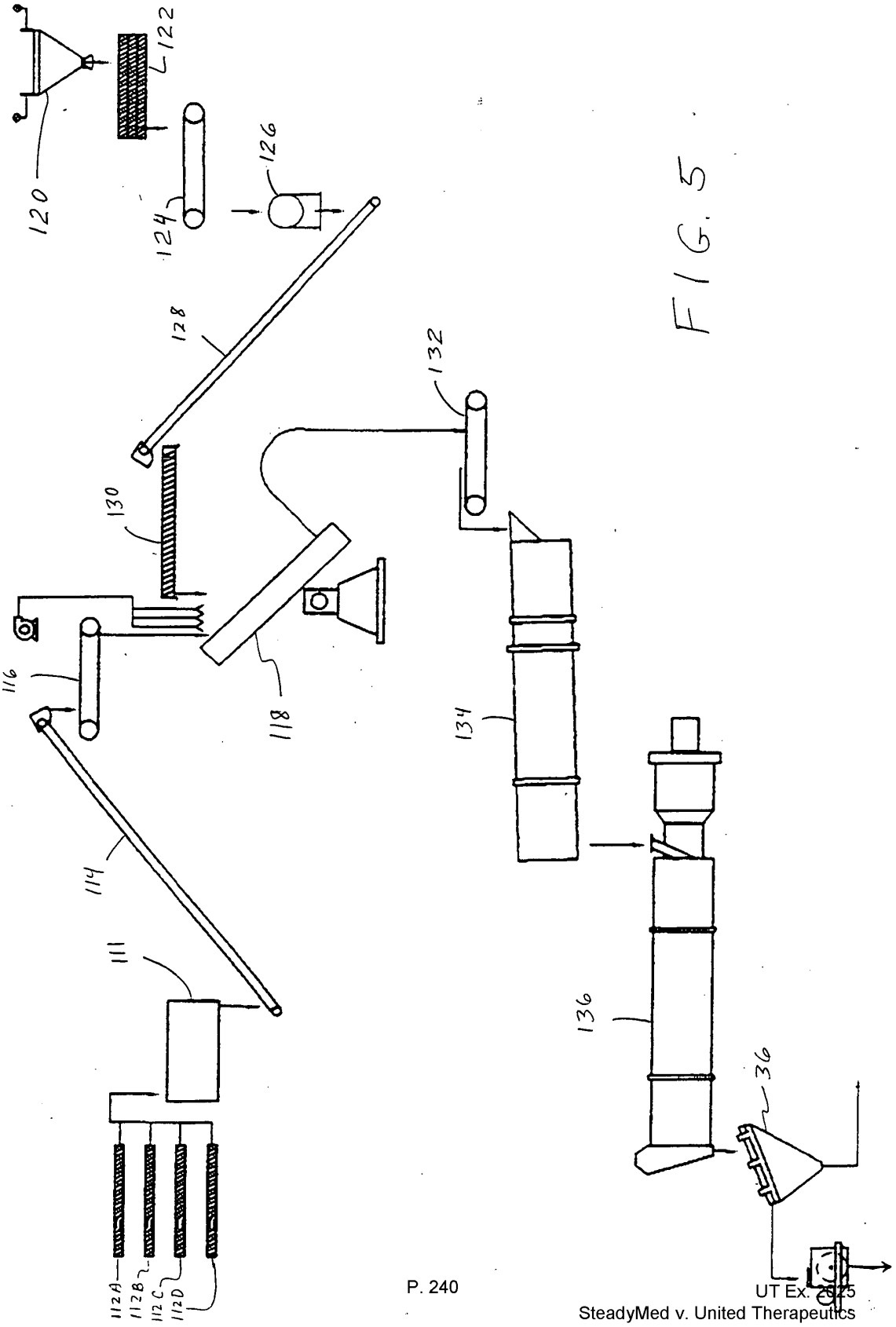


FIG. 5

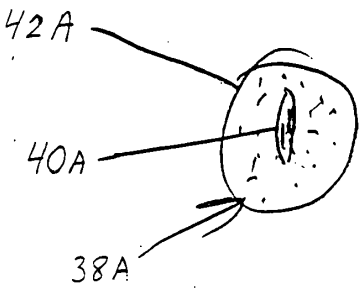


FIG. 6A

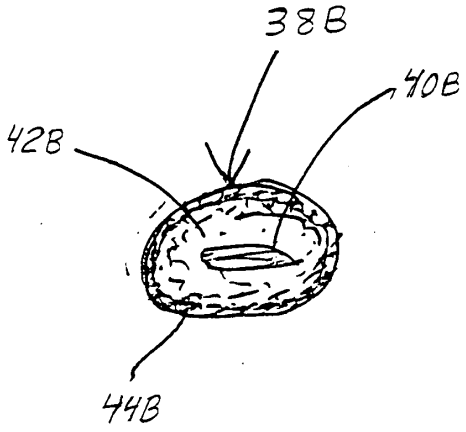
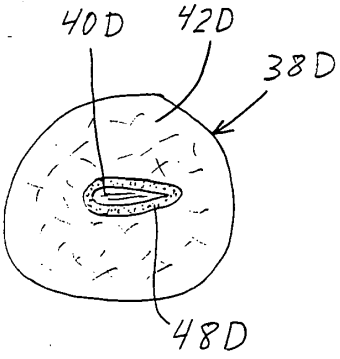


FIG. 6B



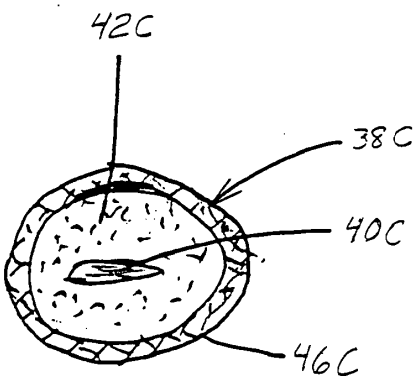
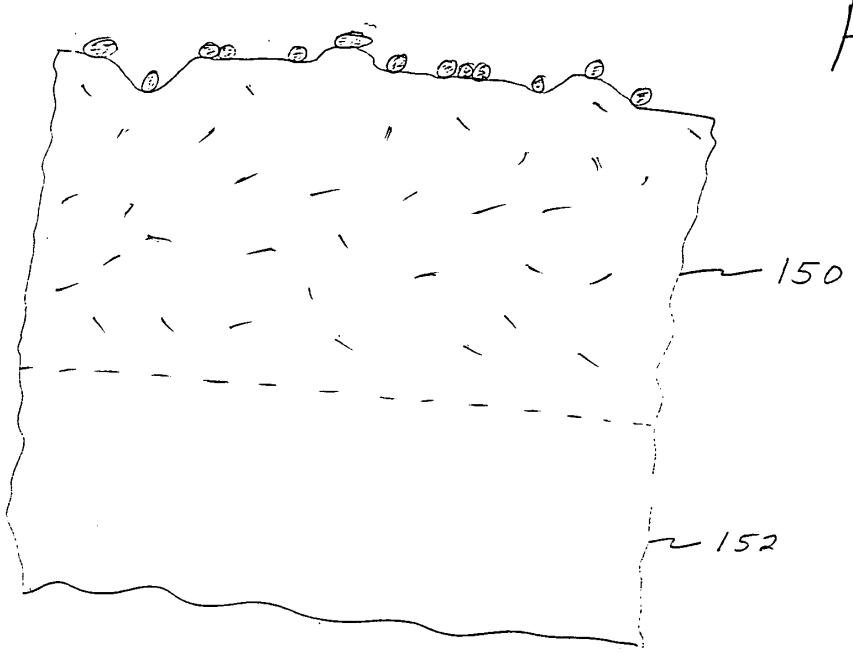


FIG. 6C

FIG. 6D

FIG. 7



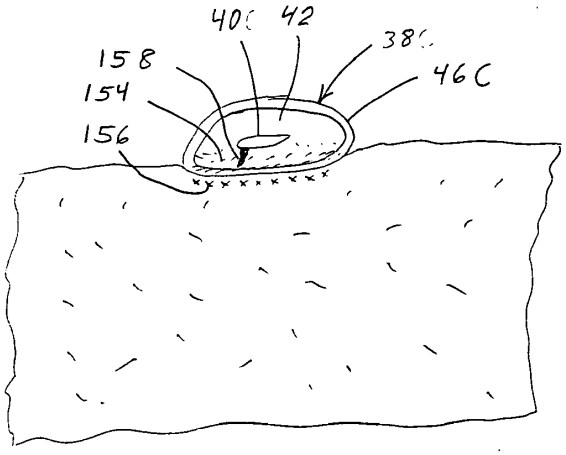


FIG. 8

PATENT APPLICATION

TITLE: SEEDING TREATMENTS

By: Daniel Paul Madigan
804 S. Madison
Green Bay, WI 54301
Citizenship: USA

Michael Dennis Krysiak
3554 Highland Center Drive
Green Bay, WI 54311
Citizenship: USA

Ronald Dean Eichhorn
1524 ½ Cedar Street
Green Bay, WI 54302
Citizenship: USA

Glen H. Wesenberg
920 Laverne Drive
Green Bay, WI 54311
Citizenship: USA

"Express Mail" mailing number EM 469 259 847 US

Date of Deposit June 10, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Jerry F. Janssen
(Typed or printed name of person mailing paper or fee)

Jerry F. Janssen
(Signature of person mailing paper or fee)

TDW, JSK

A - This application claims benefit to US provisional 60/52,287 filed 11-97.

SEEDING TREATMENTS

Field of the Invention

This present invention relates to improvements in seed and seed-related products, processes for making such products and processes for establishing and improving seed beds and seed bed germination. As additional benefits, this invention is directed at improving soil productivity through enhancements in soil fertility, soil condition/tilth, and control of soil moisture. Further, the invention relates to productive use of certain types of abundantly available manufacturing waste, which waste is currently being disposed of in landfills.

Background of the Invention

Agricultural growers, gardeners, landscape operators, flower growers, and the like produce a wide variety of cultivated crops. Many such crops are grown from seed. The sizes, shapes, and physical characteristics of the various kinds of seeds are as varied as the number of crops produced therefrom.

Producers of such cultivated crops encounter a variety of challenges in handling and distributing such seed, as well as with sowing of such seed in suitable growing media. Certain seed may desirably be sowed by a broadcast method if the seed were compatible with broadcast application. For example, grass seed for lawns is desirably broadcast, but the low density and generally non-aerodynamic shape of some grass seed can limit the range of such broadcast, and make such seed susceptible to being blown about

by wind, or washed away by surface water, even if initially well placed in a good seeding application.

Another difficulty encountered in sowing seed is that the seed may be so small as to be difficult to handle, thereby to place properly-spaced seeds at a desired spacing as tc make cost-effective use of the seed, thereby to produce a crop of the related plants without using any more seed than necessary, thus to gain maximum benefit from the amount of seed used.

While small seed may be efficiently handled by industrial equipment especially designed for handling such seed, typically the user of such seed also handles various other types of seed; and may be unable to justify the cost of such specialty seed-handling equipment. Rather, the seed user typically has a limited range of seed handling equipment which must be capable of being used and/or adapted to handle and apply all the types of seeds being used by that user. Where the seed itself can be adapted to the equipment, specialty seed can be handled without need for any specialized equipment.

Even where the seed may be sown by hand, such as in seedling or bedding trays or pots, some seeds are so small as to be difficult for the sower/user to effectively manipulate and control by hand. Typical of such difficult-to-handle seeds are seeds of lettuce, carrots, the cabbage family, ground cherries, and alfalfa. Many flower seeds are equally small and/or difficult to handle and/or manipulate, for example poppy seed.

When seed is planted, the seed has immediate use for moisture to aid in germination of the seed, and subsequent early development of the resulting young plant. Where moisture is not readily available to the seed when planted, the seed may lie in a dormant state for some period of time before germinating. While the seed is thus dormant, awaiting suitable moisture, the seed is subject to a variety of hazards which may destroy its viability. The seed may be attacked by worms, parasites, and other pests. The seed may be

eaten by foraging animals including insects and larvae. The seed may be overheated by a hot sun. The seed may lie dormant without germinating for so long that any plant emerging therefrom will have insufficient time to mature before the end of the growing season.

If and when the seed does germinate, the seedling plant has a continuing need for a proper balance of moisture and oxygen, as well as for such plant nutrients as nitrogen, phosphorous, and potash, as well as the micronutrients, in relatively predictable quantities. To the extent the proper balance of such materials is available to the young plant, a healthy young plant may be produced, with optimum potential for maximum crop production, assuming germination occurs at a seasonably-desirable time.

To the extent one or more such materials is not available to the seed and/or the young plant, plant growth, plant health, and ultimately maturity, may be adversely affected. For example, the soil may be too dry to support germination, or optimum germination. Or while the soil may in general have a desired moisture content, moisture content at a macro level can vary widely. Thus, while the soil in general may have a desirable moisture content, the microcosm of the soil adjacent an individual seed may be too dry, or too wet, to support any germination, or optimum germination.

Similarly, the soil may be generally depleted of one or more plant nutrients needed by the germinated seedling. Or while the soil may in general have desired nutrient levels, the nutrient levels at a macro level can vary widely. Thus, the microcosm of the soil adjacent an individual seed may be too low in one or more nutrients to support a desired level of plant growth, or so high as to be toxic to a desired level of plant growth.

Further, plant nutrient chemicals may be present in the soil, but so tied up chemically in the soil as to be unavailable, or poorly available, relative to the quantities and use rates needed for desired plant growth. Or the soil may become so hard, dry, and/or caked shortly after the seed germinates that the seedling

plant has difficulty penetrating such soil, difficulty becoming associated suitable nutrients, and/or difficulty taking up such nutrients because of insufficient moisture availability.

After the plant has further developed such that the plant roots extend deeper into the soil, conditions of the soil near the surface are less critical. However, until such time as the roots so penetrate, conditions of the soil at and near the top surface of the soil may be critical.

Soil fertility generally relates to uptake of plant nutrients from the soil by plants. Uptake is generally the result of two factors, the presence of plant nutrients in the soil, and the availability of the plant nutrients for plant uptake. Presence of plant nutrients in the soil is generally a function of the combination of (a) the basic level of soil fertility, (b) depletion by previous crop production and (c) replenishment with fertilizer. Availability of a plant nutrient physically present in the soil for plant uptake is in general related to solubility of the respective nutrient or nutrient combination in a solvent for the nutrient, which solvent is present in the soil, such solvent as water, along with any other material affecting solvation of the plant nutrient into the water or other solvent.

Plant nutrients are routinely depleted from the soil by crop production, and are routinely added back, or otherwise replenished, to the soil by conventional inorganic fertilizers.

In order for plant nutrients in the soil to be available for uptake by plants, the nutrients must be held in the soil without excessive leaching, but must not be held so tightly that the nutrients cannot be released for plant uptake. Thus, nutrient availability requires a balance between holding tightly enough to retain the nutrient in the root zone, without leaching, but not so tight as to make the nutrient unavailable for plant uptake. Thus, the general "condition" or "tilth" of the soil is instrumental in

determining the efficiency with which plant nutrients are utilized for plant nutrition.

A properly conditioned soil has advantageous soil chemistry in combination with advantageous soil texture. Thus, in addition to providing specific plant nutrients, soil users also use products that modify basic soil chemistry, and soil texture.

Basic soil chemistry is modified by adding to the soil, for example, calcium products to provide pH control, and flyash or like products to provide pH control as well as micronutrients.

Soil texture is generally modified by adding to the soil organic matter such as manures, sludges, wood and other plant products and by-products, and the like. While such materials have good soil conditioning properties, plant nutrient value of such materials is fixed and is generally so low that other "fertilizer"-type products must in general be used in addition to the organic matter in order to preserve plant nutrient values in the soil.

The primary object of this invention is to provide solid plant seed capsule products that supply both soil conditioning properties and the seed, which can benefit from such conditioned soil, in a given seed capsule particle.

It is a further object to provide a plant nutrient material, in the seed capsule particle, in amount beneficial to the seedling emerging from the seed, and higher than a naturally-occurring amount of such nutrient in such soil conditioning material, so as to have enhanced chemical nutrient qualities over use of the soil conditioning material alone.

In another aspect, a further object is to provide soil conditioning and optionally nutrient qualities to seed products that reach the soil as the result of fulfilling objectives separate from providing soil fertility or soil conditioning.

Still another object is to provide seed capsules containing fertility-enhancing elements having a high level of plant food

nutrients in combination with a high level of soil conditioning properties.

Still another object is to encapsulate a seed in a soil conditioning material using materials rich in plant nutrients as part of the encapsulating agent.

Yet another object is to provide a seed product which reduces the tendency for light weight seeds to be washed away by surface water runoff.

Still another object is to provide a seed product which obviates the typical practice of adding straw as a mulch over e.g. grass seed, to protect the seed from being washed away by surface water, from heat of the sun, and to hold moisture in the soil.

A further object is to provide products wherein a single seed capsule product particle provides enhanced soil texture and enhanced soil nutrient value at nutrient levels traditionally needed by newly-germinated seedlings, optionally with higher levels of plant nutrient suitably spaced from the seed itself so as to not be toxic to seedling growth, optionally in combination with time-release technology.

Yet another object is to provide fertility-enhancing seed capsule products having a suitable level of plant food nutrients in combination with a high level of organic matter as soil conditioning material.

Summary of the Invention

The invention generally addresses a combination seed capsule, comprising at least one viable seed, having an outer surface and acting as a core or pseudo-core of said combination seed capsule; and a coating of a composition comprising a soil conditioning material mounted proximate, including disposed outwardly of the outer surface of said seed.

In general, the coating provides at least one of (i) enhancing broadcast flight properties of the combination seed capsule; (ii) reducing susceptibility to deleterious affects of weather on the combination seed capsule; (ii) enhancing resistance of the combination seed capsule to attack by animals, weeds, or spore-formers; (iv) staged germination of ones of the seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in the seeds; (v) enhancing control of moisture about the seed thereby to assist in seed germination; (vi) release of plant nutrients into soil onto which the combination seed capsule is placed; (vii) soil conditioning effect to soil onto which the combination seed capsule is placed; (viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released; (ix) higher embryo emergence and survival rate in a population of the seed capsules, thereby reducing required seed planting density for a desired plant population density; and (x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

While a wide variety of seeds may be used, in general such seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

Preferably, the coating comprises the soil conditioning material in combination with at least one ingredient effective to

reduce susceptibility of the seed capsule to deleterious affect of at least one of animals, weeds, and spore-formers. In some embodiments, the ingredient for reducing such susceptibility of the seed capsule is selected from the group consisting of herbicides, fungicides, for example metalaxyl, and a bitter substance.

In some embodiments, the combination seed capsule further comprises a second coating, separate from the first coating, and comprising at least one ingredient effective to reduce susceptibility of the seed capsule to deleterious effect of at least one of animals, weeds, and spore-formers.

Some embodiments are effective to provide a plant nutrient at a desirable controlled distance from a plant seedling emerging from the seed, in an amount beneficial to the plant seedling.

In other embodiments, the second coating material is intermingled with the first coating material in an outer portion of the first coating, and generally displaced from the seed.

The second coating material can comprise a plant nutrient, beneficial in location and in amount of availability, to a plant seedling emerging from the seed. The second coating composition can comprise an inorganic form of a plant nutrient and can be selected from the group consisting of nitrogen, phosphorus, and potassium. The second coating composition can comprise urea or an inorganic form of a plant nutrient and can be selected from the group consisting of e.g. ~~urea~~, monammonium phosphate, diammonium phosphate, superphosphate, triple superphosphate, dicalcium phosphate, and potash or a ~~micro~~nutrient such as sulfur, manganese, copper, boron, iron, magnesium, or chromium.

A population of the seed capsules can comprise coatings having a range of properties affecting germination rate of the seeds, thereby to stage germination of the seeds in the population over a period of time longer than the range of germination times inherent in uncoated ones of the seeds. Such properties can be, for

example, a range of hardnesses, or a range of thicknesses, of the coatings.

The coating can comprise a first layer of the soil conditioning material, and a second layer comprising an inorganic, and/or organic, fertilizer, and/or at least one ~~micro~~nutrient, such as, for example, sulfur, manganese, copper, boron, iron, magnesium, or chromium.

A preferred soil conditioning material is a sludge composition, such as a fiber-containing by-product of a paper making operation, or sewage sludge.

The seed capsule can comprise a water-leachable plant nutrient, and/or a leach-retardant composition, such as wax, effective to retard leaching of the leachable plant nutrient out of the combination seed capsule.

In some embodiments, in a population of the combination seed capsules, the coatings in ones, but less than all, of the population, comprise ingredients effective to retard effective penetration of a seed-germinating environment to the seed for germination thereof.

In embodiments preferred for some applications, the seed capsule comprises an inner layer on the outer surface of the seed, and an outer layer, the inner layer enhancing properties of the seed for acting as nucleus in an agglomeration operation agglomerating the coating onto the inner layer.

In some embodiments, the coating comprises an admixture of the soil conditioner and a plant nutrient.

In preferred embodiments, the coating remains generally disposed about the seed, and preferably but not necessarily remains generally intact about the seed, until the seed germinates.

The invention further comprises a plant growing medium extending over an area, the plant growing medium having a root zone, and a top surface of the root zone generally corresponding with a top surface of the plant growing medium, the plant growing

medium having a first overall soil condition and texture; and a population of seed capsules disposed over the top surface of the plant growing medium, the seed capsules comprising individual seeds, having outer surfaces, and coatings of soil conditioning material disposed outwardly of the outer surfaces of the seeds, the coatings of the seed capsules providing localized germination and growth environments, at and adjacent the seeds, having texture, and nutrient and water holding properties for supporting seedling health, superior to respective properties as provided overall in the root zone of the plant growing medium.

The invention yet further comprises a method of providing plant micronutrients to soil, the method comprising placing onto the soil a population of combination seed capsules, each comprising at least one seed, and a coating comprising a plant ~~micro~~nutrient material.

The coating can comprise a first coating comprising the plant ~~micro~~nutrient, and a second coating, separate and distinct from the first coating, and comprising a soil conditioning material.

The invention yet further comprehends a method of providing a seed bed having enhanced growing conditions for growing seed, the method comprising coating a population of the seeds with a coating material, and thereby providing coatings thereon of such material, the material tending to stabilize, in the seed capsules, or in soil on which the seed capsules are disposed coating compositions which tend to hold, moisture adjacent the seeds in the seed capsules or in soil adjacent the seed capsules, in such quantities and for such times as to enhance growing conditions for the seeds; and placing the population of seeds on soil effective to support germination of the seeds which are in the seed capsules.

In some embodiments, the seed capsules comprise inner layers on the outer surfaces of the seeds, and outer layers, the inner layers enhancing properties of the seeds for acting as nuclei in

agglomeration operations agglomerating the coatings onto the inner layers.

The invention yet further comprehends a method of making a population of combination seed capsules, each comprising a seed, and a coating of a soil conditioning material, the method comprising pre-coating the seed with a material which enhances the ability of the seed to act as a nucleus in an agglomeration operation, to form a pre-coated substrate; and subsequently coating the pre-coated substrate with a soil conditioning material. A preferred pre-coating material comprises dicalcium phosphate.

In general, the pre-coating step typically results in an overall increase in the density of pre-coated seed combination. The pre-coating step can be accomplished by, for example, spraying the pre-coating material onto the seed, and subsequently driving off such as by drying, as necessary, any solvent or other liquid carrier used for application of the coating material to the seed.

In yet other expressions, the invention comprehends a method of providing an enhanced seed germination environment in combination with placement of a controlled amount of plant nutrients in controlled proximity to each seed, the method comprising providing a population of seeds, coated with a soil conditioning material which tends to enhance germination of the seeds, and with plant nutrient composition effective to enhance growth of plant embryos emerging from the seeds; and placing the population of seeds on soil effective to support germination of the seeds. In such method, the coating material can include a second ingredient comprising plant nutrient moieties.

Brief Description of the Drawings

FIGURE 1 is a transverse cross-sectional view of a coating drum suitable for spray-coating substrate seed according to the present invention.

FIGURE 2 is a partially cut away view showing a length of the drum of FIGURE 1.

FIGURE 3 is a schematic representative flow diagram illustrating a first manufacturing process for producing combination seed capsule product of the invention.

FIGURE 4 is a block diagram illustrating a second manufacturing process for producing combination seed capsule product of the invention.

FIGURE 5 is a schematic representative flow diagram illustrating a third manufacturing process for producing combination seed capsule product of the invention.

FIGURES 6A, 6B, 6C, and 6D show cross sections of seed capsules of the invention.

FIGURE 7 illustrates a cross-section of the soil root zone, and a representative population of seed capsules at the top surface of the soil.

FIGURE 8 illustrates a single seed capsule on the soil surface, and the micro-environment developing about the seed capsule.

DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

The following is a detailed description of the illustrated embodiments of the present invention which provides combination seed capsule products that provide for a combination of efficient and proper seed placement in the soil, soil conditioning properties at the specific site of the seed, plant nutrients at or near the specific site of the seed, ingredients effective to reduce deleterious effects of spore-formers and animals, and/or other various physical benefits/properties of the combination seed capsule not previously available in a single product.

In general, at least one seed substrate and at least one soil conditioning material are selected as raw materials, and are combined to make a combination soil conditioning seed capsule product of the invention.

The invention can operate with any of a wide variety of soil conditioning materials such as municipal or other sewage sludge, scrubber sludge, paper mill sludge, fly ash, dust, animal waste, other organic materials, and mineral soil conditioning materials.

The soil conditioning material can be a solid material having a melting temperature so high that handling such material in the melt state is impractical and/or undesirable in view of the limited temperatures at which the seed will remain viable. For example, the soil conditioning material may be combustible at a temperature lower than its melt temperature, or will melt only above temperatures which can be tolerated by the seed, such that viability of the seed would be destroyed if melting were attempted in an environment which exposed the seed to such temperatures. Thus, handling such material in the melt state is impractical, whereby other methods of handling the soil conditioning material may be desired.

Solid sewage sludge, sawdust, and solid animal waste are representative of soil conditioning materials which cannot be

readily melted. In the alternative, some soil conditioning materials such as sewage sludge, paper mill sludge, sawdust, and solid animal waste can be suitably comminuted and then dissolved or suspended in water or other solvent composition for processing purposes, optionally along with other soil conditioning materials and/or inorganic chemical fertilizer materials, and the solvent subsequently driven off to make a resulting solid product.

~~Inorganic~~ Chemical fertilizers generally are distributed in commerce as solid state materials. Such material is generally produced in manufacturing steps either in solution or in the melt state to meet a specified narrow range of size, hardness, and plant nutritional characteristics, distinct to the application of each such product. Examples of such fertilizers include nitrogen, phosphorus, and potassium containing products such as urea, monoammonium phosphate, diammonium phosphate, superphosphate, triple super phosphate, dicalcium phosphate, potash, and the like. The ~~inorganic~~ chemical fertilizer can be a mixture or other physical combination of known inorganic fertilizer chemicals, and may include desired amounts of ~~micro~~nutrients such sulfur, manganese, copper, boron, iron, zinc, and the like.

In preferred embodiments of this invention, a precursor seed capsule, having one or more coatings of the soil conditioning and/or other material thereon may first be prepared as a solid or semi-solid particle or agglomerate. The soil conditioning raw material may be a particulate powder, or may be fibrous, or may be a suspension of a powder or fibrous material in a liquid carrier, and is preferably coated onto the substrate seed to form a seed capsule or other agglomeration of particles, fibers, or the like. Where the soil conditioning material is, for example, sewage sludge, the sewage sludge raw material can be obtained as a slurry that may be bound together as with a binder, preferably an organic binder, when dried. The slurry may be spray-applied to the substrate seeds, for example to a rolling bed of such seeds, in

combination with a flow of air to evaporate water from the thus-applied coating. Such sewage sludge, or paper mail sludge, need not be reacted or otherwise treated with any acid, caustic, or any other chemical before being applied and/or dried, or partially dried, either in preparation for, or after, the slurry application of the sludge to the seed substrate.

Specifically, the sewage sludge or paper mill sludge used herein as soil conditioning raw material need not be treated to transform such sludge into colloidal form. Thus, the sludge preferred for use herein is generally non-colloidal in nature, and is distinguished by its non-colloidal nature from conventional sludges which are specifically treated to provide the colloidal characteristics thereto.

Natural lignin, lignosulfonates, and the like, may serve as suitable binders where the soil conditioning material is, for example, paper mill sludge, raw wood, sewage sludge, or other organic or inorganic material. In the case of, for example, calcium chloride or other inorganic additives, such materials may be added to the primary coating, e.g. onto or into the sludge coating, by well-known processes.

Soil conditioning material used herein may be devoid of such conventional plant nutrients as nitrogen, potassium, and phosphorous, or may have such limited plant nutrient value, or may be so unbalanced in nitrogen, phosphorous, and potassium content, that the soil conditioning material may not, by itself, be a desirably complete material for use as the only ingredient in the seed coating. Thus, such soil conditioning material may have limited application herein where basic level of soil fertility is seriously degraded. However, all soil conditioning materials contemplated herein beneficially modify soil to which they are applied, in some way other than direct provision of nitrogen, phosphorous, and/or potassium or other plant nutrients. By use of soil conditioner in intimate association with the seed, this

invention not only enhances soil condition of the growth medium/soil to which it is applied, it also provides soil conditioning value to the seed so coated, and in intimate association with the seed, irrespective of the general tilth condition of the growth medium into or onto which the seed capsule is applied.

Further to preferred embodiments, typically a first coating material (e.g. soil conditioning material) is readily converted into liquid state such as liquid suspension, and is provided to the process as a liquid. As a general statement, the first coating material may be sprayed onto the substrate seed, then is converted back to solid state on the thus-created seed capsules or seed capsule precursors. In the alternative, the coating material may be mixed with the seed in an (e.g. ribbon) blender, or may be otherwise coated onto the substrate seed in an agglomeration process according to well-known conventional agglomeration principles.

Regarding the coating process, the coating material can accumulate as a single or multiple layer coating on the outside of the seeds to form a population of combination seed capsules. The layer or layers of coating material can be a homogeneous or heterogeneous mixture of the desired elements. Further, such population of combination seed capsules can have a range of hardnesses and thicknesses for improved seeding treatments.

Cooperating inner and/or outer layers may be used e.g. to control direct contact between the seed and moisture. Suitable materials and processes therefore are taught in USA Patent 3,698,133 Schreiber and 4,759,151 Gerber, and are thus well known in the art.

In some embodiments, a second coating material may penetrate into the layer of soil conditioning coating material. Such penetration may comprise a generally uniform distribution of the second coating material throughout the first coating material, or

may represent a more stratified or otherwise heterogeneous distribution of second coating material in or on the first coating material.

In other embodiments, the coating materials may be mixed into a heterogenous layer. Such layer or layers of heterogenous material can then be coated upon the outside surface of the seed.

Where the liquid state of a coating material was obtained by slurrying or otherwise combining the coating material with water, the liquid fraction is reduced after application of the liquid-state material to the substrate seed, or to the growing seed capsule, to effect solidifying of the coating material after application of the coating material to the substrate seed. The liquid fraction is reduced by driving off the liquid carrier, as by medium or low temperature air, or vacuum or other flash drying, after or during application of the coating material to the substrate seed. The resulting solid seed capsule, comprising the seed coated with the e.g. sludge coating material, is then recovered as a combined soil conditioning seed capsule product of the invention.

Spraying of the liquid coating material can be accomplished by a variety of known processes such as, but not limited to, pneumatic, hydraulic, or electrostatic spraying processes. The temperature and pressure of the material being sprayed depends on the material selected, and the viscosity and other parameters of the respective material in the respective liquid state. While high atomization is desired, such is not critical. The liquid coating material need only be atomized sufficiently to provide a generally uniform coating on the substrate seeds, as determined after the coating and solidification steps in fabricating the seed capsule product are completed.

Indeed, the uniformity of coating or coating thickness about the seed is typically not critical so long as the seed is not on or immediately adjacent an outside surface of the capsule such that

the seed may fall out, or be easily broken out, of the capsule, or easily removed by dissolution of materials at and near the surface of the seed capsule. In addition, the seed should not be so near the outside surface of the capsule as to be in a nutrient layer having such high concentration of nutrient as to be toxic or otherwise detrimental to viability or growth of a plant emergent from the seed.

Spray application of the coating is suitably controlled to achieve the required addition of the spray material, liquid and/or powder, coating to the substrate seed or precursor seed capsule. An illustrated method of applying the liquid material to the substrate seed or precursor seed capsule is by using a rotating drum spray-coating apparatus. Other apparatus and methods, for example a tilted pan coating process, can be used to apply the soil conditioning material and optionally an ~~inorganic~~ chemical fertilizer material onto the substrate seed. The coating operations can be batch operations or continuous operations.

As illustrated in FIGURES 1, 2, and 4, spray apparatus can operate within a rotating drum disposed in a generally horizontal orientation. The drum may incorporate internal lifting flights which lift free-flowing (e.g. seed and growing seed capsule) particles in the drum and then let the particles fall to the bottom of the drum as a continuously falling curtain or cascade. In some embodiments, the interior of the drum is either clean and free from any flighting, or has only mixing fingers or flights that expand the area covered by the bed, that keep the bed rolling as the drum rotates, and that generally improve mixing, rather than lifting particles to the top of the drum and then releasing them in a falling cascade. However, such lifting of particles to the top of the drum, and corresponding falling cascade or falling curtain, are not excluded from processes of the invention. Rather, both such finger mixing, and such lifting coupled with falling cascade or curtain, are included within the scope of the invention.

Stationary spray nozzles are positioned within the drum to project the sprayed material onto the rolling bed, and optionally onto any curtain or cascade of falling particles. For a continuous process, the drum is preferably inclined at a small angle from horizontal, such as, without limitation, about 0.25 inch to about 0.38 inch from the horizontal for each foot of length of the drum, so that rotation of the drum causes the particles to move from the inlet end of the drum to the discharge end, while maintaining a relatively uniform bed thickness. The optimum degree of incline varies with each set-up and may thus be outside the above range. The important parameter is that the incline contribute to maintaining a bed of seed and seed capsule particles having sufficient uniformity that the spray material can be effectively applied to the particles passing through the drum. The particles are then discharged at the discharge end of the drum.

FIGURES 1 and 2 show schematically a first embodiment of processing equipment which may be used to produce seed capsules of the invention. Such processing equipment includes a drum and sprayer combination suitable for continuously producing coated seed capsules in accord with the invention. Use of the illustrated drum and sprayer combination is not critical, however, as other drum and sprayer combinations, or other coating methods such as pan coating methods, are also suitable. Referring to FIGURES 1 and 2, drum **10** has an inlet end **12** for receiving the substrate seed material or materials, or partially formed or pre-coated seed capsule precursors. Drum **10** has a discharge end **14** through which agglomerated or otherwise coated seed capsule product particles are discharged over discharge retaining ring **16**. A variable speed rotary drive (not shown) is provided for supporting and rotating the drum **10** in a counterclockwise direction as viewed in FIGURE 1 at controlled, and changeable drive speeds. Conventional slope adjustment apparatus (not shown) is provided for routine and ongoing adjustment the slope of the drum from horizontal.

Air is preferably supplied from discharge end **14** as shown in FIGURE 2, and flows countercurrent to the direction of travel of the seed substrate material. Since the contemplated coating materials are generally applied to the seed in liquid, or semi-liquid, or other moist form, and since some coating materials may thus tend to form clumps or otherwise self-agglomerate when exposed to ambient moisture conditions, air supplied at discharge end **14**, and elsewhere in the process for contact with the coated seed and seed capsules, is preferably dried in order to cost-effectively remove an optimum amount of the moisture from the coating material and to assist in maintaining suitably low moisture content in the thus coated and dried seed capsules.

A first stationary spray assembly **28** extends longitudinally within drum **10** above and adjacent the bed **20** of seed and/or seed capsules. First spray assembly **28** includes pipe **29** and nozzles **30**. A second spray assembly **32** extends longitudinally within drum **10** generally adjacent first spray assembly **28**. Second stationary spray assembly **32** includes pipe **33** and nozzles **34**, which transport the material to be sprayed. Nozzles **30** and **34** are connected to pipes **29** and **33** respectively, and project sprays of liquid or otherwise particulate coating material toward the bed of seeds and/or seed capsule precursors. The description of spray assemblies **28**, **32** as stationary means that the spray assemblies do not rotate with drum **10**. However, the positions of either nozzles **30**, **34** or pipes **29**, **33**, or both, can be adjusted within the drum for proper direction of the respective spray or sprays onto the bed of seeds and/or seed capsules or seed capsule precursors.

A stationary protective cover **24** is mounted over the spray assemblies. Seeds and/or seed capsules falling from the inner surface of the drum and the flights, above the spray assemblies, fall onto the cover, and are deflected away from the spray assemblies, as shown in FIGURE 1. Thus, cover **24** protects the

pipes and nozzles from the falling seeds and seed capsules falling onto and fouling the pipes and spray nozzles.

As drum **10** rotates, flights **22** lift and mix the seeds, seed capsule precursors, and seed capsules, but do not generally carry the bulk of the seeds and seed capsules up to the top of the drum. Some small amount of seeds, seed capsule precursors, and seed capsules will be carried upwardly to the top of the drum by even a drum devoid of any flights. Thus, all drums experience some amount of seeds and seed capsules falling from the upper part of the rotating drum whereby cover **24** is beneficial for protecting spray assemblies **28** and **32**.

Preferred flights **22** are primarily directed toward enhancing mixing of the bed **20** of seeds and seed capsules, continually refreshing the surface of the bed with a newly-emergent supply of seeds and seed capsules, rather than lifting and subsequently dropping the seeds and seed capsules which may be fragile when initially coated. To that end, each flight **22** preferably, but without limitation, has a leading surface **23A** extending at an obtuse angle "A1" of at least 90 degrees with respect to the inner surface of the drum. A more preferred angle "A1" is about 100 degrees to about 150 degrees. Trailing surface **23B** of flight **22** can be virtually any angle, with the inner surface of the drum, which angle does not interfere with the operation of adjacent leading surfaces **23A**.

Additional retaining rings can be added to the assemblage shown in the drawings, in order to provide that height "H" to the retaining ring which will provide and maintain the optimum configuration of bed **20** inside drum **10**.

As noted above, inlet end **12** of the drum may be raised above discharge end **14**. When in use, the drum rotates continuously. Seeds or previously thinly-coated or partially-coated seed capsules are continuously fed into inlet end **12** and thus added to rolling bed **20**. Flights **22** continuously mix the bed as the drum rotates,

refreshing the bed surface with newly fed seeds, or seeds and seed capsules newly brought to the surface by the continuous rotation of the drum in combination with the mixing action of the flights. Spray assembly **28** sprays the desired coating material (e.g. sewage sludge, paper mill sludge, or other coating composition, onto the continuously moving and mixing surface of bed **20** from a plurality of nozzles **30** distributed along the length of pipe **29**, and similarly along the length of drum **10**, adding the sprayed material to the seeds and seed capsules in bed **20**. After receiving the spray coating from spray assembly **28**, the seed capsules are discharged through discharge end **14**. In some embodiments, the seed capsules pass through a cooling chamber, not shown, integral in drum **10**, before being discharged through discharge end **14**.

In general, as the seeds traverse the drum, from inlet to discharge, nozzles **30** atomize the liquid or other coating material and spray such atomized coating material as e.g. droplets of the coating material onto the seeds in the bed. The result is that the seeds become generally uniformly coated with one or more layers of the coating material such that the coating material becomes an integral part of the respective seed capsules fabricated in the drum. As the coating material solidifies on the seeds, the coating material tightly bonds to the respective portions of the seeds.

As the seeds and seed capsules roll and mix with rotation of the drum, the incline of the drum causes the seeds and seed capsules to travel from inlet end **12** toward discharge end **14**.

In the alternative, or where a coating material is not readily self-bonding to the seed material, a binder material can be provided toward the inlet end of the drum at spray assembly **32**, through pipe **33** and nozzles **34**. In such embodiment, the binder is preferably sprayed onto the seeds closer to inlet end **12** rather than along the entire length "L" of the drum. The coating material is then preferably sprayed onto the seeds downstream from the inlet end, and preferably relatively downstream of nozzles **34**. Thus, the

seeds receive a first coating of the binder, and a subsequent second coating of e.g. liquid soil conditioning coating material overlying the binder.

Binder material applied as e.g. through spray assembly **32** may contain additional coating components such as e.g. flyash, lime, gypsum, or the like, as one or more components for assisting in adding bulk and thickness to an inner binder layer prior to any, or the majority of, the application of the organic coating material (e.g. sewage sludge or paper mill sludge).

In some embodiments, binder and liquid soil conditioning coating material are applied at similar locations along length "L" of the drum whereby binder and soil conditioning coating material may become intermingled/mixed before reaching the seeds, or on the seeds. For example, liquid soil conditioning coating material may be sprayed onto the seeds along the full length of the coating chamber in drum **10** while spraying of the binder material onto the substrate seeds is done relatively closer to or adjacent the inlet end of the coating chamber of the drum. Thus, a first binder layer may underlie or be mixed with the soil conditioning coating material, and may be overlain by a second layer of the soil conditioning coating material. Thus, in this embodiment, the binder layer may typically be a combination of binder material and coating material.

Further, it is contemplated that the soil conditioning coating may be applied first, followed by application of binder or inorganic fertilizer or sealer coating, in which case the binder or inorganic fertilizer or sealer may serve as an outer shell, temporarily trapping the inwardly-disposed materials inside the seed capsule. In the alternative, the soil conditioning coating may be applied first, followed by application of the binder, and wherein the binder penetrates through the soil conditioning coating, either physically or chemically, to the underlying substrate seed and there provides the binding property.

Additional spray assemblies can be provided, spraying additional materials (e.g. inorganic fertilizer materials) onto the substrate seed. Thus, e.g. 6 spray assemblies can spray 6 different coating materials onto the substrate seed. For example, a first spray material can be a binder or primer material intended primarily to enhance bonding of subsequent sprays to the substrate seed. Continuing the example, a second spray can be a combination of binder and finely comminuted particulate material such as lime and/or flyash. A third spray may be a soil conditioning material such as a paper mill sludge or a municipal sewage sludge. Fourth, fifth, and/or sixth sprays can add nitrogen, phosphorous, and/or potassium plant nutrient ingredients, alone or in combination, or as combinations. In this manner, the soil conditioning properties of the seed capsule can be established, and the plant nutrient level of the seed capsule can be enhanced to provide substantially any level of major and/or minor plant nutrients desired in the seed capsule, at substantially any relative ratios of the respective plant nutrients, and wherein the preferably primarily soil conditioning coating provides desired soil conditioning properties in the resulting product, initially for use by the specific seed contained therein, and ultimately as additive to the overall tilth of the growth medium such as soil into or onto which the seed capsule is eventually planted.

A preferred, and rather simplistic, embodiment of the invention is provided by spraying a soil conditioning liquid suspension of sewage sludge or paper mill sludge onto seeds to be encapsulated to make seed capsules. By controlling the amount of the soil conditioning sludge, or by controlling the residence time of the seeds in the drum, a desired thickness of soil conditioning coating can be provided in the resulting coated product.

Typical dried sewage sludge, as a raw material, contains about 2-6% nitrogen, up to about 2% phosphorous, and generally no potassium, and thus has little or no market value as a fertilizer

(plant food) product per se. However, by adding e.g. urea, the nitrogen content can be raised if desired, especially as a coating on or adjacent the outside surface of the seed capsule, whereby the combination fertility-enhanced, soil conditioning, seed capsule product has real market value as a comprehensive, self-contained, value-added, seed capsule product. Such product thus contains the seed, a soil conditioning composition which operates somewhat as a seed incubator providing a beneficial germination environment, and a starter quantity of fertilizer selected in quantity and placed in location so as to provide improved, ideally optimum, amounts of plant nutrients at optimum location for use by the newly-emerged embryonic plant at the germination stage of seed development.

Starting with a sludge coating having 2% by weight nitrogen, sufficient urea may be added to bring the nitrogen content to, for example, 5%, 7%, 8% or 10% nitrogen, or more, depending what analysis is desired. Starting with a sludge coating having 6% nitrogen, sufficient urea may be added to bring nitrogen content to, for example, 10%, or whatever other analysis is desired. Phosphorous and/or potassium components and/or materials having combinations of plant nutrient elements (e.g. NPK) can, similarly, be added to the sludge, either before, after, or during addition of the urea. In addition, nitrogen, potassium, and/or phosphorous-containing materials can be combined with the sludge prior to the sludge being applied to the seed.

It should be understood that the more porous the established soil conditioning coating, or e.g. the outer surface of such coating, the more any subsequent spray material penetrates the established coating. All such penetration is contemplated in use of the term "coating" herein.

In some preferred embodiments, the overall coated combination seed capsule product comprises seed capsules wherein substantially the entirety of the soil conditioning material is confined to a contiguously-defined portion of the seed capsule. In such

embodiments, the structures of the finished product seed capsules comprise coatings of contiguously arranged elements of the soil conditioning material, generally arrayed entirely or substantially entirely about the seed, which coatings may be overlain by an additional layer, optionally discontinuous, of organic or inorganic chemical fertilizer. Further coating layers of either soil conditioning material or organic or inorganic chemical fertilizer can be applied over the additional layer.

In addition, or in the alternative, other layers of other materials whether soil conditioning materials, organic or inorganic fertilizers, or other materials, can be applied to the substrate seed before applying the above mentioned layer of soil conditioning sludge. Thus, the substrate seed can be coated with a layer of a calcium compound e.g. calcium chloride, calcium carbonate, or dicalcium phosphate, or with a sulfur moiety, and/or a further layer of urea, all with optional use of binder materials.

Further to the structure of the seed capsules of the invention, the coatings on the seed capsules need not generally represent a uniform mixture of the inorganic chemical fertilizer and the soil conditioner. Rather, in a typical seed capsule a core substrate seed is overlain or encapsulated by a soil conditioning material, and is generally free from a second overlying soil conditioning coating material, and wherein the inorganic fertilizer content at the seed/coating interface is relatively higher so as to represent a second coating material such as an inorganic fertilizer coating, as compared to the inorganic fertilizer content at locations at and adjacent the seed.

The second coating can, and preferably does, in some embodiments, penetrate into voids or other interstices in an underlying e.g. soil conditioning coating. However, preferably most if not all elements of the underlying e.g. soil conditioning coating material are generally interconnected with each other without intervening coating material of the second layer, except

for an optional binder used to hold the first coating material together as a unitary structure, separate from any structure and bonding provided by the second coating material.

While the combination seed capsule can comprise discontinuities in the soil conditioning sludge coating layer, in combination with an inorganic fertilizer material in such seed capsules, such compositions are less preferred.

Regarding the coating process, FIGURE 4 illustrates in flow sheet form a manufacturing process for producing seed capsules of the invention, using the coating drum **10** as described above. It should be understood, however, that other equipment such as a pan pelletizer, a paddle mixer, or the like can be used in place of the rotary drum to obtain combination seed capsules of the invention.

The coating process operates according to conventional and generally well known agglomeration principles, as described by Wolfgang B. Pietsch in an article entitled "The Agglomerative Behavior Of Fine Particles." Such coating process uses water and heat, along with physical and/or chemical adhesives and like properties, to bind or agglomerate a plurality of types of particles and/or materials into coated seed capsules, each typically containing an individual seed.

To obtain agglomerates from relatively smaller particles of raw materials, binding forces must act within the individual developing agglomerate particles. According to known agglomeration principles, five different binding mechanisms are known to be useful for building agglomerate particles including solid bridges, interfacial attractions and capillary pressure, adhesion and cohesion, attraction between solid particles, and form-closed bonds.

At elevated temperatures, solid bridges can form by diffusion of molecules from one particle to another at the points of contact. Heat can be introduced from an external, secondary source or created during agglomeration by friction and/or energy conversion.

Solid bridges can also be built up by chemical reaction, crystallization of dissolved substances, hardening binders, and solidification of melted components.

Capillary pressure and interfacial attraction forces in liquid bridges can create strong bonds that disappear if the liquid evaporates and no other binding mechanisms take over.

Highly viscous bonding media such as tar and other high molecular weight organic liquids can form adhesive and/or cohesive bonds very similar to those of solid bridges. Thin adsorption layers are immobile and can contribute to such bonding together of fine particles under certain circumstances.

Typical short-range forces of the van der Waals electrostatic or magnetic type can cause attraction between solid particles whereby the particles stick together if such particles are sufficiently close to each other. Decreasing particle size clearly favors such attraction between solid particles.

Fibers, little platelets or bulky particles can interlock or fold about each other resulting in "form-closed" bonds.

Now referring to FIGURE 3, in some embodiments of the coating/agglomeration process, it is desirable to pre-coat the seeds prior to implementing agglomeration principles to produce the above described coating of soil conditioning material. Such embodiments comprise light-weight and/or elongate shaped seeds (i.e. grass seeds), or other similar type of seed which may not readily or inherently serve as a nucleating agent in a conventional agglomeration process with the respective soil conditioning material which is desired to be coated on the seed. Pre-coating the grass seed, for example, enhances the agglomeration of paper sludge as a coating material, of binder and/or of other coating substances, by increasing the weight of the pre-coated grass seed and by providing a more filled in, more rounded shape to such long and narrow seeds. The increased weight and more filled in shape of the grass seed enables more effective, more efficient, processing

of the seed in coating apparatus such as that illustrated in FIGURES 3 and 4.

Referring to FIGURE 3, the form and composition of such pre-coating, when needed, can vary according to the weight, shape, composition, and surface properties of the seeds, and according to the binder, if any, the soil conditioning coating or coating materials to be applied, and any other inorganic or organic coating material to be applied.

The seeds, whether pre-coated or not, are received within the rotary drum where the soil conditioning material is spray coated onto the substrate seeds to obtain combination seed capsules.

Before coating the seeds with a soil conditioner, the organic soil conditioner material (e.g. paper sludge) is preferably processed through a dryer such as a rotary drum dryer, as needed, to reduce the amount of moisture in the organic soil conditioner material to less than about 8% water by weight. Such drying is an essential step where the material is otherwise above the nominal 8% effective water content, to enable grinding the sludge to a size less than US Standard 20 mesh screen, and to prevent the particles from agglomerating with each other. Certain of the coating materials, e.g. fly ash, because of their physical properties, need not be dried before being ground to a suitable size for participating in the agglomeration operation.

The seeds, whether pre-coated or not pre-coated, and the one or more soil conditioners, are received within a mixer where growth enhancers such as time release agents and/or other environmental conditioners may be added to form a combination seed capsule. The thus pre-coated seeds are then received into a pan pelletizer, a rotary drum, or the like, where binders such as lignin, lignosulphonates, molasses, sodium silicate, wax, monammonium phosphate, or urea can be added and thereby coated onto the pre-coated seeds. Other materials which can be added to the seed capsule at the e.g. rotary drum include anti-fungal coatings such

as with metalaxyl fungicide, for example, Apron® and/or Subdue®, available from Novartis, Inc. of Greensboro, North Carolina.

The such-coated seeds are then passed into a rotary or other dryer in order to obtain a seed capsule containing 5% or less water. The maximum water fraction in the coating can vary according to the composition of the coating material, so long as the resultant seed capsules remain suitably structurally strong and so long as a population of such coated seed capsules remains free flowing in solid condition. The process for fabricating the seed capsules must maintain a temperature sufficiently low that the seeds are not heated so hot that viability of the seeds, for germination purposes, is not dramatically compromised. It is generally preferred that the temperature of the seeds be suitably controlled such that any binder and/or coating material, or other materials applied to the seeds, cool at a controlled rate while bonds form between the seeds, or seed capsule precursors and the one or more soil conditioning and/or other coating materials. Such temperatures of all materials are suitably controlled to avoid decomposition of the respective materials, loss of viability of the seeds, or breakage of seed capsules or seed capsule precursors, or coatings or coating or other materials during such processing. The temperature at the rolling seed bed inside drum **10** generally can range from about 130 degrees F to up to at least 230 degrees F for seed residence times up to at least 1 hour. At drum operating temperatures of less than 130 degrees F, drying time can become excessive. At temperatures above 230 F, the viability of the seed may be at risk, depending on the sensitivity of the seed, residence time, and other influential parameters.

The above stated temperature range is illustrative and not limiting, and will vary depending on the seed, the coating materials, and the specific process parameters of a particular coating system and coating operation. Thus, maximum e.g. drum coating temperatures can be less than 130 degrees F or more than

230 degrees F. However, the stated range is preferred, including all temperatures within such range such as, for example, 150 degrees F, 180 degrees F, 210 degrees F, and the like.

Referring to the drum of FIGURES 1 and 2, and to the pan pelletizer block in FIGURE 3, the seeds are fed continuously to an inlet as at inlet end **12** of drum **10**. Combination seed capsules, produced as described above, are released from a discharge locus such as discharge end **14** of the drum to a sizing apparatus **36** in which the seed capsules are sized through conventional sizing elements. Suitably-sized seed capsules are discharged from the sizing apparatus as product for distribution. Undersize seed capsules are fed back into mixer as shown in FIGURE 3. Oversized seed capsules are fractured and screened for reprocessing.

The recovered seed product can be further coated with any of the coating materials described above, such as urea or other inorganic or organic fertilizer, and/or with growth enhancers or other desirable materials. Further, other types of coating materials such as water repellants can be coated onto the discharged seed capsules for the purpose of importing additional desirable properties to the seed capsules.

In the process of coating porous organic materials such as sewage sludge or paper mill sludge as is optional in the invention, with a second material which is applied for other than imparting soil conditioning properties, for example an inorganic fertilizer, the general size of the coated seed capsule may be the same after applying the second material (e.g. inorganic fertilizer) as the size of the previously-coated seed capsule, or may be similar in size. Namely, the quantity of coating material added to the seed capsule can be so small as to not materially affect seed capsule size, or the coating material can be received into an e.g. porous interior of the soil conditioning coating of the seed capsule, or both.

It is contemplated that the operation and functions of the invention have become fully apparent from the foregoing description of elements, but for completeness of disclosure, the usage of the invention will be briefly described.

EXAMPLE 1

A coating drum as illustrated in FIGURES 1, 2 and 4 is used to place a coating of paper mill sludge on grass seed. Raw material grass seed about 4-6 millimeters long and about 0.5-1.0 millimeter thick, is continuously fed to pre-treater **11**, where the seed is blended with powdered lime, powdered flyash, and a lignosulfonate binder, to form partially-developed seed capsules comprising seeds coated with relatively thinner coatings of the recited mixture of coating materials. The partially-developed seed capsules are continuously fed to inlet end **12** of drum **10**, to form a bed **20** of the partially-developed seed capsules. The drum rotates continuously. The rolling of the drum, and the associated mixing affect of the flights, provide a constantly changing top surface of the bed. A paper mill sludge slurry is supplied in pipe **28** at pressure sufficient to atomize the liquid sludge slurry. A liquid sludge slurry is thus sprayed from nozzles **30** onto the top surface of the bed of partially-developed seed capsules, applying a sludge coating on those partially-developed seed capsules which are at the upper surface of the bed at any given point in time.

The resulting seed capsules, of paper mill sludge coated seeds, have a coating of soil conditioning sludge thick enough to make the material a product marketable for its soil conditioning content as well as for the seeds contained therein. Increased levels of nitrogen and/or other plant nutrients can be added by, without limitation, providing sprays of the other desired materials, preferably subsequent to at least the initial sludge slurry spray. Other materials can be included in one or more of

the sprays e.g. to retard or enhance moisture permeation into or out of the combination product in accord with the anticipated storage and/or use environment of the product.

EXAMPLE 2

FIGURE 5 illustrates the equipment used in this EXAMPLE 2. As seen therein, grass seed, lime, flyash, and calcium lignosulfonate binder are fed to ribbon blender **111** by respective screw feeders **112A**, **112B**, **112C**, **112D** respectively. Ribbon blender **111** encapsulates the seed with a thin layer of the mixture of lime, flyash, and lignosulfonate to thereby make partially-formed seed capsules. The partially-formed seed capsules are discharged from the ribbon blender and conveyed by conveyor **114** and belt feeder **116** to a tilted-pan pelletizer **118**, which rotates about a fixed axis.

Paper mill sludge is received into a weigh hopper **120** at about 60% by weight water, and is fed by screw feeder **122** and belt **124** to pin mixer **126**. The pin mixer breaks down the fiber and fiber clusters of the sludge into loose separate fibers, and discharges the resultant material onto conveyor **128** which transports the material to screw feeder **130**, and thence into the tilted pan pelletizer.

In the tilted pan pelletizer, the partially-formed seed capsules, (seeds being coated with lime, flyash, and lignosulfonate) are mixed with the comminuted paper mill sludge and thereby coated with the sludge. By operation of the tilted rotating pan pelletizer, the larger seed capsules generally rise to the top of the bed of seed capsules in the pan, and as additional material (sludge and partially-formed seed capsules) are added to the pan, the larger seed capsules overflow the lower edge of the rotating pan, onto vibrating feeder conveyor **132**.

The vibrating feeder conveyor feeds the seed capsules into granulator **134** (e.g. rotating drum) where the seed capsules may be

(e.g. spray) coated with inorganic fertilizer or other desired material.

From the granulator, the seed capsules flow into dryer **136** and are dried to a final product moisture of about 2-3% by weight water. The resultant product is then screened and sized as before, with undersized and oversized product seed capsules being recycled for further processing.

Urea and other liquid ~~inorganic~~ chemical fertilizers can, as indicated, be used as binders to bind together soil conditioning coatings which are not readily self-bonded together. In such embodiments, the urea or other liquid fertilizer composition serves as the binder or glue which holds together the soil conditioning material which is used as the coating. Other binding materials may be used either alone or in combination with the ~~inorganic~~ chemical fertilizer. Any plant nutrient components of the binder/glue composition contribute to the plant nutrient value, e.g. nitrogen, phosphorous, and/or potassium, provided by the so-made seed capsules. Thus, a binder/glue, or a multiplicity of binders/glues, properly selected as to nutrient value can provide, in the finished product, significant contribution to any desired fertility analysis.

A primary purpose of soil conditioning products is to condition the soil in terms of properties other than direct provision of plant nutrients.

The primary purpose of conventional inorganic chemical fertilizer products is to directly provide plant nutrients. It is well known that highly purified forms of inorganic chemical materials are more concentrated than desired in close or intimate proximity with seed, in the growing medium. Thus, inorganic chemical fertilizers can be diluted in concentration and still have sufficient nutrient content to be highly useful additives in soil conditioning seed capsules of the invention. It is common practice

to modify and thus dilute inorganic chemical fertilizer products with filler materials that do not provide plant nutrients, in order to provide less concentrated fertilizer products. To the inventor's knowledge, such diluents, however, do not include soil conditioning products, especially not organic soil conditioning products.

It is conventionally known to apply commercially available soil conditioning materials and inorganic fertilizers, in separate applications, to a given common plot of soil to assist the soil in growing a crop. For example, it is known to make a first broadcast or other placement of lime to control pH of the soil, followed by a second broadcast and/or row-applied placement of granular inorganic chemical fertilizer. It is also known to make sequential applications of a soil conditioning material such as fresh or aged manure followed by inorganic fertilizer, all of which may be separate from the step of applying seed. And where seed is indeed applied in the same step, the seed and soil conditioner are not intimately bound in controlled positioning with respect to each other in common in individual particles of the product so applied, as in the invention.

To the inventor's knowledge, it is not known to apply soil conditioning material and inorganic chemical fertilizer in a common carrier/particle. Nor is it known to apply seed in a seed capsule wherein the seed is intimately combined with a soil conditioning material in a common particle, optionally with an inorganic fertilizer component in controlled positioning with respect to the seed in the same capsule as a seed-soil conditioning particle.

In those embodiments of the invention comprehending both soil conditioning and inorganic fertilizer in the same seed capsule/particle, the ratio of soil conditioning material to inorganic chemical fertilizer material can vary, from, for example, about 80% by weight up to less than 100% by weight soil conditioning material, with corresponding greater than 0% up to

about 20% by weight inorganic chemical fertilizer. Generally, the invention as practically applied, however, is somewhat more narrowly defined, because the practical benefits of the invention are achieved at more balanced combinations of the soil conditioning material and the inorganic chemical fertilizer.

Thus, a preferred amount of soil conditioning material is about 90% by weight to about 98% by weight soil conditioning material, in combination with about 2% by weight to about 10% by weight inorganic chemical fertilizer. To the extent the soil conditioning material is present in amount less than about 80% by weight, the corresponding 20% by weight organic fertilizer in such close and intimate proximity to the seed may be toxic to the seed. To the extent the inorganic fertilizer is present in an amount of less than 2% by weight, the beneficial fertility affects of the fertilizer may not be perceived.

To the extent the inorganic fertilizer can be confined in a layer displaced from the seed, a higher level of inorganic fertilizer may be used while limiting risk of a toxic response from the seed. Referring now to FIGURES 6A-6D, in the embodiment of FIGURE 6A, seed capsule **38A** comprises a seed **40A** coated with a single generally homogeneous coating **42A**. Coating **42A**, as illustrated in FIGURE 6A, may comprise only the soil conditioning material (e.g. paper mill sludge or sewage sludge), or may comprise both the soil conditioning material and an inorganic fertilizer or other inorganic material generally dispersed in coating **42A**.

In FIGURE 6B, seed capsule **38B** comprises a seed **40B** coated with a first layer **42B** of soil conditioning material. A second coating material is shown penetrated part-way through the first layer **42B**, thus to make a combination outer layer **44B** comprising the combination of the material of layer **42A** and the material of the second material, such as inorganic fertilizer.

In FIGURE 6C, seed capsule **38C** comprises a seed **40C** coated with a first layer **42C** of soil conditioning material. A second

generally separate and distinct layer **46C** of a second coating material (e.g. inorganic fertilizer) is disposed outwardly on the underlying first layer **42C**. Layer **46C** generally does not penetrate layer **42C**, whereby higher levels of inorganic fertilizer may be used because of the effective displacement distance between the seed and the second layer **46C**. The second layer may be prevented from penetrating the first layer by applying e.g. an intervening layer which repels the second layer, for example wax, lignin, or the like.

In FIGURE 6D, seed capsule **38D** comprises a seed **40D** coated with a pre-coating layer **48D** of dicalcium phosphate to densify and configure the seed capsule precursor for the primary coating steps in drum **10** or pan pellitizer **118**. Layer **42D** of soil conditioning material is disposed outwardly of pre-coating layer **48D**. Other materials such as at layers **44B** or **46C** can be added to any of the embodiments, including that of FIGURE 6D to provide the properties associated therewith.

In alternative embodiments, seed capsules can comprise a seed coated with at least one heterogenous layer. The heterogenous layer comprises at least two different materials substantially commingled, uniformly or non-uniformly, within a single layer. Such materials can include, for example, soil conditioning material and inorganic fertilizer, ~~micro~~nutrients, herbicides, fungicides, binders and/or any other layer material contemplated by the present invention.

While the soil conditioning material/sewage sludge or paper mill sludge may contain a nominal amount of nitrogen and lesser quantities of phosphorous, potassium, and micronutrients, these small levels of plant nutrient content are generally not high enough for the plant nutrients to be considered a primary commercial asset. Yet only small nutrient amounts are desired so close to the seed. Thus, in some uses, the nutrient content of the sludge may be fully acceptable as the sole coating material on the

seed in making suitable and acceptable seed capsules of the invention.

Products of the invention offer a new combination of properties, namely readily available excellent soil conditioning properties in combination with the seed in a seed capsule wherein size and density of the seed capsule are controlled to the desired size and weight.

One of the properties offered by soil texture conditioners such as sewage sludge and paper mill sludge is that of maintaining soil condition by retaining moisture in the soil, retarding leaching of soil nutrients from the root zone, and attenuating hardening, clumping, or other hard agglomeration characteristics of the soil, which harder soils are more difficult for plant roots to penetrate than are softer soils. Thus, improving the soil texture condition, soil tilth, increases the efficiency with which plant nutrients are retained and used for plant nutrition, as well as generally improving the environment of the soil to accommodate, and readily receive, root growth.

When soil conditioning materials and plant nutrients are applied separately to the soil, as in the prior art, the ratio of applied plant nutrients to applied soil conditioning material typically varies widely according to variations in the uniformity of the two applications of the two materials. Further, the soil conditioning material is generally not closely associated with the plant nutrient-containing fertilizer in the soil, and certainly neither soil conditioner nor the fertilizer are controllably-closely associated with the seed, such that nutrient absorption benefits provided by the soil conditioning material are not assuredly associated with respective particles of inorganic chemical fertilizer materials, and neither the soil conditioning material nor the inorganic fertilizer is controllably and intimately associated with the seed as in a common capsule or other particle as in the invention.

Rather, where soil conditioning and fertilizer materials are applied in separate applications and/or in applications separate from the application of the seed, the bulk of the soil conditioning material and the bulk of the inorganic chemical fertilizer are generally at least somewhat separated from each other in space, and physically separated from the seeds, such that potential cooperative benefit of the soil conditioning material as relates to solvation and up-take of soil moisture and/or of the inorganic chemical fertilizer by the seed are not obtained, and/or are not obtained in controlled close association with the seed.

When the soil conditioning material, the inorganic chemical fertilizer materials, and the seed are separately applied to soil with different sets of equipment, the respective rates of application vary such that the desired ratios between the quantities of the several materials are applied somewhat non-uniformly. The variances from uniformity will be different for each of the applications, thus adversely skewing the relative ratios of the materials with respect to each other at different locations in the e.g. field. Further, when applied separately to the soil, the seed and the soil conditioner are not necessarily in intimate contact with each other as they are when both materials are combined into a single combined seed capsule product as in the invention. Nor is the seed in closely controlled proximity (e.g. within the same capsule) with the inorganic fertilizer. In reality, then, any fertilizer added to the soil but not in close proximity to the seed applied to the same soil during e.g. the same growing season, is of reduced value or no value to that application of seed, whereby little or no value is realized, during that growing season, from the application of such material to the soil.

The amounts of soil conditioning material and inorganic fertilizer added to the soil at any given time represent a small fraction of the "soil" in the plant growing zone (root zone). Thus, in the conventional practice of providing separate

applications of plant nutrients and soil conditioning material, in addition to the seed, only small fractions of the newly applied soil conditioning material and plant nutrient come into proximate cooperating relationship with each other and with the seed. Thus, the seed and any plant newly emergent from the seed are benefitted only to the extent the overall average root zone of the soil is benefitted by the applied soil conditioning material

Even were combinations of soil conditioner, inorganic chemical fertilizer, and seed are to be applied as separate and distinct physical product particles, using a single application apparatus and a single application process, the individual particles of soil conditioner, individual particles of inorganic chemical fertilizer, and individual particles of seed would be separated from each other to a significant degree, during the application process, such that the benefits of intimate association with each other in the soil would be lost. Indeed, the seed benefits from intimate contact with a substantial quantity of soil conditioner, but can tolerate intimate contact with only limited concentrations of fertilizer chemicals. Rather, fertilizer chemicals should in general be displaced from, but controllably located close to the seed.

In an uncontrolled application of fertilizer by an application separate from application of the seed, as in the prior art, some of the seed might be expected to be placed so close to some of the inorganic fertilizer as to be damaged by the toxic affect of such close association. Thus, the benefit of intimate contact between organic soil conditioning material, inorganic chemical fertilizer, and seed, is reduced and largely lost because of low levels of intimate association between the soil conditioning material and the seed, and unpredictable, uncontrolled levels of association between the seed and the inorganic chemical fertilizer, outside the combination of the invention, of soil conditioning coating of the seed, and optional addition of inorganic fertilizer at controlled

location with respect to the seed, all in the same seed capsule, as taught herein.

By combining an organic soil conditioning material in the same seed capsule with the seed, highly effective levels of soil conditioner are assuredly associated with the seed as the seed germinates and begins to grow. Where suitable levels of plant nutrient fertilizer are incorporated into the same seed capsule, growth of the newly-germinated plant is further enhanced. In either case, the soil conditioning materials can and do tend to retain moisture and nutrients in the soil in the defined area of the seed capsule by a variety of mechanisms, providing an extended time period during which nutrients can be taken up by the plants. For example, organic soil conditioning material may retain moisture, reducing moisture drainage from the soil, such that the rate of leaching of the nutrients is, in general, reduced. Further, the soil conditioning material may absorb or otherwise physically or chemically attach to plant nutrient materials in the chemical fertilizer material, thus further retarding leaching of the plant nutrient away from the seed.

While applicant cannot place an exact time period on the increase in the extent to which the soil conditioning materials retard leaching of the plant nutrients from proximity with the seed, thereby holding the plant nutrients available for up-take by the plant, any increase in time during which the nutrients are held in the soil proximate the newly-emerging plant is beneficial to meeting the nutritional needs of the plant being so fed.

By incorporating soil conditioning materials and optionally plant nutrient fertilizers, in the seed capsules, the invention offers an efficiency of application of soil conditioning materials in proximity to the seeds most beneficially affected thereby, in a beneficial association never before available. Optional addition of plant nutrients to the same seed capsule provides a largely self-contained microcosm of seed, soil conditioner, and inorganic

fertilizer in intimate yet controlled spatial relationship with each other, whereby the controlled spacings provide enhanced plant growth benefit. Namely, soil conditioning materials and plant nutrients are somewhat beneficial to each other for the overall cooperative achievement of soil fertility in the presence of the newly emerging plant which is dependent on such plant nutrients, and on moisture retained by the soil conditioner for uptake of such plant nutrients.

While soil conditioning materials do perform a number of highly interdependent tasks, one such task is in assisting in maintaining the plant nutrients in the root zone where they can be effectively used by the plants when needed. Another such task is in assisting in making the soil soft and friable in the root zone whereby the newly-emerged and very tender plant roots more readily penetrate the soil as they grow.

Where both soil conditioner and fertilizer are incorporated with the seed into the seed capsule, the soil conditioner assists in strategically maintaining the combination of soil conditioner and plant nutrients in close and controlled proximity to each other and to the seed in the soil. Such strategic placement virtually assures that the soil conditioning material and inorganic chemical fertilizer are bound to each other, in proximate relationship with the seed, for a time, such that wherever the seed capsule may land when the seed is sown, the seed will have the initial benefit of both soil conditioner and plant nutrients in intimate proximity with itself, irrespective of any condition of the surrounding growth medium. Thus, in the invention, soil conditioning material and optionally inorganic chemical fertilizer, are inherently bound to each other, and to the seed, as by the coating process, and inherently assist the seed in achieving desired germination and strong early growth.

By incorporating the soil conditioning material in the same seed capsule with the seed, the invention ensures that the seed has

benefit of intimate relationship with a beneficial amount of soil conditioner material. The seed thus receives the advantage of the beneficial amount of soil conditioner material irrespective of the overall tilth of the soil and irrespective of the overall level soil conditioner, e.g. soil texture conditioner, in the root zone of the soil with which the seed capsule becomes associated for seed and plant growth purposes.

Referring to FIGURE 7, a population of seed capsules **38** are disposed at the top surface of a cross section of soil. Root zone **150** of the soil is generally defined to that depth of the soil which typically receives roots of growing plants, and is generally defined within 20-30 inches of the top surface of the soil. Generally, and preferably, the root zone should have a soft texture, rich in organic and/or other soil conditioning material in order to provide good tilth, and desirable moisture and nutrient holding properties. Underlying root zone **150** is subsoil **152** which typically contains little organic matter.

It is a well known agricultural phenomenon that, in soil used for intensive crop production, the root zone tends, over time, to become relatively depleted of organic soil conditioning material, illustrated at **154** in FIGURE 7, negatively affecting soil tilth and texture. While wholesale addition of organic soil conditioning material can improve the overall tilth of the soil, FIGURE 7 illustrates application of the invention wherein the texture of the material immediately adjacent the seed, namely coating **42**, provides beneficial properties attributable to soil having desirable texture.

FIGURE 8 illustrates that coating **42** draws moisture **154** from the soil, into the capsule, where the moisture is available to assist in germination of seed **40**. In the process, traverse of the moisture through second coating **46C** releases plant nutrient material into the moisture, as well as downwardly into the soil adjacent the seed capsule, as illustrated at **156**. Thus, the root

158 emerging from the seed emerges into an initial growth medium, coating **42**, having texture, moisture, and plant nutrient highly advantageous to early plant growth. As root **158** advances further downward, the upper portion of the underlying soil under the capsule where the seed first enters the soil, has also been beneficially affected to the good of the plant by plant nutrients **156**, and by moisture attracted or held in the vicinity of the capsule, as a result of the presence of the soil conditioning material in the capsule.

The relative amounts of the soil conditioning material and the inorganic chemical fertilizer material in the seed capsule vary significantly in accord with the specific application, and any specific interactivity desired of the soil conditioning material and inorganic chemical fertilizer. For example, in a particular combination of soil conditioning material and inorganic fertilizer a particular plant crop to be nourished by the product may require a higher amount of plant nutrient, or a specific analysis of plant nutrients, in order to be properly fed at and shortly after the stage of germination.

Thus, for a given specific application of combination seed capsule (with fertilizer) product of the invention, the relative amount of inorganic chemical fertilizer, and the fertilizer analysis, may be increased or decreased from some "standard" in the interest of achieving a functionally adequate feeding of the newly germinated seedlings. Namely, the NPK etc. nutrient levels provided in a given seed capsule product of the invention can be set and controlled at the fertilizer manufacturing plant in accord with the respective NPK etc. nutrient needs of the seed to be supported, or of the soil or other growth medium to which the combination fertilizer of the invention is to be applied.

In any embodiments, whether or not specifically discussed here, the fabricated seed capsules are kept sufficiently cool, and are kept sufficiently dry, to avoid the seed capsules sticking to

each other, caking, and the like, and to prevent premature germination of the seed. Where liquid is used to obtain the coating material in liquid state, sufficient liquid is removed during or shortly after the coating step to avoid the seed capsules sticking to each other, or caking, or the like. Where the seed capsules are made by process other than the process described here, the details of the process will determine proper cooling, drying, or other steps to provide a finished, dry, solid seed capsule or like product. A dry such product generally has moisture content less than 10% by weight, preferably less than 5% by weight, most preferably less than 3% by weight.

As suggested by the description hereinabove, the processes of the invention are generally carried out to make combination seed improvement products solely by using physical processes such as coating and drying. While some minor chemical reactions may inadvertently accompany such physical processes, the invention does not rely on any chemical reaction for achievement of the objectives thereof. Rather the invention is focused on a physical combination of starting materials, which physical combination results in mutual benefits of the two starting materials (seed and soil conditioner, and optional inorganic chemical fertilizer) functioning intimately together, in primarily physical and physico-chemical relationship, to produce an overall increase in benefits of plant germination and early plant growth with such combination seed improvement products.

The relative amounts of seed and coating material depend on the overall benefits desired to be achieved from the coating operations. In general, the seed will comprise from about 0.1% to about 75% of the overall weight of the seed capsule. the coating material thus represents about 25% to about 99.9% by weight of the seed capsule. Where the seed content is low, the general benefit of the product is that of soil conditioning, with some seed application. Such product is well suited for application to e.g.

a healthy lawn for general improvement of soil condition, and modest fill-in of bare spots with seed.

Another benefit of low seed content by weight, especially with quite small seeds, is in creating a larger size seed capsule, and thereby facilitating the handling of such seed in commonly-used seed handling machines such as grain drills or seed broadcast machines.

Typically, however, a higher seed content is preferred so as to have major impact on the number of plants which are caused to germinate by application of such product. Thus, for a seed about 0.5-1.0 mm thick and about 4-7 mm long, a preferred fraction of seed is about 1% to about 50%, preferably about 1.5% to about 20%, more preferably about 2% to about 10% by weight seed, with respective amount of soil conditioner and optionally fertilizer. For example, in a preferred product of the invention, an above mentioned grass seed about 0.5-1.0 mm thick and about 4-7 mm long, when coated produces a seed capsule about 4 mm across and about 6-9 mm long. Smaller, or larger, seed capsules may be made and used as desired.

The size and density of the seed capsules can be readily controlled using conventional sizing equipment and processing parameters of the coating process, so as to provide a uniform product of a wide range of sizes and densities. With the size and density of any seed thus controllable, the size and density may be selected and specified for enhancing control and efficiency of seed handling and/or distribution. For example, tiny seeds such as lettuce, carrots, cabbage, and alfalfa, may be sized and weighted for easy and assured handling and distribution, whether by hand or by machine. Seeds which are non-aerodynamic, or which are so light as to be blown around, such as grass seed, can be made heavy and compact enough as to assuredly remain on location where sown after being planted. For example, non-aerodynamic seeds, after treatment according to the invention, can be broadcast-applied using

conventional equipment such as is used to broadcast apply granular fertilizer over e.g. 40 foot wide application paths.

Where time controlled germination is desirable, a population of combination seed capsules, having at least one soil conditioner and one or more nutrients, can be planted in conjunction with non-coated seeds. As a result, non-coated seeds will germinate at an earlier stage than the population of combination seed capsules. Such staggering of germination times allows, for example, the non-coated seeds to use the available soil nutrients with less competition (i.e. less seeds using limited nutrient supply). At a later time, when the coated seeds germinate, such seeds can use the nutrients leached from their combination seed capsules to germinate.

Where e.g. small such seeds are desirably planted in close proximity with each other, and wherein a relatively larger size seed capsule is desired for ease of handling such that the large size seed capsule would potentially interfere with such close placement of the seeds with respect to each other, then and in such situation, multiple seeds may be employed in individual seed capsules, e.g. generally uniformly distributed throughout the seed capsule, so as to provide for sufficiently close spacing of the seeds from each other.

Paper mill sludge, as is suggested as a coating material herein, is a resultant by-product of papermaking, typically from e.g. a de-inking process in the paper mill.

By utilizing paper mill sludge and/or sewage sludge as taught herein, one contemplates beneficially and suitably disposing of significant quantities of industrial waste which otherwise is disposed of by landfilling.

Where the product of the invention is applied as to a residential or like lawn, as in an agricultural field, the seed is applied to the soil in intimate combination (seed capsule) with the soil conditioner, such that the soil conditioner serves as moisture

retainer and sun shield. In addition, the seed capsule is much heavier and dense than the seed itself, whereby the seed capsule provides substantial protection against the seed being washed away in surface water run-off. Thus, the coating about the seed serves many of the functions typically performed by the conventionally-used straw mulch. Accordingly, product of the invention can be used to seed new lawns without any need for use of straw or any other mulch material.

Where seed is desirably used to fill in bare spots in the lawn, such seed, especially fertility-enhanced seed capsules, may be applied desirably in one of two ways. First, the coated seed capsule product may be applied only to perceived bare spots, without use of straw. The soil conditioner in the seed capsules serve the functions of the straw as described above, but perform better than straw because of the close association between the seed and the soil conditioner.

In the alternative, the coated seed capsule product may be broadcast generally over the entire lawn. Where the lawn is already healthy with thick grass growth, the soil conditioner and fertilizer will benefit the existing grasses, with minimal germination and growth of new seed from the seed capsules. Where the existing grass is thinner, the seeds in the seed capsules will have room and light to grow, whereby the combined properties of seed, soil conditioner, and fertilizer, in intimate relationship with one another, will be efficaciously used.

Where seed capsules of the invention are used to establish a new lawn, the soil conditioner in the seed capsules serve the functions of the straw as described above, obviating the need for straw in establishing the lawn seeding.

Those skilled in the art will now see that certain modifications can be made to the apparatus and methods herein disclosed with respect to the illustrated embodiments, without departing from the spirit of the instant invention. And while the

invention has been described above with respect to the preferred embodiments, it will be understood that the invention is adapted to numerous rearrangements, modifications, and alterations

To the extent the following claims use means plus function language, it is not meant to include there, or in the instant specification, anything not structurally equivalent to what is shown in the embodiments disclosed in the specification.

CLAIMS

Having thus described the invention, what is claimed is:

1. A combination seed capsule, comprising:

(a) at least one viable seed, having an outer surface and acting as a core or psuedo-core of said combination seed capsule; and

(b) a coating of a composition comprising a soil conditioning material mounted proximate, including disposed outwardly of the outer surface of said seed.

2. A combination seed capsule as in Claim 1, said coating providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

3. A combination seed capsule as in Claim 1 wherein said seed is selected from the group consisting of grass, vegetables, grains, and flowers.

4. A combination seed capsule as in Claim 1, said coating further comprising said soil conditioning material in combination with at least one ingredient effective to reduce susceptibility of

said seed capsule to deleterious affect of at least one of animals, weeds, and spore-formers.

5. A combination seed capsule as in Claim 4 wherein said at least one ingredient to reduce susceptibility of the seed capsule is selected from the group consisting of herbicides, fungicides, and a bitter substance.

6. A combination seed capsule as in Claim 5 wherein said fungicide comprises metalaxyl.

7. A combination seed capsule as in Claim 1, said coating comprising a first coating, said combination seed capsule further comprising a second coating, separate from said first coating, and comprising at least one ingredient effective to reduce susceptibility of said seed capsule to deleterious effect of at least one of animals, weeds, and spore-formers.

8. A combination seed capsule as in Claim 1, effective to provide a plant nutrient at a desirable controlled distance from a plant seedling emerging from said seed, in an amount beneficial to said plant seedling.

9. A combination seed capsule as in Claim 1, said coating comprising a first coating, said combination seed capsule further comprising a second coating of a second coating material intermingled with said first coating material in an outer portion of said first coating, and generally displaced from said seed.

10. A combination seed capsule as in Claim 9 wherein said second coating material comprises a plant nutrient, beneficial in location and in amount of availability, to plant seedling emerging from said seed.

11. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient and is selected from the group consisting of nitrogen, phosphorus, and potassium.

12. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient and is selected from the group consisting of urea, monammonium phosphate, diammonium phosphate, superphosphate, triple superphosphate, dicalcium phosphate, and potash.

13. A combination seed capsule as in Claim 9 wherein said second coating composition comprises an inorganic form of a plant nutrient is selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

14. A population of combination seed capsules of Claim 1, said population of seed capsules comprising coatings having a range of properties affecting germination rate of said seeds, thereby to stage germination of said seeds in said population over a period of time longer than the range of germination times inherent in uncoated ones of said seeds.

15. A population of combination seed capsules as in Claim 14 wherein said range of properties comprises at least one of (i) a range of hardnesses and (ii) a range of thicknesses, of said coatings.

16. A combination seed capsule as in Claim 1, said coating comprising a first layer of said soil conditioning material, and including a second layer comprising an inorganic fertilizer.

17. A combination seed capsule as in Claim 1, said coating comprising a first layer of said soil conditioning material, and including a second layer comprising at least one micronutrient.

18. A combination seed capsule as in Claim 17 wherein said micronutrient is selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

19. A combination seed capsule as in Claim 1, said soil conditioning material comprising a sludge composition.

20. A combination seed capsule as in Claim 1, said soil conditioning material comprising a fiber-containing by-product of a paper making operation.

21. A combination seed capsule as in Claim 1, said seed capsule comprising a water-leachable plant nutrient, and a leach-

retardant composition effective to retard leaching of said leachable plant nutrient out of said combination seed capsule.

22. A population of combination seed capsules of Claim 1, said coating in ones, but less than all, of said population, comprising an ingredient effective to retard effective penetration of a seed-germinating environment to said seed for germination thereof.

23. A combination seed capsule as in Claim 1, said seed capsule comprising an inner layer on the outer surface of said seed, and an outer layer, said inner layer enhancing properties of said seed for acting as nucleus in an agglomeration operation agglomerating said coating onto said inner layer.

24. A combination seed capsule as in Claim 1 wherein said coating comprises an admixture of said soil conditioner and a plant nutrient.

25. A combination seed capsule as in Claim 1 wherein said coating remains generally disposed about said seed until said seed germinates.

26. A plant growing system, comprising:

(a) a plant growing medium extending over an area, said plant growing medium having a root zone, and a top surface of said root zone generally corresponding with a top surface

of said plant growing medium, said plant growing medium having a first overall soil condition and texture; and

(b) a population of seed capsules disposed over the top surface of said plant growing medium, said seed capsules comprising individual seeds, having outer surfaces, and coatings of soil conditioning material disposed outwardly of the outer surfaces of said seeds,

said coatings of said seed capsules providing localized germination and growth environments, at and adjacent said seeds, having texture, and nutrient and water holding properties for supporting seedling health, superior to respective said properties as provided overall in the root zone of said plant growing medium.

27. A growing system as in Claim 26, said coatings remaining generally disposed about said seeds until respective ones of said seeds germinate.

28. A growing system as in Claim 26, said coatings providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

29. A growing system as in Claim 26 wherein said seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

30. A growing system as in Claim 26, said coatings further comprising said soil conditioning material in combination with at least one ingredient effective to reduce susceptibility of said seed capsules to deleterious affect of at least one of animals, weeds, and spore-formers.

31. A growing system as in Claim 26, said coating comprising a first coating, said combination seed capsules further comprising a second coating, separate from said first coating, and comprising at least one ingredient effective to reduce susceptibility of said seed capsules to deleterious effect of at least one of animals, weeds, and spore-formers.

32. A growing system as in Claim 26, effective to provide plant nutrients at desirable controlled distances from plant seedlings emerging from said seeds, in amounts beneficial to said plant seedlings.

33. A growing system as in Claim 26, said coatings comprising first coatings, said combination seed capsules further comprising second coatings of second coating materials intermingled with said first coating materials in outer portions of said first coatings, and generally displaced from said seeds.

34. A growing system as in Claim 33 wherein said second coating materials comprise plant nutrients, beneficial in location and in amount of availability, to plant seedlings emerging from said seeds.

35. A growing system as in Claim 26, said population of seed capsules comprising coatings having a range of properties affecting germination rates of said seeds, thereby to stage germination of said seeds in said population over a period of time longer than the range of germination times inherent in uncoated ones of said seeds.

36. A growing system as in Claim 26, said coatings comprising first layers of said soil conditioning material, and including second layers comprising inorganic fertilizer.

37. A growing system as in Claim 26, said soil conditioning material comprising a sludge composition.

38. A growing system as in Claim 26, said soil conditioning material comprising a fiber-containing by-product of a paper making operation.

39. A growing system as in Claim 26, said seed capsules comprising inner layers on the outer surfaces of said seeds, said inner layers enhancing properties of said seeds for acting as nucleus in an agglomeration operation agglomerating said coatings onto said inner layers.

40. A growing system as in Claim 26 wherein said coatings comprise admixtures of said soil conditioner and plant nutrient.

41. A method of providing plant micronutrients to soil, the method comprising placing onto the soil a population of combination seed capsules, each comprising at least one seed, and a coating comprising a plant micronutrient material.

42. A method as in Claim 41, the coating comprising a first coating comprising the plant micronutrient, and a second coating, separate and distinct from the first coating, and comprising a soil conditioning material.

43. A method as in Claim 41, the coating providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) enhancing control of moisture about said seed thereby to assist in seed germination;

(vi) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vii) soil conditioning effect to soil onto which said combination seed capsule is placed;

(viii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released;

(ix) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density; and

(x) assisting in stabilizing moisture content in soil on which such seed capsule is disposed.

44. A method as in Claim 41, the coating providing a plant nutrient at a desirable controlled distance from a plant seedling emerging from the seed, in an amount beneficial to the plant seedling.

45. A method as in Claim 41, the coating comprising a first coating, the combination seed capsule further comprising a second coating of a second coating material intermingled with the first coating material in an outer portion of the first coating, and generally displaced from the seed.

46. A method as in Claim 45 wherein the first coating comprises plant micronutrient material and the second coating

comprises plant nutrient material comprising at least one of nitrogen, phosphorus, and potassium.

47. A method as in Claim 41 wherein the micronutrient composition comprises a plant nutrient selected from the group consisting of sulfur, manganese, copper, boron, iron, magnesium and chromium.

48. A method as in Claim 41, the coating comprising a first layer of the soil conditioning material, and including a second layer comprising an inorganic fertilizer.

49. A method as in Claim 41, the coating comprising a sludge composition.

50. A method as in Claim 41, the coating comprising a fiber-containing by-product of a paper making operation.

51. A method as in Claim 41, the seed capsule comprising an inner layer on an outer surface of the seed, and an outer layer, the inner layer enhancing properties of the seed for acting as nucleus in an agglomeration operation agglomerating the coating onto the inner layer.

52. A method as in Claim 41 wherein the coating comprising an admixture of soil conditioner and a plant nutrient.

53. A method as in Claim 41 wherein the coating remains generally disposed about the seed until the seed germinates.

54. A method of providing a seed bed having enhanced growing conditions for growing seed, the method comprising:

(a) coating a population of the seeds with material, and thereby providing coatings thereon of such material, tending to stabilize, in the seed capsules, or in soil on which the seed capsules are disposed coating compositions which tend to hold, moisture adjacent the seeds in the seed capsules or in soil adjacent the seed capsules, in such quantities and for such times as to enhance growing conditions for the seeds; and

(b) placing the population of seeds on soil effective to support germination of the seeds which are in the seed capsules.

55. A method as in Claim 54, the coatings providing at least one of

(i) enhancing broadcast flight properties of said combination seed capsule;

(ii) reducing susceptibility to deleterious affects of weather on said combination seed capsule;

(iii) enhancing resistance of said combination seed capsule to attack by animals or spore-formers;

(iv) staged germination of ones of said seed capsules, having seeds, under a given set of conditions, over a period of time longer than the range of germination times inherent in said seeds;

(v) release of plant nutrients into soil onto which said combination seed capsule is placed;

(vi) soil conditioning effect to soil onto which said combination seed capsule is placed;

(vii) staged release of plant nutrients into soil onto which said combination seed capsule is placed, over a period of time longer than the range of times inherent in the chemical composition so released; and

(viii) higher embryo emergence and survival rate in a population of said seed capsules, thereby reducing required seed planting density for a desired plant population density.

56. A method as in Claim 54 wherein the seeds are selected from the group consisting of grass, vegetables, grains, and flowers.

57. A method as in Claim 54, effective to provide a plant nutrient at desirable controlled distances from plant seedlings emerging from the seeds, in amounts beneficial to the plant seedlings.

58. A method as in Claim 54, the coatings comprising first coatings, the combination seed capsules further comprising second coatings of second coating materials intermingled with the first coating materials in outer portions of the first coatings, and generally displaced from the seeds.

59. A method as in Claim 58 wherein the second coating materials comprise plant nutrients, beneficial in location and in amount of availability, to plant seedlings emerging from the seeds.

60. A method as in Claim 58 wherein the second coating compositions comprise inorganic forms of plant nutrients and are selected from the group consisting of nitrogen, phosphorus, and potassium.

61. A method as in Claim 54, the population of seed capsules comprising coatings having a range of properties affecting germination rate of the seeds, thereby to stage germination of the seeds in the population over a period of time longer than the range of germination times inherent in uncoated ones of the seeds.

62. A method as in Claim 54, the coatings comprising first layers of the soil conditioning material, and including second layers comprising inorganic fertilizer.

63. A method as in Claim 54, the coatings comprising first layers of the soil conditioning materials, and including second layers comprising micronutrients.

64. A method as in Claim 54, the soil conditioning materials comprising sludge compositions.

65. A method as in Claim 54, the soil conditioning materials comprising fiber-containing by-products of paper making.

66. A method as in Claim 54, the seed capsules comprising water-leachable plant nutrients, and leach-retardant compositions effective to retard leaching of the leachable plant nutrients out of the combination seed capsules.

67. A method as in Claim 54, the seed capsules comprising inner layers on the outer surfaces of the seeds, and outer layers, the inner layers enhancing properties of the seeds for acting as nuclei in agglomeration operations agglomerating the coatings onto the inner layers.

68. A method as in Claim 54 wherein the coatings comprise admixtures of the soil conditioners and plant nutrients.

69. A method as in Claim 54 wherein the coatings remain generally disposed about the seeds until the seeds germinate.

70. A method of making a population of combination seed capsules, each comprising a seed, and a coating of a soil conditioning material, the method comprising:

(a) pre-coating the seed with a material which enhances the ability of the seed to act as a nucleus in an agglomeration operation, to form a pre-coated substrate; and

(b) subsequently coating the pre-coated substrate with a soil conditioning material.

71. A method as in Claim 70 wherein the pre-coating material comprises dicalcium phosphate.

72. A method as in Claim 70 wherein the pre-coating step results in an overall increase in the density of pre-coated seed combination.

73. A method as in Claim 70 wherein the pre-coating is accomplished by spraying the pre-coating material onto the seed.

74. A method of providing an enhanced seed germination environment in combination with placement of a controlled amount of plant nutrients in controlled proximity to each seed, the method comprising:

(a) providing a population of seeds, coated with a soil conditioning material which tends to enhance germination of the seeds, and with plant nutrient composition effective to enhance growth of plant embryos emerging from the seeds; and

(b) placing the population of seeds on soil effective to support germination of the seeds.

75. A method as in Claim 74 wherein the coating material includes therein a second ingredient comprising plant nutrient moieties.

ABSTRACT OF THE DISCLOSURE

This invention pertains to combination seed capsules wherein each seed capsule includes both moieties of at least one soil conditioner and at least one seed, and optionally, one or more inorganic chemical fertilizer, growth enhancer, binder, and/or anti-fungal agent. The combination seed capsules are made by physically combining the respective soil conditioner and seed with one other, in the absence of any requirement for chemical reactions in the process of so combining the respective materials. The combination seed capsules provide cooperative and beneficial effects of the soil conditioner and the optional inorganic fertilizer, working together in controlled intimate relation with the seed, to enhance the germination and growth processes of the seed, and the plant emergent therefrom, greater than when the soil conditioner and seed, and optionally inorganic chemical fertilizer, are applied to the soil separately; the improvement being a result of the intimate relationship of the respective materials in the combination seed capsule, whereby the respective materials cooperate with each other in support of germination and plant growth.

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

Bib Data Sheet

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/113,254	07/10/1998 RULE _	047	1649	29214

APPLICANTS

DANIEL PAUL MADIGAN, GREEN BAY, WI ;
MICHAEL DENNIS KRYSIAK, GREEN BAY, WI ;
RONALD DEAN EICHHORN, GREEN BAY, WI ;
GLEN H. WESENBERG, GREEN BAY, WI ;

**** CONTINUING DATA ***************************

THIS APPLN CLAIMS BENEFIT OF 60/052,287 07/11/1997

**** FOREIGN APPLICATIONS *********************

IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 07/28/1998

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☑ no	WI	6	75	5
35 USC 119 (a-d) conditions met ☐ yes ☑ no ☐ Met after Allowance				
Verified and Acknowledged Examiner's Signature Initials				

ADDRESS

PHILIP M. WEISS
WEISS & WEISS
500 OLD COUNTRY ROAD
GARDEN CITY ,NY ~~11530~~ 11530

TITLE

SEEDING TREATMENTS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	
1542	No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/113,254	07/10/98	047	~~1649~~ 1661	29214

APPLICANT

DANIEL PAUL MADIGAN, GREEN BAY, WI; MICHAEL DENNIS KRYSIAK, GREEN BAY, WI; RONALD DEAN EICHHORN, GREEN BAY, WI; GLEN H. WESENBERG, GREEN BAY, WI.

CONTINUING DOMESTIC DATA********
VERIFIED
AMC

371 (NAT'L STAGE) DATA********
VERIFIED
AMC

FOREIGN APPLICATIONS********
VERIFIED
AMC

I REQUIRED, FOREIGN FILING LICENSE GRANTED 07/28/98

Foreign Priority claimed 35 USC 119 (a-d) conditions met	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐yes ☒no ☐yes ☒no ☐Met after Allowance Verified and Acknowledged AMC (Examiner's Initials) _____ (Initials)	WI	6	75	5

ADDRESS

THOMAS D WILHELM
WILHELM LAW SERVICE
100 W LAWRENCE STREET
THIRD FLOOR
APPLETON WI 54911

Philip M. Weiss
Weiss & Weiss
500 Old Country Road
Garden City, New York
34751

TITLE

SEEDING TREATMENTS

FILING FEE RECEIVED	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT NO. _____ for the following:	
$1,147	P. 314	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY OF COMMERCE AND
COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

NOTICE OF FILING/CLAIM FEE(S) DUE

TO ENSURE PROPER CREDIT OF FEES, PLEASE RETURN A COPY OF THIS FEE CALCULATION SHEET WITH YOUR RESPONSE.

APPLICATION NUMBER: 113 254

Total Fee Calculation

	Fee Code	Total # Claims		Number Extra	X	Fee	Fee =	Total
	Sm./Lg.					Sm. Entity	Lg. Entity	
Basic Filing Fee	201/101							790
Total Claims >20	203/103	75	-20 =	55	X			1210
Independent Claims >3	202/102	5	-3 =	2	X			164
Mult. Dep Claim Present	204/104							
Surcharge	205/105							130
English Translation	139							
TOTAL FEE CALCULATION								2294

Fees due upon filing the application.

Total Filing Fees Due = $ 2294

Less Filing Fees Submitted - $ 0

BALANCE DUE = $ 2294

Office of Initial Patent Examination

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1997

Application or Docket Number: 113254

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	15 minus 20 =	* 55
INDEPENDENT CLAIMS	5 minus 3 =	* 2
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐ RATE	FEE	OR	OTHER THAN SMALL ENTITY RATE	FEE
	395.00	OR		790.00
x$11=		OR	x$22=	220
x41=		OR	x82=	164
+135=		OR	+270=	
TOTAL		OR	TOTAL	2169

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	
+135=		OR	+270=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	
+135=		OR	+270=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	
+135=		OR	+270=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 2.

FORM PTO-875 (Rev. 8/97) *U.S. Government Printing Office: 1997 - 430-571/69194 Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

29214
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Daniel Paul Madigan et al

Group Art Unit: Unassigned

Serial Number: Unassigned

Examiner: Unassigned

Filed: July 10, 1998

For: SEEDING TREATMENTS

CORRESPONDENCE ADDRESS

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Kindly address all correspondence regarding the above-referenced Application to the following address:

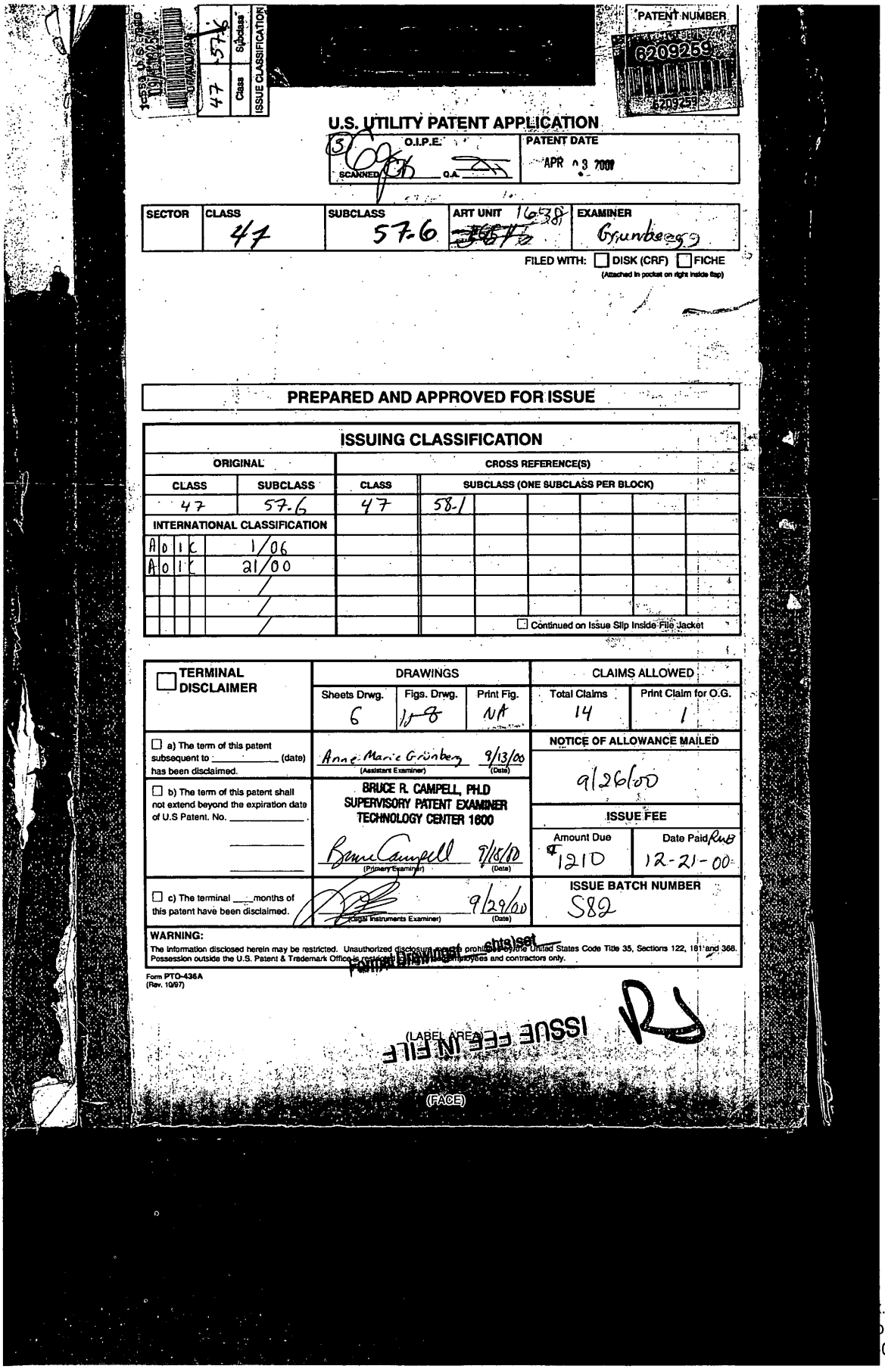
Thomas D. Wilhelm
Wilhelm Law Service, S.C.
100 W. Lawrence Street
Third Floor
Appleton, WI 54911

Phone 920-831-0100
FAX 920-831-0101

Respectfully submitted,
Daniel Paul Madigan et al

By _____
Thomas D. Wilhelm
Attorney for Applicants
(Reg. No. 28,794)

July 10, 1998
Appleton, Wisconsin



ISSUE CLASSIFICATION: Class 47 / Subclass 57.6

PATENT NUMBER 6209259

U.S. UTILITY PATENT APPLICATION

O.I.P.E.	PATENT DATE
SCANNED ___ Q.A. ___	APR 03 2001

SECTOR	CLASS	SUBCLASS	ART UNIT	EXAMINER
	47	57.6	1638	Grunberg

FILED WITH: ☐ DISK (CRF) ☐ FICHE
(Attached in pocket on right inside flap)

PREPARED AND APPROVED FOR ISSUE

ISSUING CLASSIFICATION

ORIGINAL		CROSS REFERENCE(S)						
CLASS	SUBCLASS	CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					
47	57.6	47	58.1					
INTERNATIONAL CLASSIFICATION								
A01K 1/06								
A01K 21/00								

☐ Continued on Issue Slip Inside File Jacket

☐ TERMINAL DISCLAIMER	DRAWINGS			CLAIMS ALLOWED	
	Sheets Drwg.	Figs. Drwg.	Print Fig.	Total Claims	Print Claim for O.G.
	6	8	NA	14	1

☐ a) The term of this patent subsequent to _____ (date) has been disclaimed.

☐ b) The term of this patent shall not extend beyond the expiration date of U.S Patent. No. _____.

☐ c) The terminal ___ months of this patent have been disclaimed.

Anne-Marie Grünberg (Assistant Examiner) 9/13/00 (Date)

BRUCE R. CAMPELL, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Bruce Campell (Primary Examiner) 9/18/00 (Date)

(Digital Instruments Examiner) 9/29/00 (Date)

NOTICE OF ALLOWANCE MAILED: 9/26/00

ISSUE FEE

Amount Due	Date Paid
$1210	12-21-00

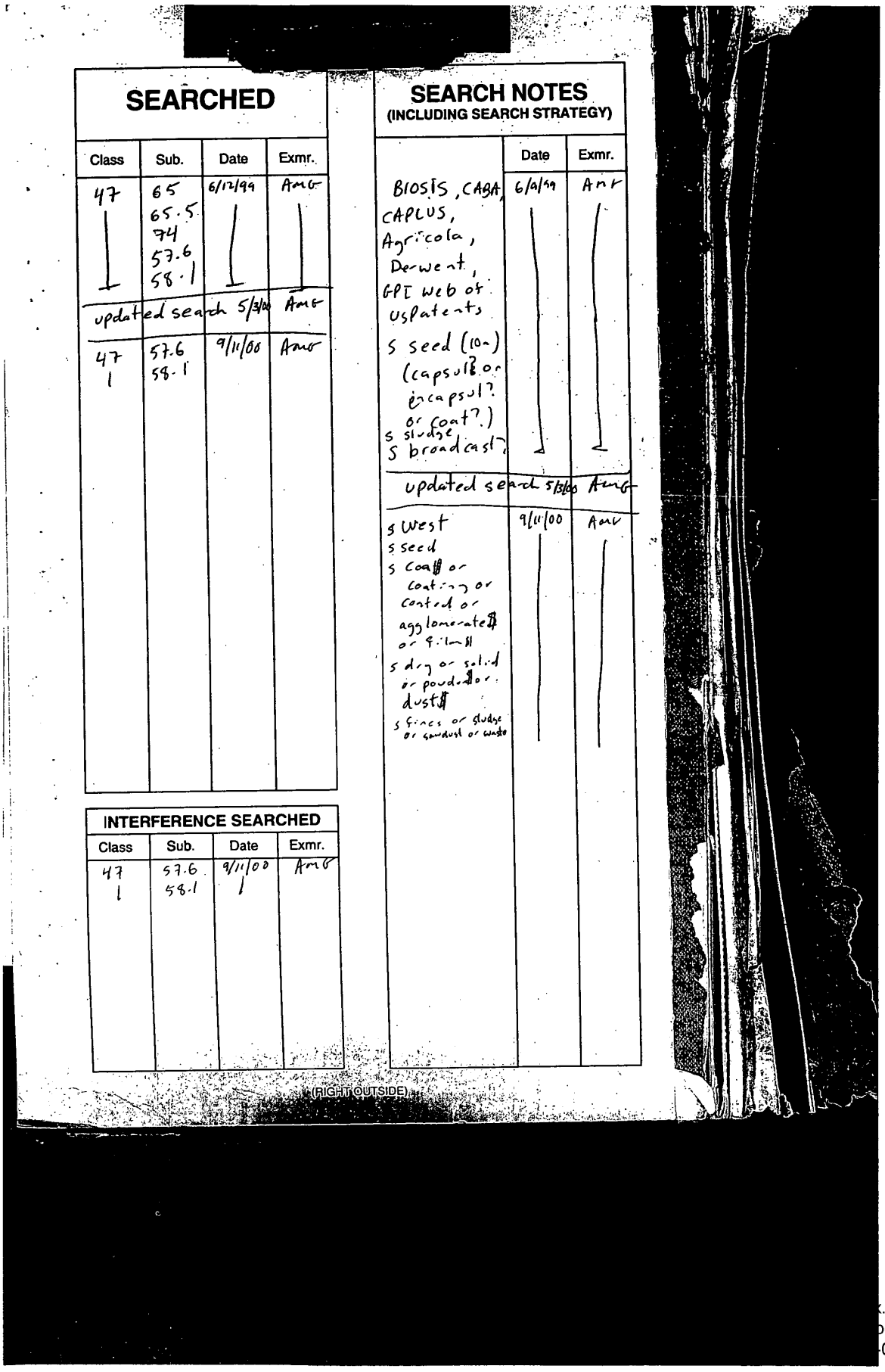
ISSUE BATCH NUMBER: S82

WARNING:
The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.

Form PTO-436A
(Rev. 10/97)

(LABEL AREA) ISSUE FEE IN FILE

(FACE)



SEARCHED

Class	Sub.	Date	Exmr.
47	65 65.5 74 57.6 58.1	6/12/99	AmG
updated search 5/3/00			AmG
47 1	57.6 58.1	9/11/00	AmG

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
47 1	57.6 58.1	9/11/00 1	AmG

SEARCH NOTES
(INCLUDING SEARCH STRATEGY)

	Date	Exmr.
BIOSIS, CABA, CAPLUS, Agricola, Derwent, GPI web of USpatents S seed (10a) (capsul? or encapsul? or coat?.) S sludge S broadcast?	6/9/99	AmG
updated search 5/3/00		AmG
S west S seed S coat? or coating or coated or agglomerate? or film? S dry or solid or powder? or dust? S fines or sludge or sawdust or waste	9/11/00	AmG

(RIGHT OUTSIDE)

ISSUE SLIP STAPLE AREA (for additional cross references)

POSITION	INITIALS	ID NO.	DATE
FEE DETERMINATION			
O.I.P.E. CLASSIFIER			
FORMALITY REVIEW		6876	

INDEX OF CLAIMS

✔ ..... Rejected
= ..... Allowed
— (Through numeral) Canceled
÷ ..... Restricted
N ..... Non-elected
I ..... Interference
A ..... Appeal
O ..... Objected

Claim	Date	Date	Date
Final / Original	6/13/19 · 5/14/20 · 9/11/20	6/13/19 · 5/14/20 · 9/11/20	

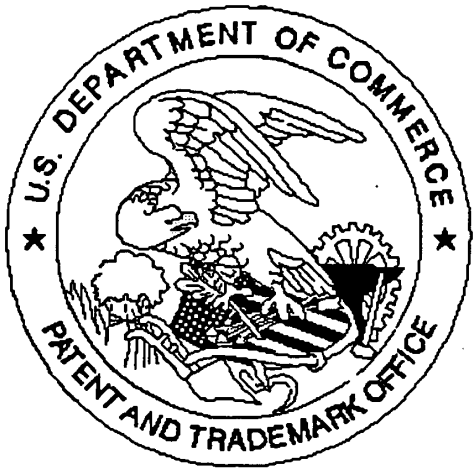
If more than 150 claims or 10 actions staple additional sheet here

(LEFT INSIDE)

PRINT OF DRAWINGS
AS ORIGINALLY FI

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

1. Application papers are not suitable for scanning and are not in compliance with 37 CFR 1.52 because:
   - ☐ All sheets must be the same size and either A4 (21 cm x 29.7 cm) or 8-1/2"x 11". Pages _____ do not meet these requirements.
   - ☐ Papers are not flexible, strong, smooth, non-shiny, durable, and white.
   - ☐ Papers are not typewritten or mechanically printed in permanent ink on one side.
   - ☐ Papers contain improper margins. Each sheet must have a left margin of at least 2.5 cm (1") and top, bottom and right margins of at least 2.0 cm (3/4").
   - ☐ Papers contain hand lettering.

2. Drawings are not in compliance and were not scanned because:
   - ☐ The drawings or copy of drawings are not suitable for electronic reproduction.
   - ☐ All drawings sheets are not the same size. Pages must be either A4 (21 cm x 29.7 cm) or 8-1/2" x 11".
   - ☐ Each sheet must include a top and left margin of at least 2.5 cm (1"), a right margin of at least 1.5 cm (9/16") and a bottom margin of at least 1.0 cm (3/8").

3. Page(s)_____ are not of sufficient clarity, contrast and quality for electronic reproduction.

4. Page(s)_____ are missing.

5. OTHER: No Declaration(s) Enclosed

J|A|C|S
ARTICLES
Published on Web 09/11/2003

Asymmetric, Stereocontrolled Total Synthesis of Paraherquamide A

Robert M. Williams,* Jianhua Cao, Hidekazu Tsujishima, and Rhona J. Cox

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received June 16, 2003; E-mail: rmw@chem.colostate.edu

Abstract: The first total synthesis of paraherquamide A, a potent anthelmintic agent isolated from various *Penicillium* sp. with promising activity against drug-resistant intestinal parasites, is reported. Key steps in this asymmetric, stereocontrolled total synthesis include a new enantioselective synthesis of α-alkylated-β-hydroxyproline derivatives to access the substituted proline nucleus and a highly diastereoselective intramolecular S_N2' cyclization to generate the core bicyclo[2.2.2]diazaoctane ring system.

Introduction

The paraherquamides[1-4] are an unusual family of fungal natural products which contain a bicyclo[2.2.2]diazaoctane core structure, a *spiro*-oxindole, and a substituted proline moiety. The parent member, paraherquamide A (**1**), was first isolated from cultures of *Penicillium paraherquei* by Yamazaki and co-workers in 1981.[1] Since then, paraherquamides B−G,[2] VM55595, VM55596, and VM55597,[3] SB203105 and SB200437,[4] and sclerotamide[5] have been isolated from various *Penicillium* and *Aspergillus* species. Marcfortines A−C are structurally similar, containing a pipecolic acid unit in place of proline.[6] Also closely related are VM55599,[3] aspergamides A and B,[7] avrainvillamide (CJ-17,665),[8] and the most recently isolated members of this family, stephacidins A and B .[9] These last six compounds contain a 2,3-disubstituted indole in place of the *spiro*-oxindole. Brevianamides A and B,[10] which contain a *spiro*-indoxyl rather than a *spiro*-oxindole, and the asperparalines, which contain a *spiro*-succinimide,[11] are also structurally comparable (Figure 1).

The paraherquamides have attracted considerable attention due to their molecular complexity, intriguing biogenesis,[12,13] and biological activity. Some members, most notably paraherquamide A, display potent anthelmintic activity and antinematodal properties.[14] Due to the appearance of drug resistance developed by helminths, broad spectrum anthelmintic agents such as the macrolide endectocides, benzimidazoles, tetrahydropyrimidines, and imidazothiazoles are beginning to lose efficacy and there has arisen an urgent need to discover new families of antiparasitic agents. The paraherquamides represent an entirely new structural class of anthelmintic compounds, and as such, they hold great potential as drugs for the treatment of intestinal parasites in animals.[15] The mode of action of the paraherquamides is, as yet, incompletely characterized, but recent work suggests that they are selective competitive cholinergic antagonists.[16]

(1) Yamazaki, M.; Okuyama E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135−136.
(2) (a) Ondeyka, J. G.; Goegelman, R. T.; Schaeffer, J. M.; Kelemen, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375−1379. (b) Liesch, J. M.; Wichmann, C. F. *J. Antibiot.* **1990**, *43*, 1380−1386. (c) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492−497.
(3) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355−1363.
(4) Banks, R. M.; Blanchflower, S. E.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1997**, *50*, 840−846.
(5) Whyte, A. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **1996**, *59*, 1093−1095.
(6) (a) Polonsky, J.; Merrien, M.-A.; Prangé, T.; Pascard, C.; Moreau, S. *J. Chem. Soc., Chem. Commun.* **1980**, 601−602. (b) Prangé, T.; Billion, M.-A.; Vuilhorgne, M.; Pascard, C.; Polonsky, J.; Moreau, S. *Tetrahedron Lett.* **1981**, *22*, 1977−1980.
(7) Fuchser, Jens. Beeinflussung der Sekundarstoffbildung bei *Aspergillus ochraceus* durch Variation der Kulturbedingungen sowie Isolierung, Strukturaufklarung und Biosynthese der neuen Naturstoffe. Ph.D. Thesis, University of Göttingen, Germany, 1995. K. Bielefeld Verlag: Friedland, 1996 (Prof. A. Zeeck).
(8) (a) Fenical, W.; Jensen, P. R.; Cheng, X. C. U.S. Patent 6,066,635, 2000. (b) Sugie, Y.; Hirai, H.; Inagaki, T.; Ishiguro, M.; Kim, Y.-J.; Kojima, Y.; Sakakibara, T.; Sakemi, S.; Sugiura, A.; Suzuki, Y.; Brennan, L.; Duignan, J.; Huang, L. H.; Sutcliffe, J.; Kojima, N. *J. Antibiot.* **2001**, *54*, 911−916.
(9) Qian-Cutrone, J.; Huang, S.; Shu, Y.-Z.; Vyas, D.; Fairchild, C.; Menendez, A.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Gao, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556−14557.
(10) (a) Birch, A. J.; Wright, J. J. *J. Chem. Soc., Chem. Commun.* **1969**, 644−645. (b) Birch, A. J.; Wright, J. J. *Tetrahedron* **1970**, *26*, 2329−2344. (c) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999−3008.
(11) (a) Hayashi, H.; Nishimoto, Y.; Nozaki, H. *Tetrahedron Lett.* **1997**, *38*, 5655−5658. (b) Hayashi, H.; Nishimoto, Y.; Akiyama, K.; Nozaki, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 111−115.
(12) (a) Porter, A. E. A.; Sammes, P. G. *J. Chem. Soc., Chem. Commun.* **1970**, 1103. (b) Baldas, J.; Birch, A. J.; Russell, R. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 50−52. (c) Birch, A. J. *J. Agric. Food Chem.* **1971**, *19*, 1088−1092. (d) Kuo, M. S.; Wiley, V. H.; Cialdella, J. I.; Yurek, D. A.; Whaley, H. A.; Marshall, V. P. *J. Antibiot.* **1996**, *49*, 1006−1013.
(13) (a) Stocking, E. M.; Sanz-Cervera, J. F.; Williams, R. M.; Unkefer, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 7008−7009. (b) Williams, R. M.; Sanz-Cervera, J. F.; Sancenón, F.; Marco, J. A.; Halligan, K. M. *Bioorg. Med. Chem.* **1998**, *6*, 1233−1241. (c) Stocking, E. M.; Williams, R. M.; Sanz-Cervera, J. F. *J. Am. Chem. Soc.* **2000**, *122*, 9089−9098. (d) Stocking, E. M.; Martinez, R. A.; Silks, L. A.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 3391−3392. (e) Stocking, E. M.; Sanz-Cervera, J. F.; Unkefer, C. J.; Williams, R. M. *Tetrahedron* **2001**, *57*, 5303−5320. (f) Stocking, E. M.; Sanz-Cervera, J. F.; Williams, R. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1296−1298.
(14) (a) Ostlind, D. A.; Mickle, W. G.; Ewanciw, D. V.; Andriuli, F. J.; Campbell, W. C.; Hernandez, S.; Mochales, S.; Munguira, E. *Res. Vet. Sci.* **1990**, *48*, 260−261. (b) Shoop, W. L.; Egerton, J. R.; Eary, C. H.; Suhayda, D. *J. Parasitol.* **1990**, *76*, 349−351. (c) Shoop, W. L.; Eary, C. H.; Michael, H. W.; Haines, H. W.; Seward, R. L. *Vet. Parasitol.* **1991**, *40*, 339−341. (d) Shoop, W. L.; Michael, B. F.; Haines, H. W.; Eary, C. H. *Vet. Parasitol.* **1992**, *43*, 259−263. (e) Shoop, W. L.; Haines, H. W.; Eary, C. H.; Michael, B. F. *Am. J. Vet. Res.* **1992**, *53*, 2032−2034. (f) Schaeffer, J. M.; Blizzard, T. A.; Ondeyka, J.; Goegelman, R.; Sinclair, P. J.; Mrozik, H. *Biochem. Pharmacol.* **1992**, *43*, 679−684. For a review, see: (g) Geary, T. G.; Sangster, N. C.; Thompson, D. P. *Vet. Parasitol.* **1999**, *84*, 275−295.

10.1021/ja036713+ CCC: $25.00

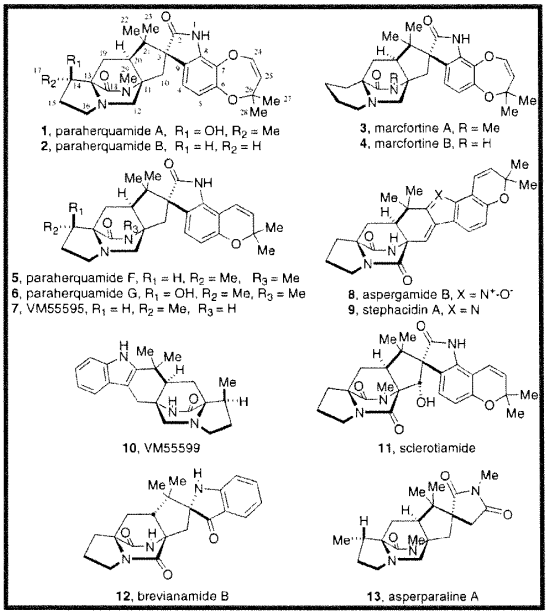
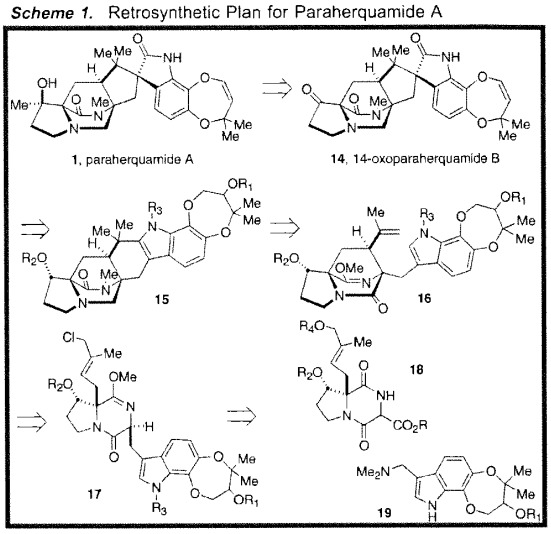


Figure 1. Structures of some paraherquamides and related compounds.

The small quantities of paraherquamide A that can be isolated from cultures for biological study have slowed the development of these agents. Recently, Lee and Clothier reported the interesting semisynthetic conversion of marcfortine A (**3**), a metabolite more readily available by fermentation, into paraherquamide A via paraherquamide B (**2**).[17] Following synthetic studies on brevianamide B (**12**),[18] our laboratory reported the first total synthesis of a member of the paraherquamide family, *ent*-paraherquamide B, in 1993, in which a diastereoselective intramolecular S_N2' cyclization reaction was used to construct the core bicyclo[2.2.2]diazaoctane ring system.[19] We have further exploited this reaction strategy, and we described the first total synthesis of paraherquamide A in 2000.[20] Herein, we detail a full account of this work.

Scheme 1. Retrosynthetic Plan for Paraherquamide A



Synthesis of an α-Alkylated-β-Hydroxyproline

Despite the apparent similarity in the structures of paraherquamides A and B, synthesis of the former turned out to be a significantly more challenging endeavor owing to the presence of the unusual β-hydroxy-β-methyl proline residue. In the semisynthesis of paraherquamide A from marcfortine A (**3**), the final step was addition of methylmagnesium bromide to 14-oxoparaherquamide B (**14**).[17] We planned to use this same methodology to complete our total synthesis and to construct **14** using a similar strategy to that used for paraherquamide B, that is, coupling of suitably functionalized indole (**19**) and diketopiperazine (**18**) units and then an intramolecular S_N2' cyclization followed by palladium-mediated closure of the seventh ring, and finally oxidation and rearrangement of the 2,3-disubstituted indole to the *spiro*-oxindole of 14-oxoparaherquamide B[19] (Scheme 1).

New methodology was now required to prepare a suitably functionalized α-alkylated-β-hydroxyproline residue. A variety of methods were investigated for the asymmetric construction of this class of compound, leading to the development of a potentially general synthetic method which uses dianion alkylation of the readily available *N*-*t*-BOC-β-hydroxyproline ethyl ester derivative **12** with net retention of stereochemistry.[21] This methodology has now successfully been applied to a concise asymmetric and stereocontrolled total synthesis of paraherquamide A.

Epoxide **20**, which is commercially available or made by epoxidation of isoprene with *m*CPBA, was treated with *n*-Bu_4NI and TBSCl to provide iodide **21** as a mixture of geometrical isomers (*E*:*Z* ≈ 6:1) in 58% overall yield. Diester **22** was prepared in two steps from ethyl glycinate and ethyl acrylate, and then a Dieckmann cyclization was conducted, using a slight modification of the procedure described by Rapoport,[22]

(15) (a) Blizzard, T. A.; Marino, G.; Mrozik, H.; Fisher, M. H.; Hoogsteen, K.; Springer, J. P. *J. Org. Chem.* **1989**, *54*, 2657−2663. (b) Blizzard, T. A.; Mrozik, H.; Fisher, M. H.; Schaeffer, J. M. *J. Org. Chem.* **1990**, *55*, 2256−2259. (c) Blizzard, T. A.; Margiatto, G.; Mrozik, H.; Schaeffer, J. M.; Fisher, M. H. *Tetrahedron Lett.* **1991**, *32*, 2437−2440. (d) Blizzard, T. A.; Margiatto, G.; Mrozik, H.; Schaeffer, J. M.; Fisher, M. H. *Tetrahedron Lett.* **1991**, *32*, 2441−2444. (e) Lee, B. H.; Clothier, M. F.; Johnson, S. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 553−554. (f) Lee, B. H.; Clothier, M. F.; Dutton, F. E.; Nelson, S. J.; Johnson, S. S.; Thompson, D. P.; Geary, T. G.; Whaley, H. D.; Haber, C. L.; Marshall, V. P.; Kornis, G. I.; McNally, P. L.; Ciadella, J. I.; Martin, D. G.; Bowman, J. W.; Baker, C. A.; Coscarelli, E. M.; Alexander-Bowman, S. J.; Davis, J. P.; Zinser, E. W.; Wiley, V.; Lipton, M. F.; Mauragis, M. A. *Curr. Top. Med. Chem.* **2002**, *2*, 779−793. (g) Lee, B. H.; Clothier, M. F. U.S. Patent 5,750,695, 1998.

(16) (a) Robertson, A. P.; Clark, C. L.; Burns, T. A.; Thompson, D. P.; Geary, T. G.; Trailovic, S. M.; Martin, R. J. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 853−860. (b) Zinser, E. W.; Wolfe, M. L.; Alexander-Bowman, S. J.; Thomas, E. M.; Davis, J. P.; Groppi, V. E.; Lee, B. H.; Thompson, D. P.; Geary, T. G. *J. Vet. Pharmacol. Ther.* **2002**, *25*, 241−250.

(17) (a) Lee, B. H.; Clothier, M. F. *J. Org. Chem.* **1997**, *62*, 1795−1798. (b) Lee, B. H.; Clothier, M. F.; Pickering, D. A. *J. Org. Chem.* **1997**, *62*, 7836−7840.

(18) Williams, R. M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 808−821.

(19) Cushing, T. D.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **1996**, *118*, 557−579.

(20) Williams, R. M.; Cao, J.; Tsujishima, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2540−2544.

(21) Williams, R. M.; Cao, J. *Tetrahedron Lett.* **1996**, *37*, 5441−5444.

(22) (a) Blake, J.; Willson, C. D.; Rapoport, H. *J. Am. Chem. Soc.* **1964**, *86*, 5293−5299. For more regioselective methods, see: (b) Yamada, Y.; Ishii, T.; Kimura, M.; Hosaka, K. *Tetrahedron Lett.* **1981**, *22*, 1353−1354. (c) Sibi, M. P.; Christensen, J. W.; Kim, S.-G.; Eggen, M.; Stessman, C.; Olen, L. *Tetrahedron Lett.* **1995**, *36*, 6209−6212.

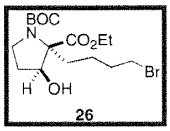
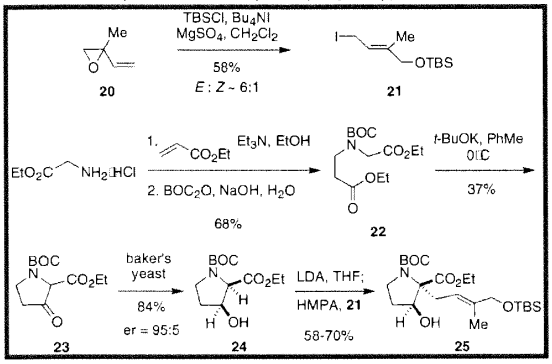


Figure 2. Assignment of relative stereochemistry of **25**.

Scheme 2. Synthesis of α-Alkylated-β-Hydroxyproline **25**



to yield racemic β-ketoester **23** (Scheme 2). Baker's yeast reduction afforded the optically active β-hydroxyester **24** with an enantiomeric ratio of ca. 95:5 as described by Knight et al.[23] Alkylation of the dianion of **24** with substituted allyl iodide **21** proceeded with retention of stereochemistry and excellent diastereoselectivity under the conditions previously developed.[21] The desired α-alkylated product **25** was obtained in 58−70% isolated yield with little or no *O*-monoalkylation or *O*-,*C*-dialkylation taking place. It was interesting to note during large scale synthesis of **25** that the amount of HMPA required in the alkylation reaction ranged from 1.4 to 13.6 equiv depending on the batch of **24** that was used, despite the batches being apparently identical by ^{1}H NMR, IR, TLC, and optical rotation. The reasons for this phenomenon are presently unclear.[24]

The assignment of the relative stereochemistry of **25** was obtained by comparison of the ^{1}H NMR and optical rotation data of **25** to those of **26**, which was obtained by alkylation of **24** with 1,4-dibromobutane. The relative stereochemistry of **26** was assigned unambiguously through single-crystal X-ray analysis (Figure 2).[21] The absolute stereochemistry of **25** was confirmed by Barton deoxygenation and conversion to diketopiperazine (+)**-29** as illustrated in Scheme 3. This same diketopiperazine could be obtained, as the enantiomer, from **30**. This compound has previously been converted to (+)-paraherquamide B, a substance whose absolute stereochemistry has been confirmed.[19]

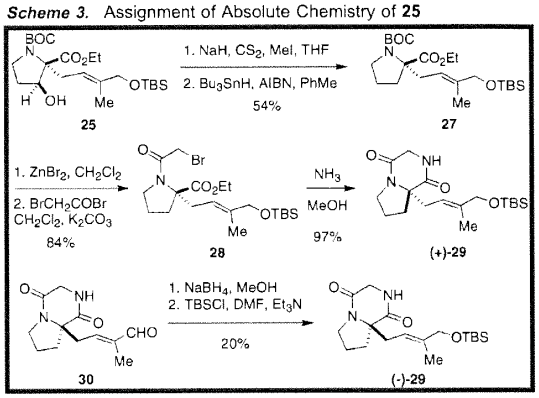
Synthesis of a Functionalized Diketopiperazine

It was necessary to convert the substituted proline (**25**) into a suitably functionalized diketopiperazine for a similar Somei−Kametani coupling reaction to that used in our total synthesis of paraherquamide B. Initial studies on this system were carried out with the secondary alcohol protected as a benzyl ether.

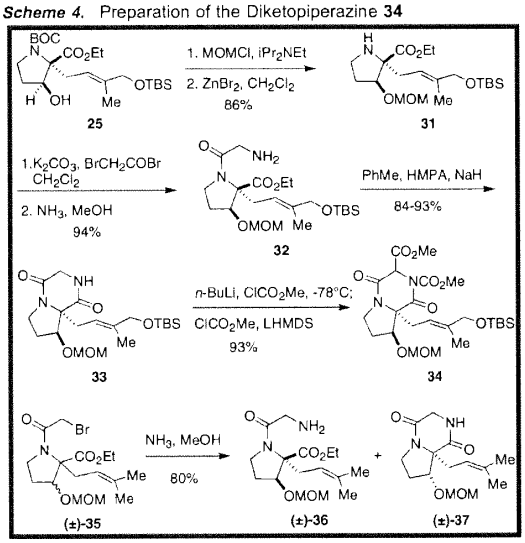
(23) (a) Cooper, J.; Gallagher, P. T.; Knight, D. W. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1313−1317. For alternative baker's yeast reductions, see: (b) Sibi, M. P.; Christensen, J. W. *Tetrahedron Lett.* **1990**, *31*, 5689−5692. (c) Bhide, R.; Mortezaei, R.; Scilimati, A.; Sih, C. J. *Tetrahedron Lett.* **1990**, *31*, 4827−4830.

(24) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1624−1654.

Scheme 3. Assignment of Absolute Chemistry of **25**



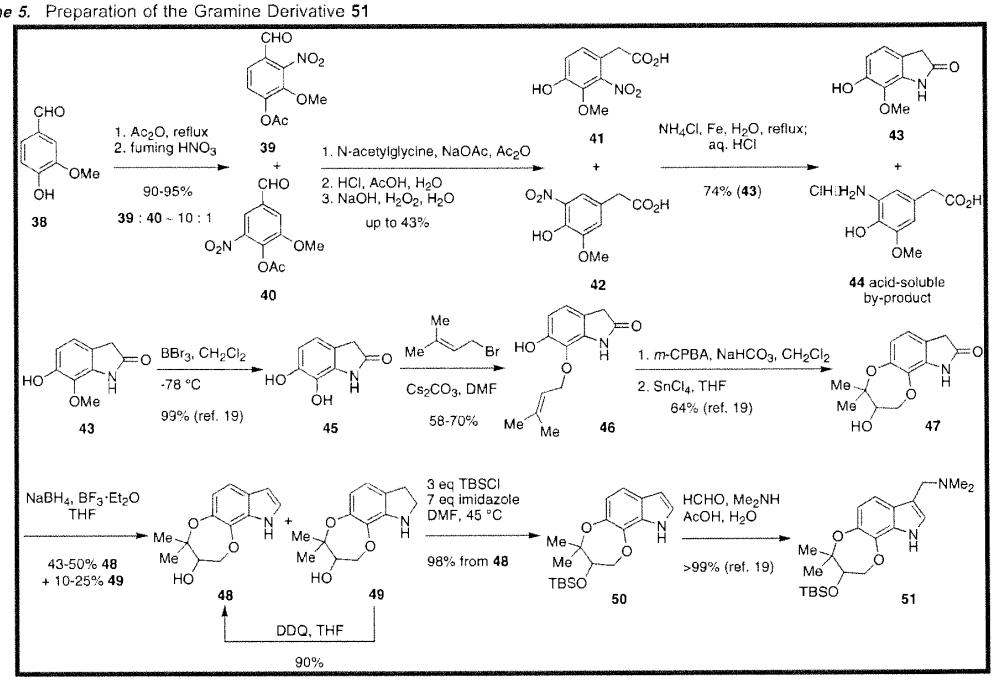
Scheme 4. Preparation of the Diketopiperazine **34**



However, because of problems with selectivity and purification later in the synthesis, the less bulky and more polar methoxymethyl (MOM) protecting group was used in the final synthetic route. After MOM protection of the alcohol, the *N*-*t*-BOC group was smoothly removed with $ZnBr_2$ in dichloromethane[25] and the exposed secondary amine (**31**) was acetylated with bromoacetyl bromide under Schotten−Baumann conditions (Scheme 4). Treatment of the bromoacetamide with methanolic ammonia afforded the corresponding glycinamide (**32**) which was directly subjected to cyclization in the presence of sodium hydride in toluene/HMPA to afford the bicyclic compound **33** in 75% overall yield from **25**. An interesting observation about the ease of closure of hydroxylated diketopiperazines was made during this study. When there is no hydroxyl substituent (e.g., in **28**) or the protected hydroxyl group is *trans* to the ester, the diketopiperazine typically forms spontaneously from the aminoester in methanol at room temperature. On the other hand, a *cis*-isomer such as **31** can be isolated as the aminoester from the amination reaction, and formation of the diketopiperazine requires much more forcing conditions. On amination of a

(25) Nigam, S. C.; Mann, A.; Taddei, M.; Wermuth, C.-G. *Synth. Commun.* **1989**, *19*, 3139−3142.

Scheme 5. Preparation of the Gramine Derivative **51**



mixture of diastereomeric bromoacetamides **35**, the aminoester **36** and the diketopiperazine **37** are produced. This is presumably because the *cis*-diketopiperazine is significantly more sterically hindered. After diketopiperazine formation, a one-pot double carbomethoxylation reaction was performed by the sequential addition of *n*-BuLi in THF followed by addition of methylchloroformate, which carbomethoxylates the amide nitrogen. Subsequent addition of more methylchloroformate followed by LHMDS afforded **34** in 93% yield as an ~6:1 mixture of *E* and *Z* isomers, with the newly created stereogenic center as a single stereoisomer (relative configuration was not assigned).

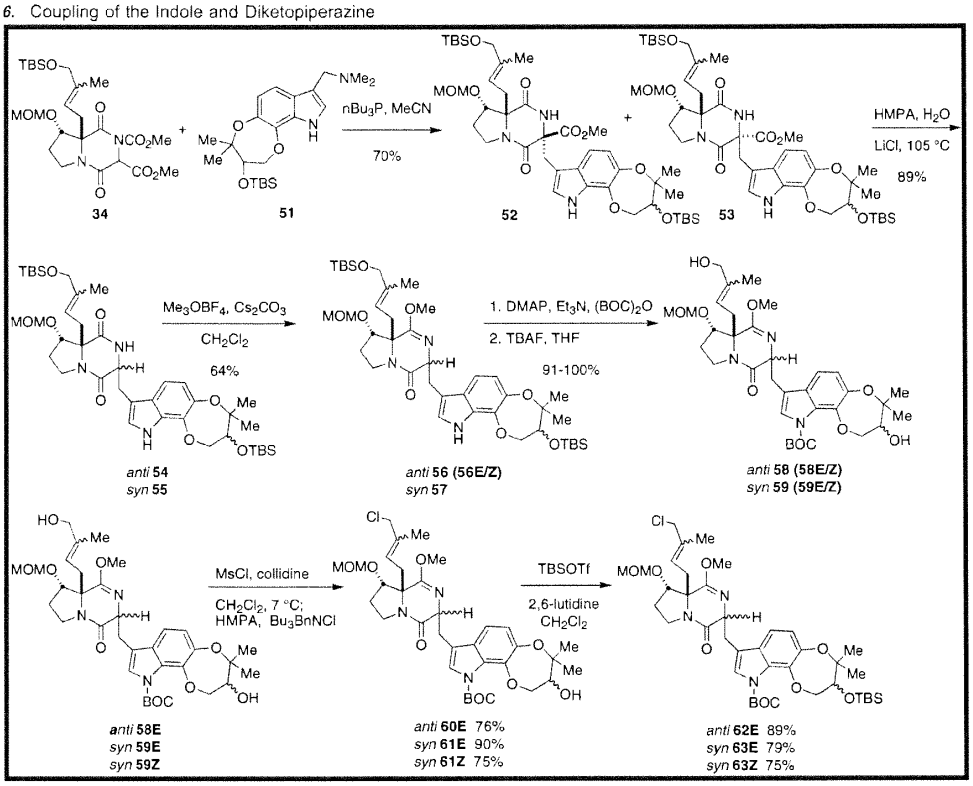
Improved Synthesis of the Gramine Derivative

With this functionalized diketopiperazine in hand, we turned our attention to improvement of the synthesis of the dioxepin-containing indole fragment that we originally described in 1990.[26] The original route provides compound **51** in 14 steps with no chromatography required until the ninth step. However, further optimization was necessary to achieve a more rapid and efficient large-scale synthesis. The route we have developed is illustrated in Scheme 5. Vanillin (**38**) was acetylated with acetic anhydride and then treated with fuming nitric acid to afford **39**, the desired regioisomer, and **40**, the undesired isomer, in an ~10:1 ratio. Initially, these regioisomers were separated by hydrolysis of the acetate group and isolation of the desired phenol isomer by crystallization.[27] Analysis of the product mixture by TLC revealed that **39** had a lower R_f and **40** had exactly the same R_f as that of the starting material, and it was possible to isolate **39** by flash chromatography. However, neither purification method proved optimal for a large-scale protocol. The new approach circumvents these problems. Instead, we directly used the mixture of nitrobenzaldehydes **39** and **40**. After a three-step transformation,[28] **39** provided the desired acid **41**, and **40** provided the undesired acid **42**. Reduction of the nitro group was originally carried out in 95% yield by hydrogenation over palladium on carbon at 40 psi and 80 °C. However, this protocol could prove awkward on a large scale, so an alternative approach was developed using iron and NH_4Cl[29] which, while the yield (74%) is more moderate, proved easier to scale-up. On reduction to the corresponding amines, the amine intermediate from **41** cyclized to oxindole **43**, but **42** was simply reduced to amino acid **44**, which cannot undergo an intramolecular cyclization reaction due to geometric restriction. On extraction of the reaction mixture, the amino acid (**44**) was removed with aqueous acid leaving the oxindole (**43**) in the organic phase. Demethylation then proceeded smoothly as already described to give **45**.[30]

Prenylation of **45** is partially selective for the 7-hydroxy position due to the greater acidity of this hydroxyl group. However, under the prenylation conditions originally developed for paraherquamide B, small amounts of the 6-prenyloxy and 6,7-diprenyloxy isomers were also formed, and the three compounds are difficult to separate by flash chromatography. In this modification of our original route, replacement of the base with Cs_2CO_3 improves the selectivity and yield of **46**.

(26) Williams, R. M.; Cushing, T. D. *Tetrahedron Lett.* **1990**, *31*, 6325−6328.
(27) Raiford, L. C.; Stoesser, W. C. *J. Am. Chem. Soc.* **1928**, *50*, 2556−2563.
(28) (a) MacDonald, S. F. *J. Chem. Soc.* **1948**, 376−378. (b) Beer, R. J. S.; Clarke, K.; Davenport, H. F.; Robertson, A. *J. Chem. Soc.* **1951**, 2029−2032.
(29) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, M. B., Jr.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakantan, R.; Reich, M. F.; Shen, R.; Tsou, H.-R.; Upeslacis, E.; Wang, Y. F.; Wu, B.; Ye, F.; Zhang, N. *J. Med. Chem.* **2000**, *43*, 3244−3256.
(30) Since this route was developed, McWhorter and Savall have published a route to **45** which is shorter and higher yielding, but the applicability of their synthesis on a large scale has not yet been demonstrated. Savall, B. M.; McWhorter, W. W. *J. Org. Chem.* **1996**, *61*, 8696−8697.

Scheme 6. Coupling of the Indole and Diketopiperazine



Extraction into base during the workup procedure also removes the diprenylated byproduct which allowed for easier purification.

A major problem in our first generation synthesis of the gramine derivative was during reduction of the oxindole to the indole, when over-reduction to the indoline occurred in variable quantities giving a ratio of 4:1 to 2:1 of indole/indoline. Attempts were made, without success, to effect a more selective reduction of the oxindole. However, the problem was solved in an indirect fashion as it proved possible to oxidize the indoline byproduct to the indole with DDQ[31] in greater than 90% yield.

Formation of TBS ethers on hindered alcohols is known to be very sensitive to the concentration of the reaction mixture. The silylation reaction was optimized by concentrating the reaction mixture to give an improved yield of 95% from 82%. Finally indole **50** was converted to the gramine derivative **51** under standard conditions. The advantages of this new approach are significant in terms of increased yield, lower cost, and faster synthesis on a large scale.

Coupling of the Indole and Diketopiperazine

Somei–Kametani coupling[32] of diketopiperazine **34** with the gramine derivative **51** in the presence of tri-*n*-butylphosphine gave a separable mixture of two diastereomers **52** and **53** in a 3:1 ratio, each as a mixture of four diastereomers (Scheme 6).[33] Decarbomethoxylation was effected by treatment of **52** and **53** individually with LiCl in hot, aqueous HMPA to provide, in both cases, a mixture of **54** (*anti*-isomer) and **55** (*syn*-isomer), which could now be separated into the *E* and *Z* isomers, each of which as a mixture of two diastereomers (epimeric at the dioxepin secondary alcohol). However, as separation of the geometric isomers proved to be difficult, the compounds were usually carried through the synthetic sequence as a mixture and separated only for analytical purposes. Protection of the secondary amide as the corresponding methyl lactim ether was accomplished by treating **54** and **55** with trimethyloxonium tetrafluoroborate and Cs_2CO_3 in dichloromethane. Model studies had shown that Cs_2CO_3 was a more efficient base than Na_2CO_3 for this reaction, as it leads to a lower incidence of TBS cleavage and *N*-methylation. Next, the indole nitrogen was protected as the corresponding *N*-*t*-BOC derivative by treatment with di-*tert*-butyl dicarbonate in the presence of DMAP, and then the silyl ethers were removed with tetrabutylammonium fluoride (TBAF) to provide **58** (*anti*) and **59** (*syn*). From this point onward, the *E* and *Z* isomers were utilized separately. Unfortunately, the Corey procedure,[34] which had been successful

(31) He, F.; Foxman, B. M.; Snider, B. B. *J. Am. Chem. Soc.* **1998**, *120*, 6417–6418.

(32) (a) Somei, M.; Karasawa, Y.; Kaneko, C. *Heterocycles* **1981**, *16*, 941–949. (b) Kametani, T.; Kanaya, N.; Ihara, M. *J. Chem. Soc., Perkin Trans. 1* **1981**, 959–963.

(33) The stereochemistry at the newly formed stereogenic centers in **52** and **53**, and in all subsequent compounds, was assigned on the basis of ^{1}H NMR data. In compounds where the indole substituent is on the same face of the diketopiperazine as the MOM ether, the signal for the methoxy group is at significantly higher field than in the situation where these two substituents are on opposite faces. This is due to the proximity of the methoxy group to the shielding effects of the aromatic system.

(34) Corey, E. J.; Kim, C. U.; Takeda, M. *Tetrahedron Lett.* **1972**, *13*, 4339–4342.

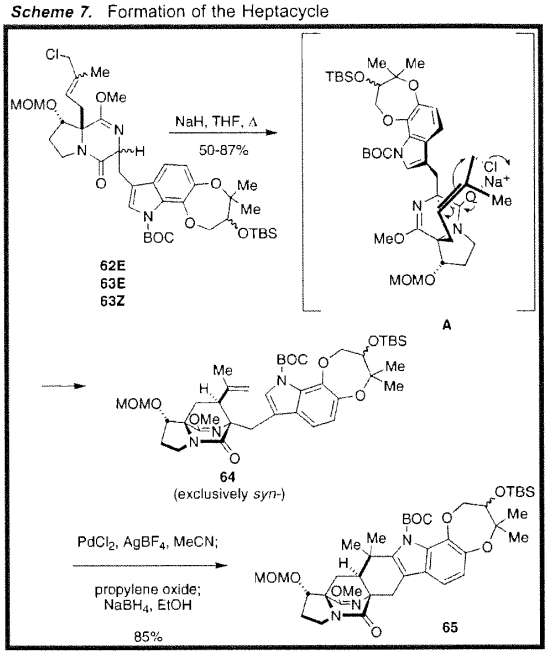
in the synthesis of paraherquamide B for conversion of an allylic alcohol to the corresponding chloride, proved unreliable when applied to the paraherquamide A system. Under the conditions used previously, cleavage of the lactim ether and chlorination at the 2-position of the indole were observed. Extensive investigation into suitable conditions was carried out, and it was eventually found that selective conversion of the primary alcohols **58** and **59** to the corresponding mesylates was possible in the presence of the hindered base collidine. Displacement of mesylate by a chloride ion under these reaction conditions was very slow so Bu_3BnNCl (as an external chloride source) and a polar solvent were added to accelerate the reaction, allowing formation of the allylic chlorides (**60** and **61**) in up to 90% yield. This is a simple, practical, and reproducible method for preparing allylic chlorides in molecules bearing labile functional groups. Finally, careful reprotection of the secondary alcohols with *tert*-butyldimethylsilyl triflate in the presence of 2,6-lutidine afforded the key allylic chlorides **62** and **63**.

S_N2' Cyclization and Closure of the Seventh Ring

The stage was now set for the critical intramolecular S_N2' cyclization, that sets the relative stereochemistry at C-20 during formation of the bicyclo[2.2.2]diazaoctane ring nucleus. Based on precedent from the paraherquamide B synthesis,[19] **63E** was treated with NaH in refluxing benzene. However, the reaction was very slow and gave the desired cyclization product **64** in only 25% yield, accompanied by products from competing pathways. The acidic proton in **63E** is more sterically hindered than in the corresponding substrate for the paraherquamide B synthesis, due to the presence of the MOM ether. Since NaH likely exists as heterogeneous clusters in benzene, it was expected that use of a more coordinating solvent may break up the clusters and render deprotonation more facile. Conveniently, use of NaH in refluxing THF afforded the desired S_N2' cyclization product **64** in 87% yield from **63E** exclusively as the desired *syn*-isomer.[35] This remarkably diastereoselective intramolecular S_N2' cyclization reaction proceeds, in a nonpolar solvent like THF, via a tight, intramolecular ion-pair driven cyclization ("closed" transition state)[36] as shown in Scheme 7. Compound **62E** also underwent the same transformation to give **64** in 82% yield. In both cases, the product was sometimes accompanied by a small amount of Boc-deprotected cyclized product which could be reprotected under standard conditions. In addition, it was interesting to note that the *Z*-isomer, **63Z**, provides the same cyclization product, again with exclusive *syn* selectivity, in 50% yield.

Closure of the seventh ring was attempted using $PdCl_2$ and $AgBF_4$ in acetonitrile followed by $NaBH_4$ to reduce the incipient heptacyclic σ-palladium adduct,[37] reaction conditions which had been successful in the paraherquamide B synthesis.[19] However, the only products isolated under the same conditions with **64** were those appearing to arise from removal of the *N*-*t*-BOC, MOM and lactim ether protecting groups, presumably by HBF_4 generated in situ. To buffer the reaction mixture, propylene oxide was added as an acid scavenger and the reaction now proceeded to give the desired 2,3-disubstituted indole (**65**) in 85% yield.

Scheme 7. Formation of the Heptacycle

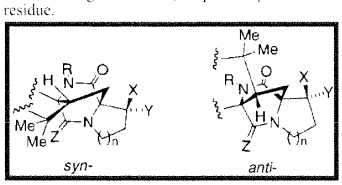


Completion of the Synthesis

Conditions could not be found which would allow direct and high-yielding conversion of the lactim ether (**65**) to the amide. However, use of 0.1 M aqueous HCl in THF gave the corresponding ring-opened amine methyl ester (**66**) which was recyclized to the bicyclo[2.2.2]diazaoctane (**67**) by treatment of **66** with catalytic 2-hydroxypyridine in hot toluene. Chemoselective reduction of the tertiary amide in the presence of the secondary amide to give **68** could be effected by treatment of the diamide **67** with the AlH_3–Me_2NEt complex followed by quenching with sodium cyanoborohydride, methanol, and acetic acid, as used in the synthesis of paraherquamide B. However, use of excess diisobutylaluminum hydride (DIBAL-H) in dichloromethane was a simpler experimental procedure and gave improved yields of **68**.[38] *N*-Methylation of the secondary amide proceeded smoothly and was followed by cleavage of the MOM ether with bromocatecholborane.[39] Oxidation of the secondary alcohol with Dess–Martin periodinane[40] and concomitant removal of the *N*-*t*-BOC group and TBS ether with TFA gave ketone **70** (Scheme 8).

The final critical oxidative spirocyclization of the 2,3-disubstituted indole was effected by a two-step procedure.

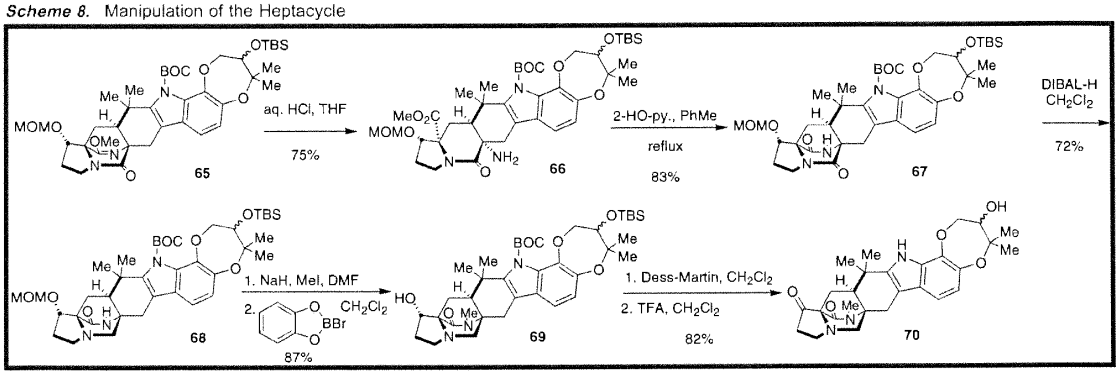
(35) The *syn*/*anti* relationship in this case refers to the relative stereochemistry between the C-20 stereogenic center (see paraherquamide numbering) and the proline residue.



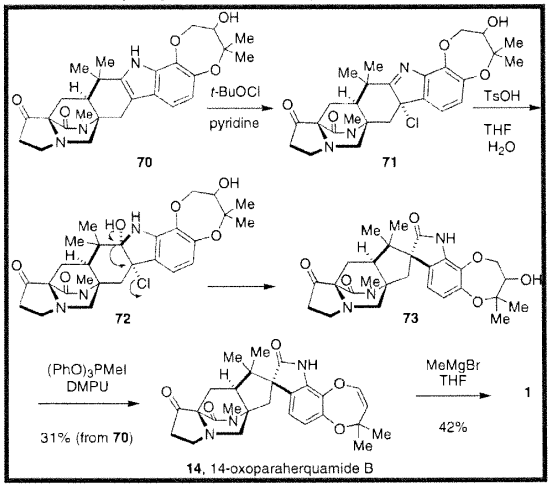
(36) (a) Denmark, S. E.; Henke, B. R. *J. Am. Chem. Soc.* **1989**, *111*, 8032–8034. (b) Denmark, S. E.; Henke, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 2177–2194.

(37) Trost, B. M.; Fortunak, J. M. D. *Organometallics* **1982**, *1*, 7–13.
(38) Fukuyama, T.; Liu, G. *Pure Appl. Chem.* **1997**, *69*, 501–505.
(39) Boeckman, R. K., Jr.; Potenza, J. C. *Tetrahedron Lett.* **1985**, *26*, 1411–1414.
(40) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

Scheme 8. Manipulation of the Heptacycle



Scheme 9. Spirocyclization and Completion of the Synthesis



Treatment of **70** with *tert*-butyl hypochlorite in pyridine provided a labile 3-chloroindolenine, from which it was found necessary to rigorously remove, by azeotroping with benzene, all of the pyridine prior to the next step. Pinacol-type rearrangement with TsOH in aqueous THF then generated the desired *spiro*-oxindole (**73**). From our investigations during the paraherquamide B synthesis, it was found that use of a sterically demanding amine such as pyridine gives the best stereoselectivity during the chlorination reaction. It is assumed that addition of chlorine to **70** proceeds from the least hindered face of the indole giving the α-chloroindolenine **71**. Hydration of the imine functionality, interestingly, must also occur from the same α-face, that is, *syn*-to the relatively large chlorine atom, to furnish the *syn*-chlorohydrin **72** which subsequently rearranges stereospecifically to the desired *spiro*-oxindole **73** (Scheme 9).

Dehydration of the seven-membered ring in **73** with methyl triphenoxyphosphonium iodide (MTPI) in DMPU afforded 14-oxoparaherquamide B (**14**) in moderate yield.[17] This intermediate has been previously obtained semisynthetically from marcfortine A by a group from Pharmacia−Upjohn, and comparison of the authentic and synthetic materials confirmed the identity of this substance. Addition of methylmagnesium bromide to the ketone group of **14** has been previously described to give paraherquamide A along with the corresponding C-14 epimer in around 50% yield.[17a] Employment of this protocol using MeMgBr with our synthetic ketone gave (−)-paraherquamide A (**1**) as the exclusive product (the C-14 epimer was not detected) in 42% yield. This product was identical to a natural sample of paraherquamide A by ^{1}H NMR, ^{13}C NMR, IR, exact mass, and mobility on TLC (R_f). A synthetic sample was recrystallized from ether and had mp 250 °C (dec), $[\alpha]_D^{25}$= −22 (c = 0.2, MeOH). Natural paraherquamide A recrystallized from ether under the same conditions yielded a sample with mp 250 °C (dec) and $[\alpha]_D^{25}$ = −21 (c = 0.2, MeOH). All intermediates up to the final product have an enantiomeric ratio of approximately 97.5:2.5; the final synthetic paraherquamide A upon recrystallization from ether is approximately optically pure.

We have reported here the first total synthesis of paraherquamide A, the most biologically potent member of this family of compounds. This asymmetric synthesis proceeds in 46 steps from commercially available materials, with the longest linear sequence being 34 steps.

The approach developed in this study makes it feasible to examine the design and synthesis of other members of the paraherquamide family and should also permit access to structurally unique paraherquamides that may have significant biological properties. The application of this methodology to the asymmetric, stereocontrolled total synthesis of other members of the paraherquamide family, and evaluation of their properties is currently under study in these laboratories.

Acknowledgment. This work was supported by the NIH (Grant CA 70375). The Japanese Society for the Promotion of Science (JSPS) is acknowledged for providing fellowship support to H.T. Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631. We also wish to thank Dr. Timothy Blizzard of Merck & Co. for providing an authentic sample of natural paraherquamide A. We also wish to acknowledge Dr. Byung H. Lee of the Pharmacia−Upjohn Co. (now Pfizer) for providing NMR spectra and an authentic specimen of 14-oxoparaherquamide B. We also wish to thank Dr. Alfredo Vazquez for assistance with purification and characterization of synthetic paraherquamide A.

Supporting Information Available: Complete experimental procedures and spectroscopic and analytical data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JA036713+

Stereocontrolled Total Synthesis of (+)-Paraherquamide B[⊥]

Timothy D. Cushing,[‡] Juan F. Sanz-Cervera,[†] and Robert M. Williams*

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received August 7, 1995[⊗]

Abstract: The convergent stereocontrolled, asymmetric total synthesis of (+)-paraherquamide B is described. Key features of this synthesis include (1) an improved procedure to effect reduction of unprotected oxindoles to indoles; (2) a complex application of the Somei/Kametani coupling reaction; (3) a high-yielding and entirely stereocontrolled intramolecular S_N2' cyclization reaction that constructs the core bicyclo[2.2.2] ring system; (4) a mild Pd(II)-mediated cyclization reaction that constructs a complex tetrahydrocarbazole; and (5) the chemoselective reduction of a highly hindered tertiary lactam in the presence of an unhindered secondary lactam, utilizing precoordination of the more reactive secondary lactam to triethylaluminum.

Introduction

The paraherquamides are complex, heptacyclic, toxic mold metabolites with potent anthelmintic activity isolated from various *Penicillium* sp. The parent and most potent derivative, paraherquamide A (**1**), was first isolated from *Penicillium parherquei* in 1980 by Yamazaki.[1] The simplest member, paraherquamide B (**2**), plus five other structurally related paraherquamides C−G (**3**−**9**) were isolated from *Penicillium charlesii (fellutanum)* (ATCC 20841) in 1990 at Merck & Co.[2,3] and concomitantly at SmithKline Beecham.[4] More recently three additional related compounds were discovered by the same group at SmithKline.[5] Interest in the paraherquamides has come from the finding that this class of alkaloids displays potent anthelmintic and antinematodal properties.[6,7]

There are essentially three classes of broad-spectrum anthelmintics currently in use: the benzimidazoles, the levamisoles/morantels, and the avermectins/milbemycins. Unfortunately, the first two groups have lost much of their utility due to the recent appearance of drug resistance built up by the helminths.[7a,8] More recently drug resistance to the avermectins has been observed in various parasites.[9] The paraherquamides represent an entirely new structural class of antiparasitic agents, which promise to play a significant role in the near future. The relatively low culture yields of paraherquamide obtained for biological study have slowed the development and potential commercialization of these agents (Figure 1).

As part of our ongoing efforts to elucidate the biosynthesis of the core bicyclo[2.2.2] ring system of the related alkaloids the brevianamides,[10] we have applied methodology originally developed for the stereocontrolled total synthesis of (−)-brevianamide B[11] to complete the first stereocontrolled total synthesis of (+)-paraherquamide B (**12**);[12] the results of this study are described in full herein.

The paraherquamides are structurally very similar to brevianamides A and B (**17** and **16**)[13] and marcfortines A−C (**13**−**15**)[14] with respect to the common core bicyclo[2.2.2] ring system that is derived from the cycloaddition of an isoprene unit across the amino acid α-carbons. This close structural similarity might imply a related biogenesis, and the structural features of these substances shall be described briefly from this standpoint. The paraherquamides and brevianamides A and B (**17** and **16**) appear to be derived from the condensation of tryptophan and proline, while the marcfortines are formed from the condensation of tryptophan and pipecolic acid. The origin of the methyl group in the pyrrolidine ring of paraherquamides A and C−E and VM55595-7 could in principle come from the methylation of proline, but it seems more likely that this amino acid residue is derived from isoleucine. The very low fermentation yield of paraherquamide B may be a manifestation of poor incorporation of cyclo-L-trp-L-pro into the subsequent biosynthetic machinery

[⊥] Dedicated to Professor Ei-ichi Negishi on the occasion of his 60th birthday.

[†] On leave from the Department of Organic Chemistry of the University of Valencia, Spain.

[‡] Present address: Tularik Inc., 270 East Grand Ave., South San Francisco, CA 94080.

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1995.

(1) (a) Yamazaki, M.; Fujimoto, H.; Okuyama, E.; Ohta, Y. *Proc. Jpn. Assoc. Mycotoxicol.* **1980**, *10*, 27. (b) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135.

(2) (a) Blizzard, T. A.; Marino, G.; Mrozik, H.; Fisher, M. H.; Hoogsteen, K.; Springer, J. P. *J. Org. Chem.* **1989**, *54*, 2657. (b) Blizzard, T. A.; Mrozik, H.; Fisher, M. H.; Schaeffer, J. M. *J. Org. Chem.* **1990**, *55*, 2256. (c) Blizzard, T. A.; Margiatto, G.; Mrozik, H.; Schaeffer, J. M.; Fisher, M. H. *Tetrahedron Lett.* **1991**, *32*, 2437. (d) Blizzard, T. A.; Margiatto, G.; Mrozik, H.; Schaeffer, J. M.; Fisher, M. H. *Tetrahedron Lett.* **1991**, *32*, 2441. (e) Blizzard, T. A.; Rosegay, A.; Mrozik, H.; Fisher, M. H. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 461.

(3) (a) Ondeyka, J. D.; Goegelman, R. T.; Schaeffer, J. M.; Kelemen, L. *J. Antibiot.* **1990**, *43*, 1375. (b) Liesch, J. M.; Wichman, C. F. *J. Antibiot.* **1990**, *43*, 1380.

(4) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492.

(5) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355.

(6) Ostlind, D. A.; Mickle, W. G.; Ewaneiw, D. V.; Andriuli, F. J.; Campbell, W. C.; Hernandez, S.; Mochaeles, S.; Munguira, E. *Res. Vet. Sci.* **1990**, *48*, 260.

(7) (a) Shoop, W. L.; Egerton, J. R.; Eary, C. H.; Suhayda, D. *J. Parasitol.* **1990**, *76* (2) 186. (b) Shoop, W. L.; Michael, B. F.; Haines, H. W.; Eary, C. H. *Vet. Parasitol.* **1992**, *43*, 259. (c) Schaeffer, J. M.; Blizzard, T. A.; Ondeyka, J.; Goegelman, R.; Sinclair, P. J.; Mrozik, H. *Biochem. Pharmacol.* **1992**, *43*, 679. (d) Shoop, W. L.; Haines, H. W.; Eary, C. H.; Michael, B. F. *Am. J. Vet. Res.* **1992**, *53*, 2032.

(8) Coles, G. C. *Pestic. Sci.* **1977**, *8*, 536.

(9) (a) Van Wyk, J. A.; Malan, F. S. *Vet. Rec.* **1988**, *123*, 226. (b) Echevarria, F. A. M.; Trindade, N. P. *Vet. Rec.* **1989**, *124*, 147.

(10) (a) Sanz-Cervera, J. F.; Glinka, T.; Williams, R. M. *J. Am. Chem. Soc.* **1993**, *115*, 347. (b) Sanz-Cervera, J. F.; Glinka, T.; Williams, R. M. *Tetrahedron* **1993**, *49*, 8471.

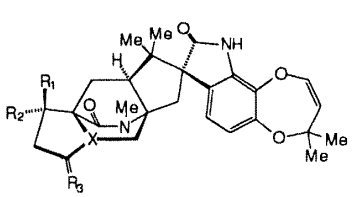
(11) (a) Williams, R. M.; Glinka, T.; Kwast, E. *J. Am. Chem. Soc.* **1988**, *110*, 5927. (b) Williams, R. M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 808. (c) Williams, R. M.; Glinka, T.; Kwast, E. *Tetrahedron Lett.* **1989**, *30*, 5575.

(12) A preliminary account of this work has appeared: Cushing, T. D.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **1993**, *115*, 9323.

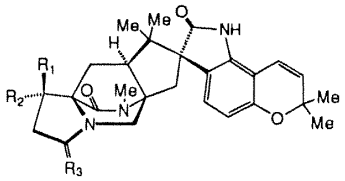
(13) (a) Birch, A. J.; Wright, J. J. *J. Chem. Soc., Chem. Commun.* **1969**, 644. (b) Birch, A. J.; Wright, J. J.; *Tetrahedron* **1970**, *26*, 2339. (c) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999. (d) Baldas J.; Birch, A. J.; Russell, R. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 50.

(14) (a) Polonsky, J.; Merrien, M. A.; Prange, T; Pascard, C. *J. Chem Soc., Chem Commun.* **1980**, 601. (b) Prange, T.; Billion, M.-A.; Vuilhorgne, M.; Pascard, C.; Polonsky, J. *Tetrahedron Lett.* **1981**, *22*, 1977.

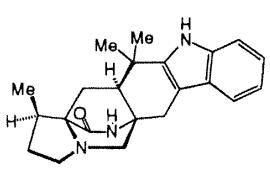
0002-7863/96/1518-0557$12.00/0



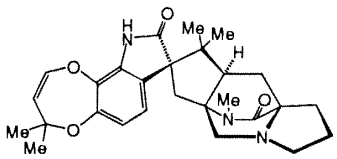
1, (−)-paraherquamide A, R_1 = OH, R_2 = Me, R_3 = H_2, X = N
2, (−)-paraherquamide B, R_1 = H, R_2 = H, R_3 = H_2, X = N
3, (−)-paraherquamide C, R_1 = R_2 = CH_2, R_3 = H_2, X = N
4, (−)-paraherquamide D, R_1 = O, R_2 = CH_2, R_3 = H_2, X = N
5, (−)-paraherquamide E, R_1 = H, R_2 = Me, R_3 = H_2, X = N
6, VM55596, R_1 = H, R_2 = Me, R_3 = H_2, X = N$^+$-O$^-$
7, VM55597, R_1 = H, R_2 = Me, R_3 = O, X = N



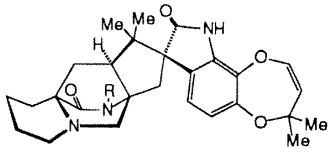
8, (-)-paraherquamide F, R_1 = H, R_2 = Me, R_3 = Me
9, (-)-paraherquamide G, R_1 = OH, R_2 = Me, R_3 = Me
10, VM55595, R_1 = H, R_2 = Me, R_3 = H



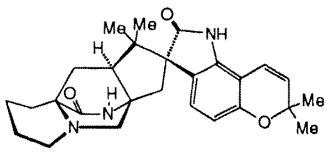
11, VM55599



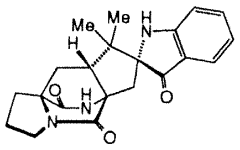
12, (+)-paraherquamide B



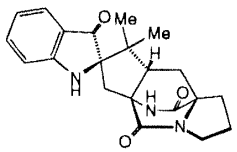
13, (−)-marcfortine A, R = Me
14, (−)-marcfortine B, R = H



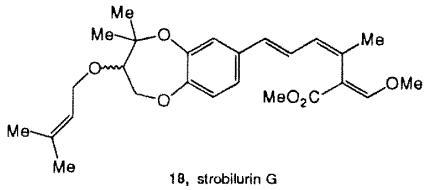
15, (−)-marcfortine C



16, (+)-brevianamide B



17, (+)-brevianamide A



18, strobilurin G

Figure 1.

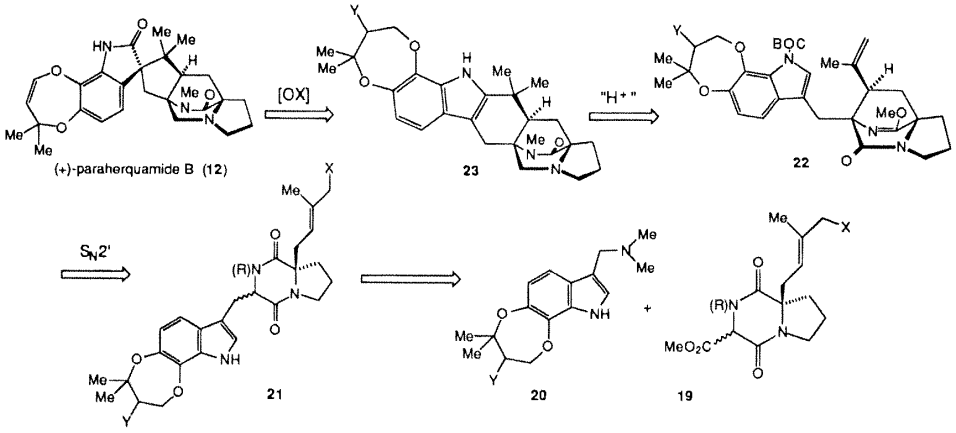
or may be the result of inefficient demethylation of the isoleucine-derived amino acid precursor.

The oxidation state of the amino acid-derived dioxopiperazine moiety remains unchanged in the case of the brevianamides, but for the paraherquamides and the marcfortines the tertiary amide residue is enzymatically reduced to a monooxopiperazine, a process that is known.[15] The tryptophan-derived indolyl side chain of the paraherquamides and marcfortines is oxidized to spiro-oxindoles while the indolyl side chain of the brevianamides oxidize to spiro-indoxyls. The paraherquamides, marcfortines, and brevianamides all incorporate one isoprene unit that forms the bridging bicyclo[2.2.2] ring structure. The paraherquamides and marcfortines differ from the brevianamides in that a second isoprene unit coupled with an oxidized form of tryptophan gives the dioxepin (or pyran) moiety. This is one of the most interesting and unique features of these compounds. The *gem*-dimethyl dioxepin ring found in paraherquamides A−E (**1**−**5**) and marcfortines A and B (**13** and **14**) is a unique ring system among natural products. A similar structural feature was discovered in the antifungal natural product strobilurin G (**18**),[16] but this dioxepin moiety lacks the double bond found in the other metabolites (Figure 1).

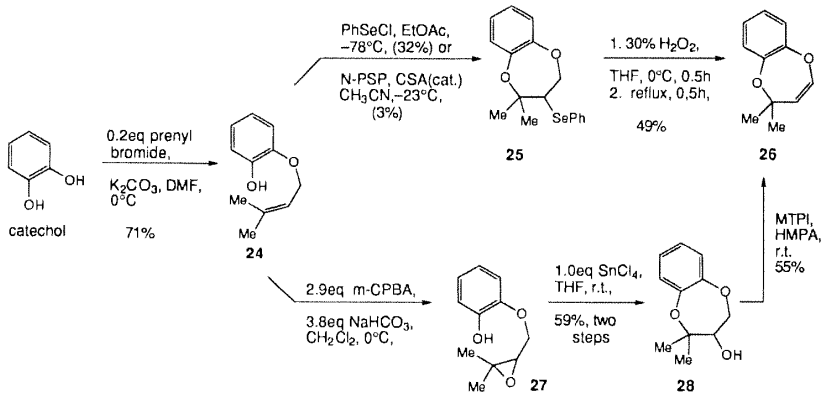
As outlined in Scheme 1, a convergent synthesis of the enantiomer of paraherquamide B (**12**)[17] was envisioned to contain four key carbon−carbon bond-forming reactions. The first task would involve the construction of a suitably α-alkylated proline derivative.[11] The second important coupling would be the Somei/Kametani-type alkylation[18] of a suitably protected gramine derivative (**20**) and the requisite piperazinedione (**19**). The third and perhaps most crucial C−C bond-forming reaction, providing the core bicyclo[2.2.2] ring system, was a stereofacially controlled intramolecular S_N2' cyclization reaction that sets the stereochemistry at C-20 (paraherquamide numbering) and concomitantly installs the isopropenyl group that will be utilized in the fourth C−C bond-forming reaction. This isopropenyl group, in turn, would be conscripted for an olefin−cation cyclization to provide the heptacyclic tetrahydrocarbazole. Standard procedures to effect this transformation involve strong protic acids,[11,19] and there was reason for concern about the reactivity of the more highly oxygenated indole (**22**) as a practical synthetic precursor to **23**. The penultimate step, a regio- and stereofacially controlled oxidative spirocyclization reaction, must be accomplished to construct the desired spirooxindole. A number of these transformations were explored during the course of the investigations on the synthesis of (−)-brevianamide B,[11] including a simple oxindole model study,[11c] which set a firm foundation for addressing some of the

(15) Bond, R. F.; Boeyens, J. C. A.; Holzapfel, C. V.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1751.
(16) Fredenhagen, A.; Hug, P.; Peter, H. H. *J. Antibiot.* **1990**, *48*, 661.
(17) The enantiomer of the natural product was selected as the target due to the large relative cost difference between (*S*)- and (*R*)-proline.
(18) (a) Somei, M.; Karasawa, Y.; Kaneko, C. *Heterocycles* **1981**, *16*, 941. (b) Kametani, T.; Kanaya, N.; Ihara, M. *J. Am. Chem. Soc.* **1980**, *102*, 3974.
(19) (a) Stoermer, D.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 564. (b) Guller, R.; Dobler, M.; Borschberg, H.-J. *Helv. Chim. Acta* **1991**, *74*, 1636. (c) Darbre, T.; Nussbaumer, C.; Borschberg, H.-J. *Helv. Chim. Acta* **1984**, *67*, 1040. (d) Delpech, B.; Khuong-Huu, Q. *J. Org. Chem.* **1978**, *43*, 4898.

Scheme 1



Scheme 2



stereochemical and regiochemical issues that would be faced in attacking the paraherquamide ring system.

Results and Discussion

Construction of the Dioxepinooxindole Ring System. The prenylated catechol ring system of the paraherquamides is an unusual oxidative cyclization product that previously has not been observed to occur in metabolites of mixed biogenetic origin. Although the parent 2*H*-1,5-benzodioxepin has been synthesized previously,[20] to the best of our knowledge there has been no reported synthesis of the corresponding isoprenyl dioxepin ring system of paraherquamide. The synthesis of this ring system was explored in a simple model study employing prenylated catechol **24** (Scheme 2).[21] It was speculated that the requisite 7-*endo-tet* cyclization reaction would be facilitated by a stabilized tertiary carbocation provided by the prenyl substituent.

The first attempt at effecting this cyclization reaction employed a phenylselenoetherification.[22] Following a procedure of Clive,[23] **24** cyclized to **25** with either PhSeCl or *N*-phenylselenophthalimide (N-PSP),[24] although in very low yield. The main byproducts came from the electrophilic addition across the double bond, electrophilic aromatic substitution of the phenyl ring by the phenyl selenide, and phenolic attack at the methylene producing the six-membered-ring product. The selenide **25** was treated with H_2O_2 and the resulting selenoxide thermally eliminated providing the unique dioxepin **26** in 49% yield.

Due to the low yield of the phenylselenoetherification, an alternative procedure involving epoxidation followed by a Lewis acid-mediated ring closure was investigated.[25] The prenylated catechol **24** was epoxidized with buffered *m*-CPBA to provide epoxide **27**, which was treated with stannic chloride to give the dioxepin **28**. A major side product in this reaction was a ketone,

(20) Guillaumet, G.; Coudert, G.; Loubinnoux, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 64.

2H-1,5-benzodioxepin

(21) Williams, R. M.; Cushing, T. D. *Tetrahedron Lett.* **1990**, *31*, 6325.

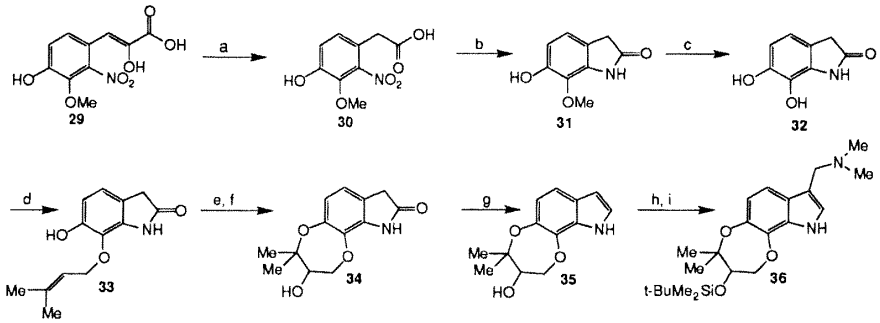
(22) (a) Nicolaou, K. C. *Tetrahedron* **1981**, *37*, 4097. (b) Nicolaou, K. C.; Magolda, R. L.; Sipio, W. J.; Barnette, W. E.; Lysenko, Z.; Joullie, M. M. *J. Am. Chem. Soc.* **1980**, *102*, 3784. (c) Clive, D. L. J. *Tetrahedron* **1978**, *34*, 1049−1132.

(23) (a) Clive, D. J. L.; Chiiattu, G.; Curtis, N. J.; Kiel, W. A.; Wong, C. K. *J. Chem. Soc., Chem. Commun.* **1977**, 725. See also: (b) Liotta, D.; Zima, G. *Tetrahedron Lett.* **1978**, *50*, 4977. (c) Tiecco, M.; Testaferri, L.; Tingoli, M.; Bartoli, D.; Balducci, R. *J. Org. Chem.* **1990**, *55*, 429.

(24) Nicolaou, K. C.; Claremon, D. A.; Barnette, W. E.; Seitz, S. P. *J. Am. Chem. Soc.* **1979**, *101*, 3704.

(25) (a) Cookson, R. C.; Liverton, N. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1589. (b) Kocienski, P.; Love, C.; Whitby, R.; Roberts, D. A. *Tetrahedron Lett.* **1988**, *29*, 2867. See also: (c) Nicolaou, K. C.; Prasad, C. V. C.; Somers, P. K.; Hwang, C.-K. *J. Am. Chem. Soc.* **1989**, *111*, 5335.

Scheme 3[a]



[a] Reagents and conditions: (a) 4.0 equiv of NaOH, 1.0 equiv of 30% H_2O_2, 81−93%; (b) H_2, Pd/C, AcOH, 92%; (c) 2.5 equiv of BBr_3, CH_2Cl_2, −78 °C, 99%; (d) 1.2 equiv of prenyl bromide, 1.1 equiv of K_2CO_3, DMF, 0 °C to room temperature, 52%; (e) *m*-CPBA, $NaHCO_3$, CH_2Cl_2; (f) 1.2 equiv of $SnCl_4$, THF, 64%; (g) 1.6 equiv of $NaBH_4$, 3.5 equiv of BF_3·OEt_2, THF, 44−50%; (h) *t*-$BuMe_2SiCl$, im, DMF, 40 °C, 83%; (i) CH_2O, $HNMe_2$, AcOH, H_2O, 99%.

resulting from a 1,2 hydride shift.[26] A number of methods were explored to effect the dehydration of the secondary alcohol of dioxepin **28**; the best result was realized with methyltriphenoxyphosphonium iodide (MTPI) in HMPA to provide **26**.[27]

With a proven method accessible for the construction of the dioxepin ring system, attention was focused on constructing the requisite gramine derivative. Oxygenated indoles are notoriously unstable and undergo facile autoxidation,[28] photooxidation,[29] dimerization, and polymerization.[30] In light of this problematic reactivity, our plan called for formation of the dioxepin ring system prior to indole (gramine) formation. The approach employed involved the formation of a suitably substituted oxindole (essentially a protected indole), followed by the construction of the dioxepin and final elaboration into the gramine derivative.

The known pyruvic acid **29** (Scheme 3)[31] (prepared in five steps from vanillin) was oxidatively decarboxylated[32] to afford the phenylacetic acid **30**, which was reductively cyclized to give the required oxindole **31**[33] in nearly quantitative yield.

At this point, a method was needed to differentiate between the two phenolic substituents for the prenylation reaction. A number of attempted selective protecting group strategies were explored, but nothing satisfactory was found; it was thus decided to forgo any protecting group for the 6-hydroxy position. Oxindole **31** was cleanly demethylated upon treatment with (clear) boron tribromide. The resulting oxindole **32** was subjected to the prenylation conditions, and the desired alkylated product **33** was obtained in 52% yield.[34,35] Both of the methods discussed above for the formation of the seven-membered ring were examined. The phenylselenoetherification procedure failed on this substrate, and only products resulting from electrophilic aromatic substitution were formed.

The alternative epoxidation/Lewis acid-mediated cyclization again proved to be successful on this substrate. The epoxidation reaction (*m*-CPBA) had to be buffered with $NaHCO_3$, to prevent the formation of the six-membered-ring tertiary alcohol. In most cases, the reaction was worked up and taken on to the next step without purification (the labile epoxide tended to cyclize to the six-membered tertiary alcohol upon contact with silica gel). The incipient epoxide product was directly treated with $SnCl_4$ in THF to provide the desired seven-membered-ring alcohol **34** (64% overall yield from **33**).

N-Alkylated oxindoles have been reported to be reduced to indoles by the use of DIBAL or $LiAlH_4$;[36] however, in the case of unsubstituted oxindoles, this reduction either fails or requires

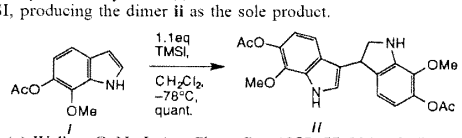
(26) For a related observation, see: Taylor, S. T.; Davisson, M. E.; Hissom, B. R., Jr.; Brown, S. J.; Pristach, H. A.; Schramm, S. B.; Harvey, S. M. *J. Org. Chem.* **1987**, *52*, 425.

(27) Hutchins, R. O.; Hutchins, M. G.; Milewski, C. A. *J. Org. Chem.* **1972**, *37*, 4191.

(28) Houlihan, W. J.; Remers, W. A.; Brown, R. K. *Indoles, Part one, The Chemistry of Heterocycles;* John Wiley & Sons, Inc.: New York, 1972.

(29) (a) Chan, A. C.; Hilliard, P. R., Jr. *Tetrahedron Lett.* **1989**, *30*, 6483. (b) d'Ischia, M.; Prota, G. *Tetrahedron* **1987**, *43*, 431.

(30) This difficulty was observed in a short synthesis of the known 6-acetoxy-7-methoxyindole (**i**). The unstable substance **i** was treated with TMSI, producing the dimer **ii** as the sole product.



i *ii*

See: (a) Walker, G. N. *J. Am. Chem. Soc.* **1955**, *77*, 3844. (b) Burton, H.; Duffield, J. A.; Prail, P. F. G. *J. Chem. Soc.* **1950**, 1062. (c) Beer, R. J. S.; Mcgrath, L.; Robertson, A.; Woodier, A. B. *J. Chem. Soc.* **1949**, 2061. (d) Beer, R. J S.; Clarke, K.; Khorana, H. G.; Robertson, A. *J. Chem. Soc.* **1948**, 2223. (e) Chan, A. C.; Hilliard, P. R., Jr. *Tetrahedron Lett.* **1989**, *30*, 6483. (f) d'Ischia, M.; Prota, G. *Tetrahedron* **1987**, *43*, 431. (g) Deibel, R. M. B.; Chedekel, M. R. *J. Am. Chem. Soc.* **1984**, *106*, 5884. (h) Heacock, R. A. *Chem. Rev.* **1959**, *59*, 181.

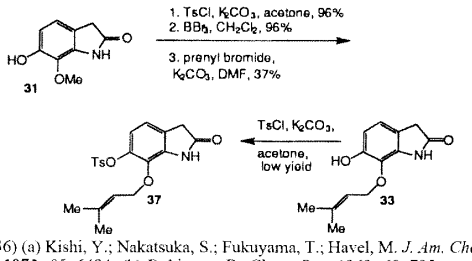
(31) (a) Beer, R. J. S.; Clarke, K.; Davenport, H. F.; Robertson, A. *J. Chem. Soc.* **1951**, 2029. (b) Bennington, F.; Morin, R. D.; Clark, L. C., Jr. *J. Org. Chem.* **1959**, *24*, 917.

(32) Kosuge, T.; Ishida, H.; Inabe, A.; Nukaya, H. *Chem. Pharm. Bull.* **1985**, *33*, 1414.

(33) This material has interesting chemical and physical characteristics. The solvent must be removed immediately after the hydrogenolysis to prevent the white product from turning to a black sludge. This oxindole **31** would also change from a white color to a metallic gray simply by drying on the vacuum pump. These decomposition properties are no doubt due to the autoxidation of the indole tautomer form.

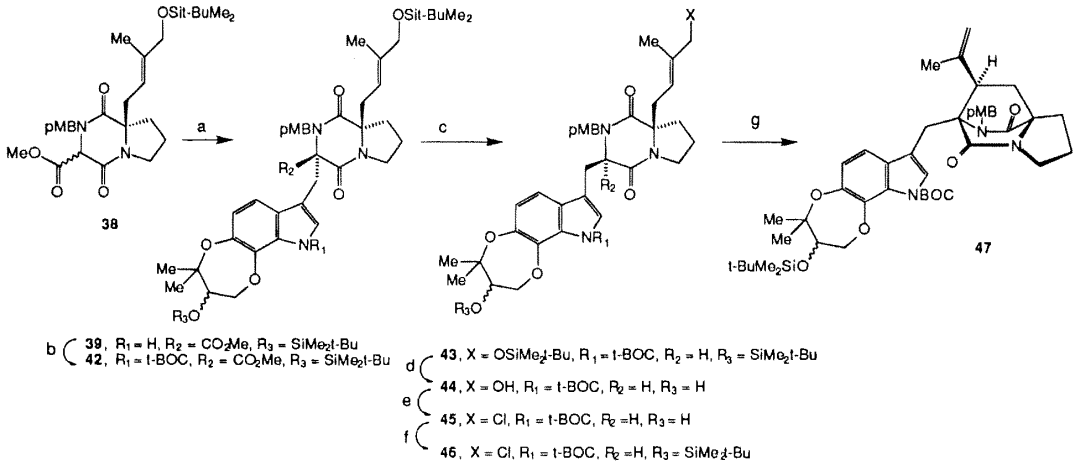
(34) The undesired regioisomer was obtained in less than 1% yield, and the bis-alkylated material was produced in only 8.3% yield. This selectivity is presumably a manifestation of the domination of inductive effects of the amide functionality directing the alkylation to the 7-position.

(35) The structure of compound **33** was confirmed by simply tosylating **33** and comparing the product (**37**) to the same substance prepared from **31**. The two independently synthesized products were identical in every way.



(36) (a) Kishi, Y.; Nakatsuka, S.; Fukuyama, T.; Havel, M. *J. Am. Chem. Soc.* **1973**, *95*, 6494. (b) Robinson, B. *Chem. Rev.* **1969**, *69*, 785.

Scheme 4[a]



[a] Reagents and conditions: (a) **36**, 0.5 equiv of PBu_3, MeCN, 51%; (b) DMAP, Et_3N, BOC_2O, CH_2Cl_2, 90%; (c) 5 equiv of LiCl, 1.5 equiv of H_2O, HMPA, 100 °C, 66%; (d) 3.0 equiv of *n*-Bu_4NF, THF, 79%; (e) 1.9 equiv of LiCl, 4.0 equiv of collidine, 4.0 equiv of MsCl, DMF, 86%; (f) *t*-$BuMe_2SiOTf$, 2,6-lutidine,CH_2Cl_2, 76%; (g) 10 equiv of NaH, benzene, 11%.

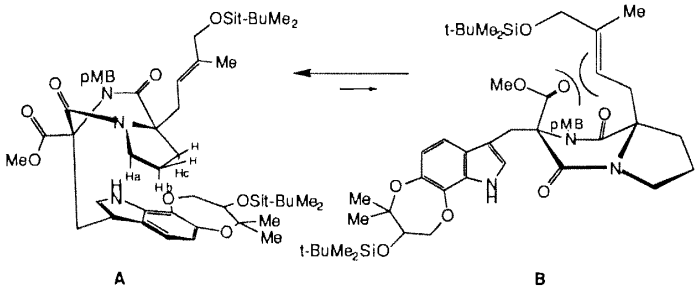


Figure 2.

more vigorous conditions. In 1972 it was reported[37] that substituted and unsubstituted oxindoles could be reduced to the corresponding indole in high yields with borane in THF at 0 °C. Oxindole **34** was subjected to these conditions (1.0 M BH_3/THF, Aldrich), but with no reaction. However, when oxindole **34** was treated with 1.6 equiv of $NaBH_4$ and 3.5 equiv of $BF_3 \cdot OEt_2$ in THF for 1 day (0 °C to room temperature), the desired indole **35** was obtained in 43−50% yield. The indole **35** was treated with a warm solution of TBDMSCl and imidazole in DMF, to provide the required O-silylated indole, which was easily converted to the gramine **36** through the well-known Mannich procedure (Scheme 3).

Construction of the Bicyclo[2.2.2] Ring System. To probe the stability of the dioxepin−indole in subsequent transformations, a model study involving the previously synthesized racemic piperazinedione **38**[38] was investigated (Scheme 4). Indole **36** was condensed with the piperazinedione **38** following the Somei/Kametani conditions[18] to give the desired *syn* product **39** (a racemic mixture of two diastereomers) in 51% yield. The relative stereochemistry of this substance was evident by an examination of the ^1H NMR spectrum. There is a large upfield shift of the proline ring protons of **39** (δ Ha, Hb, Hc; 0.03−0.19 (m), 0.43−0.52 (m), 0.62−0.72 (m) ppm). It is well-known that N-alkylated piperazinediones prefer to adopt a boat-like conformation due to the planar geometry of the amides and A-1,3 steric interactions of *N*-alkyl residues. This forces the substituents on the amino acid α-carbons to adopt either pseudoaxial or pseudoequatorial dispositions. In conformer **B** (Figure 2) the carbomethoxy group is sterically congested by the bulky isopentenyl group, favoring the alternate boat conformer (**A**), which positions the indole ring under the piperazinedione, positioning the two pyrrolidine protons Ha and Hb directly over the shielding cone of the aromatic indole ring system; the corresponding *anti*-isomer cannot adopt this type of conformation. Furthermore, a consideration of the mechanism of the Somei/Kametani reaction[18] supports the expectation that the *syn*-isomer (**39**) should be the major product. The gramine derivative (**36**) reacts with tributylphosphine to form a bulky (tributylphosphino)indole intermediate that can only approach from the less congested face of the piperazinedione enolate, away from the isopentenyl moiety.

A similar phenomenon was observed when **39** was subjected to the decarbomethoxylation procedure (LiCl, H_2O, HMPA) directly. The two main products isolated were the *syn*-isomer **40** and the *anti*-isomer **41**, in a ratio of 3.3:1.0 (Figure 3). These stereochemical assignments were made by comparing the ^1H NMR spectral data of **40** and **41**. There was a pronounced upfield shift of three pyrrolidine ring protons in compound **41**, a shift that is not observed for diastereomer **40**.

Piperazinedione **39** was first converted to the BOC-protected indole **42**, which was subsequently subjected to a decarbomethoxylation reaction supplying the *syn*-diastereomer **43** as

(37) Sirowej, H.; Khan, S. A.; Plieninger, H. *Synthesis* **1972**, 84.

(38) Williams, R. M.; Glinka, T. *Tetrahedron Lett.* **1986**, *27*, 3581.

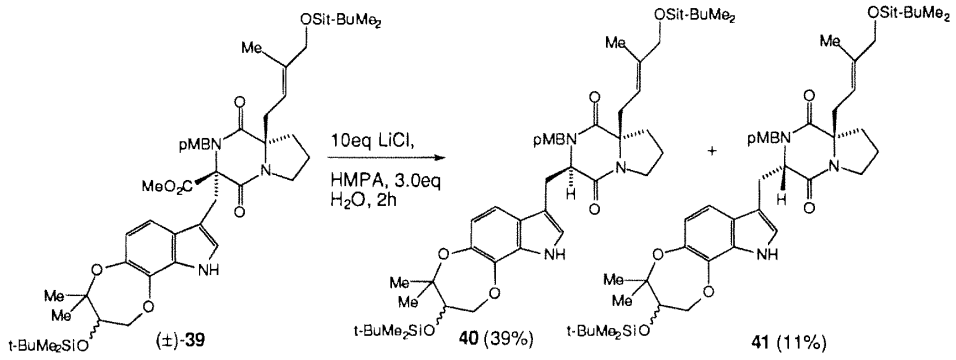
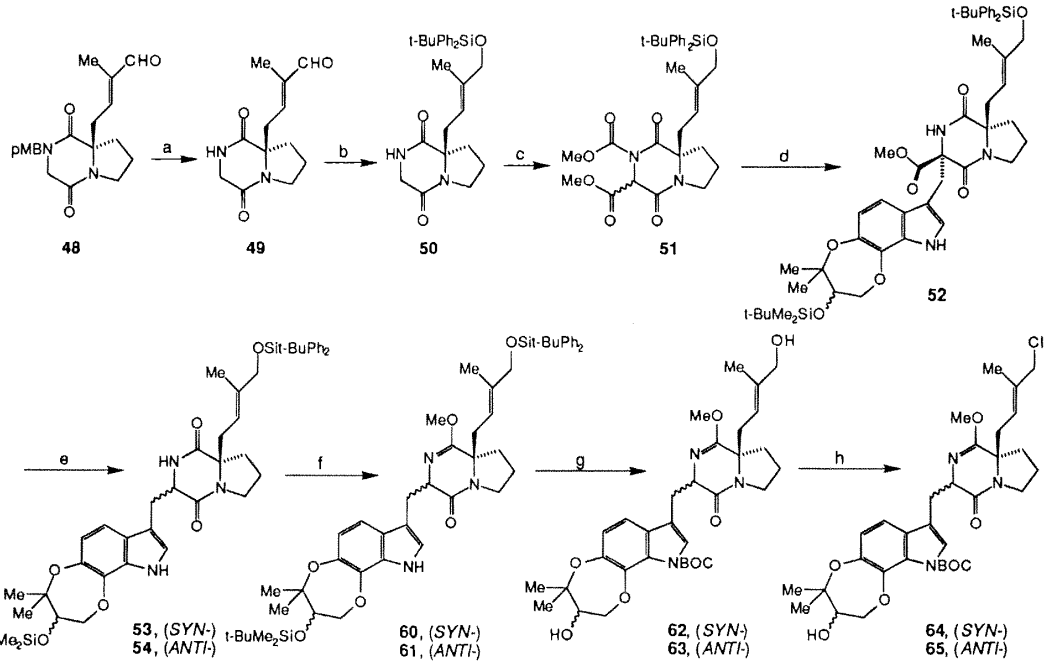


Figure 3.

Scheme 5[a]



[a] Reagents and conditions: (a) 3.8 equiv of CAN (0.33 M), 2:1 CH_3CN/H_2O, 2 h, 79%; (b) (i) 2 equiv of $NaBH_4$, EtOH; (ii) *t*-$BuPh_2SiCl$, im, DMF, 75%; (c) (i) 1.0 equiv of *n*-BuLi, 1.1 equiv of MeOCOCl, −78 °C; (ii) 2.2 equiv of $LiN(SiMe_3)_2$, 1.1 equiv of MeOCOCl, THF, −100 °C, 93%; (d) **36**, 0.5 equiv of PBu_3, CH_3CN, reflux, 73%; (e) LiCl, HMPA, 100 °C (*syn*/*anti* 3:1), 89%; (f) Me_3OBF_4, Na_2CO_3, CH_2Cl_2 (*syn*, 81%; *anti*, 62−71%); (g) (i) BOC_2O, DMAP, Et_3N, CH_2Cl_2; (ii) *n*-Bu_4NF, THF (*syn*, 90%; *anti*, 85%); (h) NCS, Me_2S (*syn*, 74−81%; *anti*, 86%).

the major product. Compound **43** (the minor, *anti*-diastereomer was not utilized) was desilylated to provide the diol **44**, which was converted to the allylic chloride **45**. Careful treatment of **45** with *t*-$BuMe_2SiOTf$, to prevent transesterification of the BOC groups,[39] gave the desired product **46** in 76% yield. Allylic chloride **46** was subjected to 10 equiv of NaH in refluxing benzene, but the reaction proved extremely sluggish. After 5 days, the desired product **47** was obtained in a poor 11% yield (19% based on recovered **46**; accompanied by extensive decomposition). The *syn*-isomer **47** was the only stereoisomer formed in this reaction; the corresponding *anti*-isomer was not detected. While this reaction demonstrated the potential viability of the stereoselective intramolecular S_N2' reaction, work on the racemic model system was halted, due to the low yield in this key transformation coupled with perceived difficulties associated with removing the *N*-*p*-methoxybenzyl group.

Total Synthesis of (+)-Paraherquamide B. Starting from the known piperazinedione **48** (prepared in eight steps from (S)-proline),[11] the enal **49** was obtained in 79% yield by treatment of **48** with a 0.33 M solution of ceric ammonium nitrate (Scheme 5).[40] The resulting product (**49**) was reduced with $NaBH_4$ and protected with *t*-$BuPh_2SiCl$ in a two-step process to give the silyl ether **50** in 75% yield. Compound **50** was then subjected to a two-step, one-pot acylation providing the required substrate **51** in 93% yield. The crude material was a mixture of epimers in a ratio of approximately 4:1 (*syn*:*anti*). Interestingly this mixture had a tendency to epimerize during column chroma-

(39) Sakaitani, M.; Ohfune, Y. *J. Am. Chem. Soc.* **1990**, *112*, 1150.

(40) Yoshimura, J.; Yamaura, M.; Suzuki, T.; Hashimoto, H. *Chem. Lett.* **1983**, 1001.

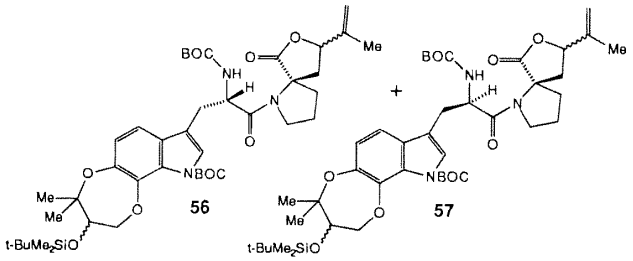
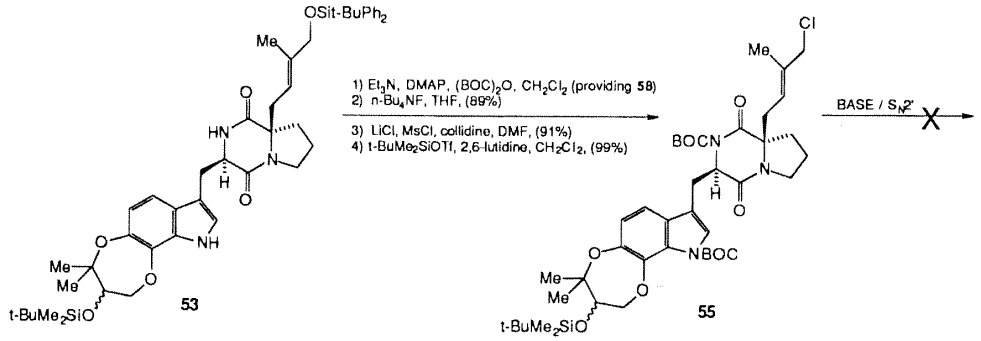


Figure 4.

Scheme 6



tography, resulting in an increase in the proportion of the *syn*-isomer. The two products were combined and condensed with the gramine **36** providing the indole **52** in 73% yield as a mixture of two diastereomers (epimeric at the secondary alcohol stereogenic center). Interestingly, the imidic carbamate group was also cleaved in the course of this reaction. Compound **52** was subjected to the decarbomethoxylation procedure, affording a 3:1 mixture of **53** (*syn*) and **54** (*anti*) in 89% combined yield.

The lactam **53** could be converted to the *N*-BOC-protected allylic chloride **55** in four steps and in good overall yield (Scheme 6), but numerous attempts to effect the S_N2' reaction on this substrate failed. These reactions were capricious and were accompanied by the occasional appearance of the spirolactones **56** and **57**, formed in low yield <5% (Figure 4). It seems likely that the failure of **55** to cyclize in the desired fashion can be attributed to nonbonding interactions between the *tert*-butoxycarbonyl group and the pendant dioxepin indole.[41,42]

These observations dictated that a suitable amide protecting group would have to be selected that was less electron withdrawing and less sterically demanding than both the *tert*-butoxycarbonyl and the *p*-methoxybenzyl groups. The loss of the lactam methoxycarbonyl group in the alkylation of **51** with the gramine **36** was presumably due to N → N acyl transfer to dimethylamine, a byproduct of the Somei/Kametani reaction. This appears to be a general reaction that was used to selectively deprotect the *N*-*tert*-butoxycarbonyl group of **58** without deblocking the *N*-*tert*-BOC-protected indole. Thus, refluxing a solution of **58** and Me_2NH in CH_3CN furnished compound **59** in 92% yield[43] (Scheme 7).

The strategy planned for the reduction of the tertiary amide called for the protection of the secondary lactam as a lactim ether,[44] and this group seemed suitable for use earlier in the synthesis and appeared compatible with the S_N2' cyclization. Thus, *syn*-isomer **53** was treated with 20 equiv (optimum) of Na_2CO_3 and 5 equiv of Me_3OBF_4 in dichloromethane for 4 h, to yield 81% of compound **60**. Even though the next two reactions could be carried out in a stepwise fashion, it proved most convenient to convert **60** directly to the protected diol **62** in a one-pot, two-step sequence. Diol **62** was then subjected to the chlorination procedure successfully used in the conversion of diol **44** to the allylic chloride **45**. Unfortunately, under these conditions, the reaction failed and the lactim ether was cleanly deblocked back to the lactam. This problem was finally solved by following the procedure of Corey,[45] which called for the addition of compound **62** to a mixture of *N*-chlorosuccinimide and dimethyl sulfide, which yielded the chloride **64** in 81% yield.

Allylic chloride **64** was reprotected with *t*-$BuPh_2SiOTf$ to provide **66** in 77−82% yield. The stage was now set to effect the S_N2' reaction. Compound **66** was refluxed in benzene with 20 equiv of sodium hydride, resulting in a very clean and high-yielding cyclization reaction furnishing the desired product **68** in 93% yield (Scheme 8).

(41) The formation of the two spiro compounds **56** and **57** is presumably due to the increased electrophilicity of the N-acylated amide. Apparently, trace moisture in the reaction mixture caused the production of hydroxide, which then hydrolyzed the reactive amide bond. The resulting carboxylic acid cyclized in an S_N2' fashion, furnishing the spiro lactones.

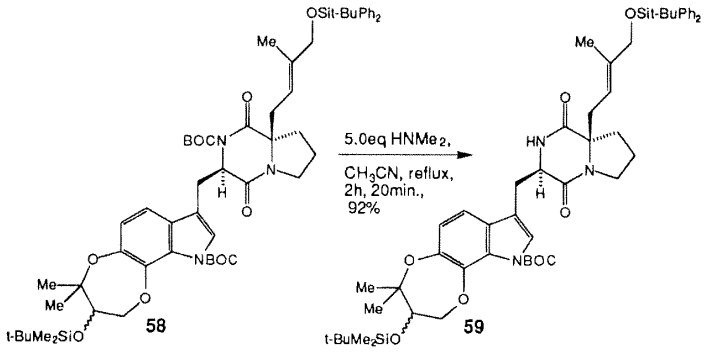
(42) (a) Giovannini, A.; Savoia, D.; Umani-Ronchi, A. *J. Org. Chem.* **1989**, *54*, 228. (b) Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424.

(43) This result is noteworthy, especially in light of a report that *tert*-butoxycarbonyl-protected amides are cleaved to the *tert*-butoxycarbonyl-protected amines with DEAEA (2-(*N*,*N*-diethylamino)ethylamine) in CH_3CN at room temperature; see: Grehn, L.; Gummarsonn, K.; Ragnarsson, U. *Acta Chem. Scand. B* **1987**, *41*, 18. However, the substrates examined in that report were all open-chain amides. Interestingly it is known that BOC-protected lactams can be cleaved by base but it is the amide bond that is broken as was observed on substrate **55**. Recently it has been reported that $Mg(OMe)_2$ will also cleave lactam carbamates including BOC-protected lactams; see: Wei, Z.-Y.; Knaus, E. E.; *Tetrahedron Lett.* **1994**, *35*, 847.

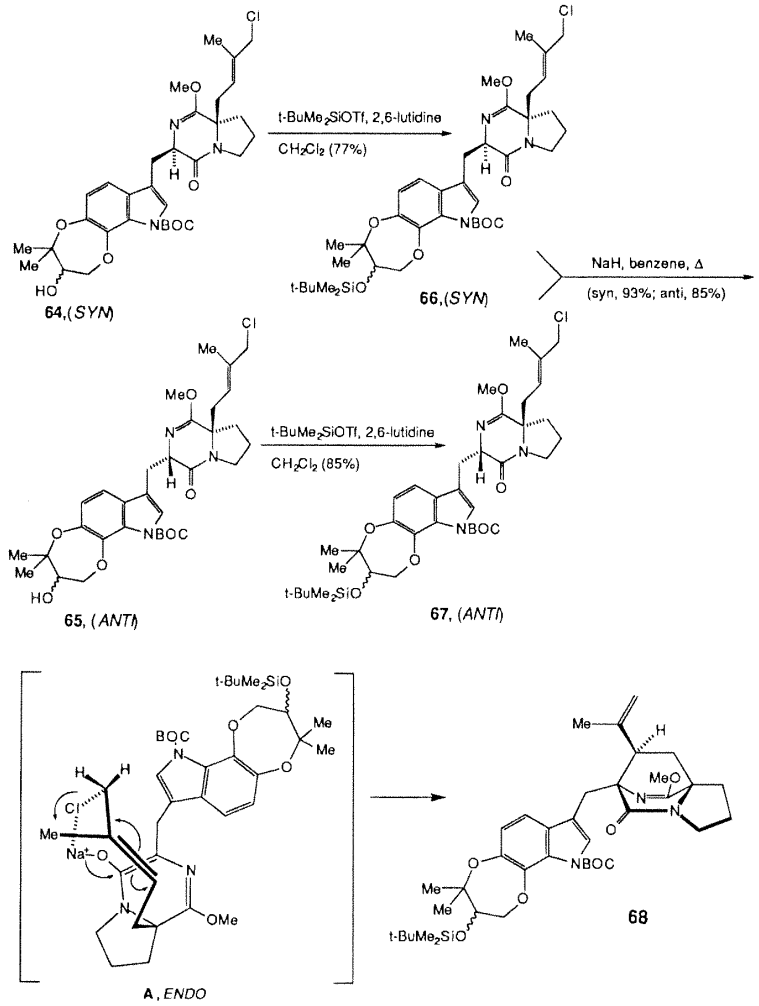
(44) Williams, R. M.; Brunner, E. J.; Sabol, M. R. *Synthesis* **1988**, 963.

(45) Corey, E. J.; Kim, C. U.; Takeda, M. *Tetrahedron Lett.* **1972**, *13*, 4339.

Scheme 7



Scheme 8



This last series of reactions was also carried out in parallel on the *anti*-isomer **54**. Following the same sequence (five steps) we obtained the fully protected chloride **67** in good yield. The chloride **67** was then refluxed in benzene with the required amount of sodium hydride to yield the same product (**68**, 85% yield) as that obtained from **66**. The yields of **68** from both routes were very high, and the undesired *anti*-diastereomer was not detected. The high degree of facial selectivity observed in the cyclizations to **68** and **47** is quite interesting and warrants some comments. It is generally accepted that S_N2' reactions

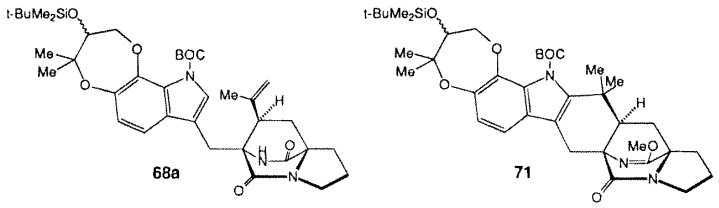


Figure 5.

favor a *syn* orientation[46] (i.e., the incoming nucleophile attacks the π-electrons from the same face as the departing leaving group, polarizing the π-system in the proper orientation for a "backside" displacement on the C−Cl bond). Alternatively, a frontier molecular orbital analysis has indicated[46a] that the stabilization imparted by a $HOMO_{Nuc}-LUMO_{allylic}$ interaction is greater for the *syn* overlap. While the greatest level of diastereoselectivity was observed with a nonpolar aprotic solvent (benzene), a fairly significant change in the relative amounts of the *syn*- and *anti*-diastereomers can be realized by simply changing the solvent to a more polar solvent such as DMF.[11] In the present system, additional stabilization for the *endo* transition state may be due to the formation of a tight contact ion pair between the chlorine atom and sodium atom of the enolate species (see **A**, Scheme 8) in the transition state for the formation of the C−C bond.[47] The poor ligating solvent benzene is not capable of effectively solvating the enolate cation nor the developing chloride anion in the transition state. It is reasonable that this type of association favors the rotamer that positions the allylic chloride moiety over the enolate, resulting in the desired *syn* stereochemistry.

With the bicyclo[2.2.2] ring system constructed in a reliable and high-yielding sequence, attention was turned to the final C−C bond-forming reaction on the indole. Due to the strongly acidic conditions that were used previously for a related cyclization reaction in the brevianamide synthesis, it was assumed that the silyl ether, the *tert*-butoxycarbonyl protecting group, and the lactim ether would be removed during this cyclization reaction. Subjecting compound **68** to the standard conditions (dilute, aqueous HCl in dioxane at 10 °C)[11,19,48] resulted in extensive decomposition, and none of the desired cyclized product was ever detected. The reaction conditions were extensively varied using different acids and temperatures, but the only recognizable products were those stemming from the loss of protecting groups. The problem might be attributed to the enhanced basicity of the indole at the 2-position (indole numbering) caused by the electron-donating oxygen atoms in the aromatic ring. If protonation at the 2-position is kinetically competitive with olefin protonation, cyclization would be precluded.

A search of the literature revealed a 1982 Trost and Fortunak paper[49] wherein $PdCl_2$ and $AgBF_4$ were utilized to effect the Heck-type cyclialkylations of various isoquinuclidine model compounds. Compound **68** was exposed to these conditions, affording the heptacycle **69** in 63−82% yields. During the course of the reaction, the lactim ether moiety was cleaved, restoring the free, secondary amide.[50] The main byproduct of this reaction was the uncyclized free lactam **68a** (Figure 5), which curiously did not cyclize to **69** when subjected to the same conditions. It was also observed that the lactim ether protected heptacycle **71** could not be deblocked to the free lactam **69** with $PdCl_2$ and $AgBF_4$ alone, implying that the cleavage of the lactim ether is due to the tetrafluoroboric acid produced in the cyclization, and that the cyclization occurs *prior* to lactim ether cleavage.

Trost and Fortunak speculated[49] that the cyclization mechanism was either a Heck-type arylation or the electrophilic aromatic substitution of a palladium-complexed olefin, and there was evidence to support both mechanistic possibilities. It is possible that the palladium chloride and the silver tetrafluoroborate react to form a powerful Lewis acid, since an incubation period involving these two reagents is needed prior to the introduction of the substrate. It was reported[49] that there is no reaction with other mixed-metal systems involving palladium chloride (e.g., boron trifluoride, aluminum chloride, stannous chloride, stannic chloride, titanium trichloride). The enhanced basicity (nucleophilicity) at the 2-position of indole **68** renders this substance perfectly disposed to undergo a Heck-type arylation reaction.

There are several reports of methods that will selectively reduce a tertiary amide in the presence of a secondary amide.[51] The secondary lactam of **69** was protected as the lactim ether **71** and treated with diborane; however, the spectral characteristics of the major products isolated were consistent with reduction of both the tertiary amide and the lactim ether. In 1991 Martin *et al.*[52] successfully used alane to reduce a tertiary amide in the presence of an oxindole (secondary amide) relying on the known rate difference for reduction between these two groups.[53] However, initial experiments with this reagent gave poor results, with the secondary amide undergoing reduction along with the tertiary amide. Compound **69** (and **71**) is sufficiently twisted such that the *gem*-dimethyl groups effectively block the β-face of the tertiary amide (Figure 6),

(46) (a) Magid, R. M.; Fruchey, O. S.; Johnson, W. L.; Allen, T. G. *J. Org. Chem.* **1979**, *44*, 359. (b) Magid, R. A. *Tetrahedron* **1980**, *36*, 1901.

(47) The idea that the stereochemical outcome of an intramolecular enolate alkylation is determined by chelation in the transition state was recently demonstrated by Denmark and Henke, who observed a marked preference for a "closed" transition state (coordination of the cationic counterion to an enolate and the developing alcohol) resulting in a *syn* product. For example, the highest *syn:anti* ratio (89:11) was obtained in toluene and the lowest *syn:anti* ratio (2:98) was obtained with a crown ether. These observations parallel the facial selectivities described herein and in ref 11 on the intramolecular S_N2' reaction; see: (a) Denmark, S. A.; Henke, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 2177. (b) Denmark, S. A.; Henke, B. R. *J. Am. Chem. Soc.* **1989**, *111*, 8022.

(48) (a) Hutchison, A. J.; Kishi, Y. *J. Am. Chem. Soc.* **1979**, *101*, 6786. (b) Guller, R.; Borschberg, H.-J. *Tetrahedron Lett* **1994**, *35*, 865.

(49) Trost, B. M.; Fortunak, J. M. D. *Organometallics* **1982**, *1*, 7.

(50) (a) Ashimori, A.; Overman, L. E. *J. Org. Chem.* **1992**, *57*, 4571. (b) Karabelas, K.; Westerlund, C.; Hallberg, A. *J. Org. Chem.* **1985**, *50*, 3896. (c) Cava, M. P.; Kevinson, M. I. *Tetrahedron* **1985**, *41*, 5061 and literature cited therein.

(51) In a recently reported synthesis of gelsemine, a tertiary lactam was reduced in the presence of a secondary lactam with DIBAH. However, this reagent failed on substrates **69**; see: Dutton, J. K.; Steel, R. W.; Tasker, A. S.; Popsavin, V.; Johnson, A. P. *J. Chem. Soc., Chem. Commun.* **1994**, 765.

(52) Martin, S. F.; Benage, B.; Geraci, L. S.; Hunter, J. E.; Mortimore, M. *J. Am. Chem. Soc.* **1991**, *113*, 6161.

(53) (a) Yoon, N. M.; Brown, H. C. *J. Am. Chem. Soc.* **1968**, *90*, 2927. (b) Marlett, E. M.; Park, W. S.; *J. Org. Chem.* **1990**, *55*, 2968. (c) Jorgenson, M. J. *Tetrahedron Lett.* **1962**, 559. (d) Another very recent synthesis of gelsemine reported the reduction of the same gelsemine precursor (as in ref 51) with AlH_3. Newcombe, N. J.; Fang, Y.; Vijn, R. J.; Hiemstra, H.; Speckamp, W. N. *J. Chem. Soc., Chem. Commun.* **1994**, 767.

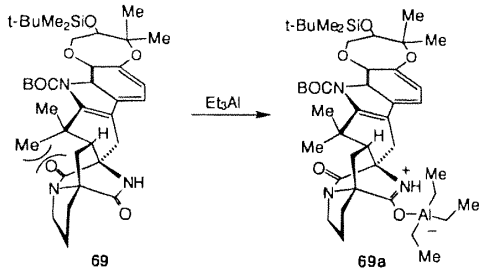


Figure 6.

leaving the α-face relatively unencumbered. However, a modification of the alane procedure[52] proved satisfactory for this transformation. The piperazinedione **69** was pretreated with $AlEt_3$, with the expectation that this Lewis acid would form a complex with the more exposed secondary lactam (**69a**, Figure 6) and leave the tertiary lactam accessible for reduction.

Following 10 min of precomplexation with $AlEt_3$, 5 equiv of AlH_3-Me_2NEt complex was added, followed by quenching with $NaCNBH_3$, acetic acid, and methanol to provide the desired amine **70** in 63% yield. Compound **70** was smoothly alkylated with methyl iodide, affording the N-methylated product **72** in 95−98% yield. Compound **72** was subsequently deblocked with 80 equiv of TFA in CH_2Cl_2 to yield the penultimate heptacycle **73** in 97% (Scheme 9).

The stage was now set for the final transformations involving the oxidative pinacol-type rearrangement and dehydration. Due to the difficulties encountered in the attempted cationic cyclization on the indole (cf. **68** → **69**), there was concern about the reactivity of the indole ring toward the electrophilic reagents that would be utilized in the oxidative pinacol-type reaction. There was the possibility that the electron-donating oxygen atoms on the indole ring would hinder the acid-catalyzed rearrangement of, for example, an intermediate chloroindolenine,[54] similarly to the way that strong acid hindered the cationic cyclization.[55] When compound **73** was treated with *tert*-butyl hypochlorite and triethylamine, there was an almost an instantaneous reaction resulting in the total disappearance of starting material and the appearance of two new components (≈1:2 ratio as evidenced by ^1H NMR analysis) that were presumed to be the expected diastereomeric chloroindolenines. When this mixture was subjected to the standard rearrangement procedure employing a refluxing solution of acetic acid, water, and methanol, these substances slowly decomposed (many bands in the PTLC).[56]

Since the tertiary amine of **73** might react with the chlorinating reagent and was thus considered to be a possible culprit in these oxidations, an attempt to effect the pinacol-type rearrangement before the amide reduction step was investigated. Thus, piperazinedione **69** was readily deblocked with TFA to provide the amide **76** in 95% yield (Scheme 10). This substance was treated with *t*-BuOCl and Et_3N in the same manner as before, producing two products **77**/**78** (≈1:4 ratio). Using a milder $MeOH/H_2O/AcOH$ system (stirring at room temperature), an oxindole compound **79** was formed in 29% yield. Although this result was encouraging, this substance appeared to possess the incorrect relative stereochemistry at the spiro-ring juncture. This assignment was supported by comparing the ^1H NMR spectra of **79** and an authentic sample of (−)-paraherquamide B (**1**). The *gem*-dimethyl signals of **79** were shifted upfield, indicating that one methyl group is in the shielding cone of the oxindole carbonyl.

After a careful reexamination of the decomposition products obtained from the attempted pinacol-type rearrangement of **73**, it was determined that there were mainly two decomposition pathways, and that they were in direct competition with the desired process. These two pathways involve the intermediacy of an oxonium-stabilized tertiary carhocation (at C-3 of the indole) that decomposes to quinone-type products. Additionally, products were isolated whose spectral characteristics were consistent with an elimination process followed by nucleophilic reaction with the solvent at the tryptophan benzylic carbon.

In the classical pinacol rearrangement there is a distinct carbonium ion intermediate, but recent studies have shown that this may in fact be more of a concerted process[57] and, furthermore, that the nature of the solvent can have an impact on which of the two processes, concerted or stepwise, will predominate. There have been conflicting reports in the literature on whether this type of rearrangement is, at all times, stereospecific.[58,59] A detailed study[59c] involving the isolation and separation of the two diastereomeric chloroindolenines derived from yohimbine demonstrated that this reaction can be entirely stereospecific. Alternatively, by increasing the solvating power of the reaction medium, each of these chloroindolenines formed two rearranged products, indicating that the reaction went (at least in part) by way of a carbocationic intermediate. This is consistent with the observed production of **79** from **77** and **78**. A less polar solvent system should minimize the side reactions involving carbocation intermediates and, at the same time, should increase the stereospecific nature of the pinacol-type rearrangement. Thus, treatment of **73** with *t*-BuOCl and Et_3N in CH_2Cl_2 provided the two chloroindolenines **74** and **75** (≈2.25:1 ratio, respectively). The solvent was removed, and the crude reaction mixture was refluxed with a solution of 95% THF, 4% H_2O, and 1% TFA, giving a 62% yield of oxindole products (43% of the desired **80** and 19% the epi product **81**).[60] The C-3-epi-isomer (**81**) was easily distinguishable from the desired isomer (**80**) by the upfield shift of the *gem*-dimethyl signals in the ^1H NMR spectrum. The relative amounts of products (**80** and **81**) indicate that the cyclization was stereospecific under these conditions. It was thus deduced that an increase in the ratio of the desired oxindole **80** to the undesired

(54) (a) Gaskell, A. J.; Radunz, H. -E.; Winterfeldt, E. *Tetrahedron Lett.* **1970**, 5361. (b) Winterfeldt, E.; Gaskell, A. J.; Korth, T.; Radnuz, H.-E.; Walkowiak, M. *Chem. Ber.* **1969**, *102*, 3558. (c) Hollinshead, S. P.; Grubisha, D. S.; Bennett, D. W.; Cook, J. M. *Heterocycles* **1989**, *29*, 529.

(55) Concern about this possible difficulty was somewhat ameliorated by the knowledge of an alternative procedure that employed OsO_4−pyridine. See: (a) Takayama, H.; Kitajima, M.; Ogata, K.; Sakai, S. *J. Org. Chem.* **1992**, *57*, 4583. (b) Takayama, H.; Odaka, H.; Aimi, N.; Sakai, S. *Tetrahedron Lett.* **1990**, *38*, 5483. (c) Takayama, H.; Masubuchi, K.; Kitajima, M.; Aimi, N.; Sakai, S. *Tetrahedron* **1989**, *45*, 1327. (d) Fu, X.; Cook, J. M. *J. Org. Chem.* **1993**, *58*, 661. See also: (e) Takayama, H.; Tominaga, Y.; Kitajima, M.; Aimi, N.; Sakai, S. *J. Org. Chem.* **1994**, *59*, 4381.

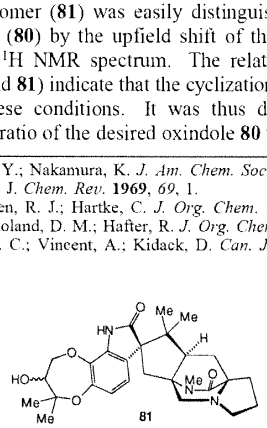
(56) Similar problems were observed during the total synthesis of isopteropodine and pteropodine; see: Martin, S. F.; Mortimore, M. *Tetrahedron Lett.* **1990**, *31*, 4557. In this system, the solution involved treating the chloroindolenines with silver perchlorate in methanolic perchloric acid. This method was attempted on substrate **73**, but unfortunately it failed to produce any desired product.

(57) Osamura, Y.; Nakamura, K. *J. Am. Chem. Soc.* **1993**, *115*, 9112.

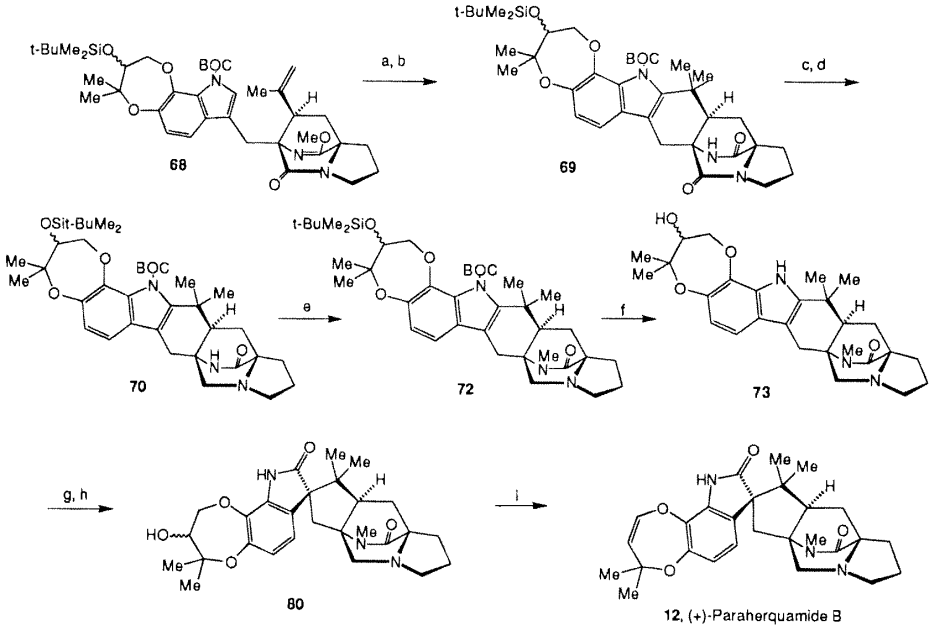
(58) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1.

(59) (a) Owellen, R. J.; Hartke, C. *J. Org. Chem.* **1976**, *41*, 102. (b) Kuehne, M. E.; Roland, D. M.; Hafter, R. *J. Org. Chem.* **1978**, *43*, 3703. (c) Awang, D. V. C.; Vincent, A.; Kidack, D. *Can. J. Chem.* **1984**, *62*, 2667.

(60)

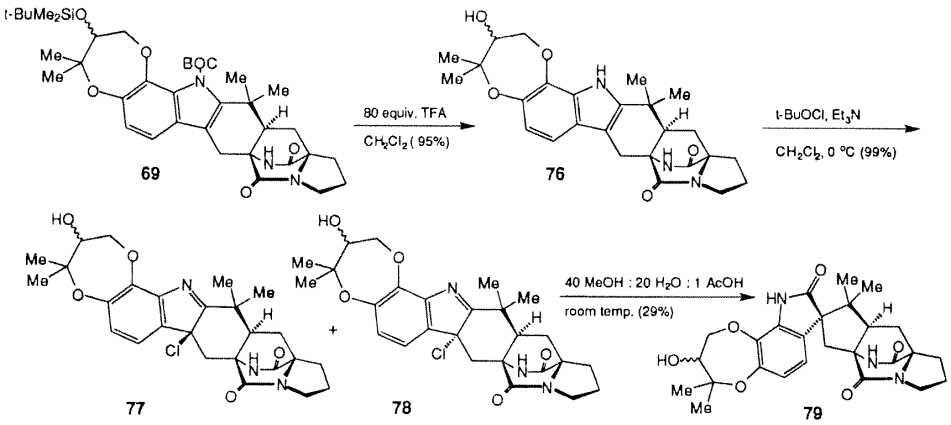


Scheme 9[a]

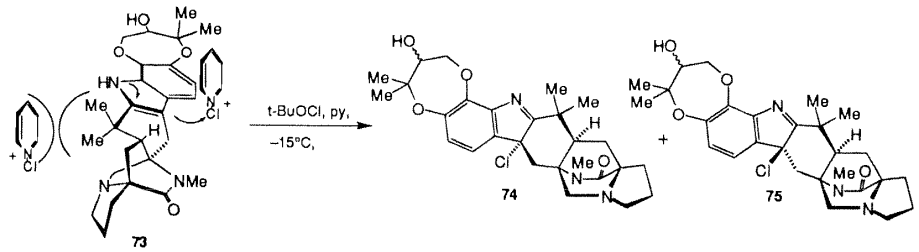


[a] Reagents and conditions: (a) $PdCl_2$, $AgBF_4$, MeCN; (b) $NaBH_4$ (63−82% from **68**); (c) 1.1 equiv of Et_3Al, 5.0 equiv of AlH_3−DMEA, THF, toluene; (d) 2.0 equiv of $NaCNBH_3$, AcOH, MeOH (65% from **69**); (e) 2.5 equiv of NaH, 2.0 equiv of MeI, DMF (98%); (f) 80 equiv of TFA, CH_2Cl_2 (96%); (g) *t*-BuOCl, pyridine, −15 °C; (h) 90% THF, 10% H_2O, pTsOH (76%); (i) MTPI, DMPU (79%).

Scheme 10



Scheme 11



isomer **81** could be achieved simply by finding a method that would increase the ratio of chloroindolenines (**74**:**75**). The α-face of **73** is considerably more hindered than the β-face, a supposition that was supported by the difficulties encountered in the reduction of **71** and **69**. Increasing the steric bulk of the chlorinating agent should favor attack on the β-face of **73**, thus

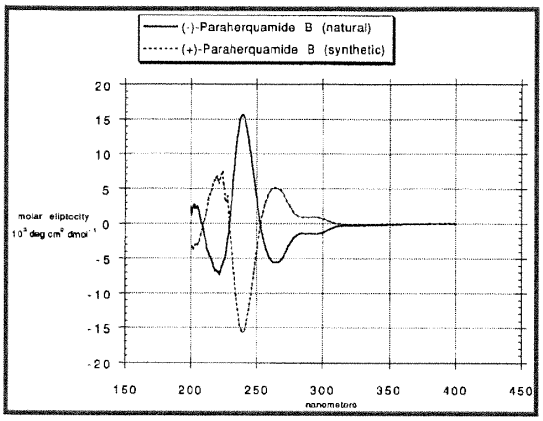


Figure 7.

providing a greater relative amount of chloroindolenine **74**. When **73** was treated with *t*-BuOCl in pyridine instead of triethylamine, the desired chloroindolenine **74** was produced in a much more stereoselective fashion. It can be speculated that *tert*-butyl hypochlorite forms a bulky complex with pyridine, delivering the chlorine more selectively to the least hindered α-face of **73** (only a small amount, ≈5%, of the undesired isomer **75** was formed under these conditions (Scheme 11)).

Employing a minor modification of the solvent system, the crude mixture of **74/75** was refluxed with a solution of 90% tetrahydrofuran, 10% H_2O containing 15 equiv of *p*-toluenesulfonic acid to give the desired oxindole **80** in 76% yield (from **73**), with only 4% of the undesired **81** being formed.

The stereospecific conversion of the chloroindolenines into the corresponding oxindoles requires that the water molecule attack the imine from the same face as the chlorine atom. *Anti* attack on the imine is not as likely because of certain stereoelectronic effects.[59c] The addition of water to the β-face of **74** situates the six-membered ring adjacent to the indole ring in a stable chair conformation that would also place the C−Cl bond and the migrating $(CH_3)_2CC$ group in an unfavorable *syn* alignment. Conversely, the addition of water to the α-face of compound **75** would result in an unfavorable boat conformation that would also place the C−Cl bond and the migrating $(CH_3)_2$-CC group in an unfavorable *syn* alignment. Thus, the major isomer **74** must either (1) suffer kinetically controlled attack by water on the same face of **74** as the chlorine atom, which aligns the migrating group and the C−Cl bond in a stereoelectronically favorable *anti* orientation, or (2) undergo reversible attack by water from either face, with only the correct carbinolamine, which aligns the migrating group and the C−Cl bond in a stereoelectronically favorable *anti* orientation, rearranging irreversibly to the oxindole.

The final dehydration reaction (MTPI, DMPU, 18 h) on the alcohol **80** produced (+)-paraherquamide B (**12**) in 79% yield (Scheme 9). This substance proved to be identical to the natural product by comparison of the ^1H and ^{13}C NMR spectra, mobility on TLC, IR spectra, mass spectra, and UV spectra. Comparison of the CD spectra of the natural (−)-paraherquamide B (**2**) and the synthetic (+)-paraherquamide B (**12**) (Figure 7) confirmed the expected enantiomeric relationship between these two products.

Conclusion

The first stereocontrolled, asymmetric total synthesis of (+)-paraherquamide B has been completed. The synthesis is convergent, starting from (S)-proline and vanillin with an overall yield of 1.4% from (S)-proline.

Key features of this synthesis include (1) a new method to effect reduction of unprotected oxindoles to indoles; (2) a complex application of the Somei/Kametani reaction that concomitantly effected a desired decarbomethoxylation; (3) a high-yielding and entirely stereocontrolled intramolecular S_N2' cyclization reaction; (4) a mild Pd(II)-mediated cyclization reaction that concomitantly deblocked a lactim ether protecting group; and (5) the chemoselective reduction of a highly hindered tertiary lactam in the presence of an unhindered secondary lactam, utilizing precoordination of the more reactive secondary lactam to triethylaluminum.

Experimental Section

General information. Melting points were determined in open-ended capillary tubes and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on either a Bruker WP-270SY 270 MHz or a Bruker AC300P 300 MHz NMR spectrometer. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.24 or TMS at δ 0.0. IR spectra were recorded on a Perkin-Elmer 1600 FT IR spectrometer. Mass spectra were obtained on a V. G. Micromass Ltd. Model 16F spectrometer. The CD spectrum was obtained on a Jasco J710 spectropolarimeter. High-resolution mass spectra were obtained from the Midwest Center for Mass Spectrometry Department of Chemistry, University of Nebraska—Lincoln, Lincoln, NE. Elemental analyses were obtained from M-H-W Laboratories, Phoenix, AZ. Optical rotations were recorded on a Perkin-Elmer 24 polarimeter at a wavelength of 589 nm using a 1.0 dm cell of 1.0 mL total volume.

Column chromatography and flash column chromatography were performed with silica gel grade 60 (230−400 mesh). Radial chromatography was performed with a Harrison Research Chromatotron Model 7924 using E. Merck silica gel 60 PF-254 containing gypsum; 1, 2, 4, and 8 mm plates were used as needed. Preparatory thin layer chromatography (PTLC) was carried out with Merck Kieselgel 60 F_{254} precoated glass plates (either 0.25 or 0.50 mm); visualization was carried out with ultraviolet light and/or heating with a solution of 5−7% phosphomolybdic acid; staining with I_2; vanillin; or Dragendorf.

All solvents were commercial grade and were distilled and dried as follows: tetrahydrofuran (THF) from sodium benzophenone ketyl; diethyl ether from sodium benzophenone ketyl; carbon tetrachloride from calcium hydride; dioxane from sodium; benzene from sodium benzophenone ketyl; dichloromethane from calcium hydride; acetonitrile from P_2O_5. DMF was dried and stored over 3 Å molecular sieves, as were benzene and toluene. HMPA was dried and stored over 4 Å molecular sieves. Dimethyl sulfide, 2,6-lutidine, triethylamine, and pyridine were all distilled prior to use. Phenylselenium chloride was purified by sublimation. *N*-Chlorosuccinimide (NCS) was recrystallized from benzene. LiCl was dried and stored in the oven. All other reagents were commercial grade and used without further treatment. Abbreviations are used throughout: *N*,*N*-dimethylformamide (DMF); acetic acid (AcOH); di-*tert*-butyl dicarbonate ($(BOC)_2O$); methyltriphenoxyphosphonium iodide (MTPI); ethyl acetate (EtOAc); *m*-chloroperbenzoic acid (*m*-CPBA); (*N*,*N*-dimethylamino)pyridine (DMAP); hexamethylphosphoramide (HMPA); ceric ammonium nitrate (CAN); methanesulfonyl chloride (MsCl); *N*-chlorosuccinimide (NCS); trifluoroacetic acid (TFA); dimethylethylamine (DMEA); imidazole (im); 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU).

2-[(3-Methyl-2-butenyl)oxy]phenol (24). To a stirred, cold (0 °C), dark solution of catechol (2.07 g, 18.8 mmol, 5.0 equiv) in DMF (65 mL) in a reaction vessel that had been flushed with Ar was added anhydrous K_2CO_3 (0.520 g, 3.76 mmol, 1.0 equiv). After 5 min, prenyl bromide (0.441 mL, 3.76 mmol, 1.0 equiv) was added dropwise. The reaction mixture was kept at 0 °C for ~6 h and stirred at room temperature for an additional 18 h. The mixture was then poured into a separatory funnel, diluted with H_2O (100 mL), and extracted five times with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by radial chromatography (eluted with 1% ethyl acetate/hexanes) to give 479 mg (71%) of **24** as a colorless oil. An analytical sample was obtained by PTLC on silica gel (eluted with hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.74 (3H, s), 1.80 (3H, s), 4.57 (2H, d, J = 6.8 Hz), 5.49 (1H, m), 5.70 (1H, s, D_2O exch), 6.82−6.92 (4H, m). IR (NaCl, neat): 3533, 2932, 1612, 1502, 1467, 1385, 1259, 1221, 1106, 997, 743 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 178 (11), 161 (11), 110 (78), 69 (67), 32 (100). Microanalysis calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92 Found: C, 73.88; H, 8.00.

(±)-3,4-Dihydro-2,2-dimethyl-3-(phenylseleno)-2*H*-benzodioxepin (25). A solution of phenylselenium chloride (117.8 mg, 0.615 mmol, 1.05 equiv) in EtOAc (4.1 mL, 0.15 M) was slowly added (~1 mmol/h) to a stirred solution of **24** (104.4 mg, 0.58 mmol, 1.0 equiv) in EtOAc (3.90 mL, 0.15 M) at −75 °C under Ar. This mixture was allowed to warm to room temperature and was stirred for a total of 17 h. The solution was poured into a separatory funnel and washed twice with H_2O and once with brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by PTLC (eluted with 1:3 hexanes/benzene) to afford 62.1 mg (32%) of **25**. An analytical sample was obtained by PTLC (eluted with hexanes, and then distilled under reduced pressure).

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.28 (3H, s), 1.76 (3H, s), 3.62 (1H, dd, J = 3.4, 10.3 Hz), 4.17 (1H, dd, J = 10.3, 12.6 Hz), 4.40 (1H, dd, J = 3.5, 12.6 Hz), 6.94−6.98 (4H, m), 7.30−7.34 (3H, m), 7.59−7.62 (2H, m). IR (NaCl, neat): 2986, 1491, 1256, 1088, 1000 cm^{-1}. HRMS (EI): *m/e* 334.0473 ($C_{17}H_{18}O_2Se$ requires 334.0472).

2,2-Dimethyl-2*H*-1,5-benzodioxepin (26). To a stirred solution of **25** (61.7 mg, 0.185 mmol, 1.0 equiv) in THF (3 mL) was added H_2O_2 (0.21 mL, 0.5 mmol, 10 equiv) at 0 °C. The resulting solution was stirred for 0.5 h and then brought to reflux temperature for 0.5 h. The mixture was poured into a separatory funnel, diluted with water, and extracted with ether. The ethereal solution was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by PTLC (eluted with 1:3 hexanes/EtOAc) to afford 16.0 mg (49%) of **26** as a pale yellow oil (see data below).

Compound **26** was also obtained from **28** as follows: To a solution of **28** (76.2 mg, 0.39 mmol, 1.0 equiv) in HMPA (2 mL) under N_2 at room temperature was added MTPI (291.5 mg, 0.64 mmol, 1.6 equiv) all at once. After being stirred for 1 day, the mixture was poured into a separatory funnel containing 1 M NaOH and was extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation gave a crude yield of 163.5 mg. The crude product was purified by radial chromatography (eluted with 1:10 EtOAc/hexanes, then 1:5 EtOAc/hexanes) to afford 46 mg (66%) of **26**.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.42 (6H, s), 4.81 (1H, d, J = 7.8 Hz), 6.30 (1H, d, J = 7.8 Hz), 6.95−7.06 (4 H, m). IR (neat): 2978, 1654, 1587, 1495, 1311, 1242, 750 cm^{-1}. HRMS (EI): *m/e* 176.0835 ($C_{11}H_{12}O_2$ requires 176.0837).

(±)-2-[(3,3-Dimethyloxiranyl)methoxy]phenol (27). To a solution of **24** (1.31 g, 7.35 mmol, 1.0 equiv) in CH_2Cl_2 (40.0 mL) under N_2 at 0 °C was added $NaHCO_3$ (803 mg, 9.56 mmol, 1.3 equiv) followed by *m*-CPBA (1.27 g, 7.35 mmol, 1.0 equiv). After 1.5 h additional $NaHCO_3$ (812 mg, 9.66 mmol, 1.21 equiv) and *m*-CPBA (1.26 g, 7.35 mmol, 0.99 equiv) were added. This mixture was kept stirring at 0 °C for 2 h, when more $NaHCO_3$ (778 mg, 9.27 mmol, 1.3 equiv) and *m*-CPBA (1.12 g, 6.49 mmol, 0.88 mmol) were added. After 2 h, the cold mixture was filtered to remove the solids. The filtrate was washed three times with 10% $Na_2S_2O_3$ and three times with brine, dried over $MgSO_4$, and evaporated to dryness to afford 1.41 g (99%) of **27**. An analytical sample was recrystallized from toluene to give a glassy solid, mp 36−37 °C.

^{1}H NMR (270 MHz) ($CDCl_3$): δ TMS 1.37 (3H, s), 1.41 (3H, s), 3.18 (1H, dd, J = 4.2, 6.3 Hz), 4.07 (1H, dd, J = 6.4, 11.0 Hz), 4.28 (1H, dd, J = 4.2, 11.0 Hz), 5.78 (1H, s, D_2O exch), 6.81−6.97 (4H, m). IR (NaCl, neat): 3413, 2966, 1590, 1502, 1267, 744 cm^{-1}. Microanal. Calcd for $C_{11}H_{14}O_4$: C, 68.02; H, 7.26. Found: C, 67.91; H, 7.39.

(±)-3,4-Dihydro-2,2-dimethyl-2*H*-1,5-benzodioxepin-3-ol (28). A flame-dried flask, flushed with Ar, was charged with dry THF (85.4 mL). Tin tetrachloride (0.85 mL, 7.3 mmol, 1.0 equiv) was then added dropwise in 5 min. After 10 min a solution of **27** (1.41 g, 7.26 mmol, 1.0 equiv) in dry THF (13.8 mL) was added slowly (dropwise) to the mixture. The reaction mixture was stirred at room temperature for 20 min, poured into saturated $NaHCO_3$, washed with brine, dried over $MgSO_4$, and evaporated to dryness. The crude product was purified by radial chromatography (eluted with 1:7 EtOAc/hexanes) to afford 842 mg (60% or 59% for two steps) of **28** as an oil. An analytical sample was obtained by PTLC (eluted with 5:1 EtOAc/hexanes, and then distilled under reduced pressure).

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.20 (3H, s), 1.53 (3H, s), 2.96 (1H, d, J = 11.3 Hz, D_2O exch), 3.58 (1H, ddd, J = 1.1, 4.0, 11.3 Hz), 4.08 (1H, dd, J = 1.1, 12.6 Hz), 4.20 (1H, dd, J = 4.0, 12.6 Hz), 6.98−7.02 (4H, m). IR (NaCl, neat): 3448, 2978, 1596, 1490, 1261 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 194 (41), 176 (19), 136 (57), 121 (100), 59 (63). HRMS (EI) *m/e* 194.0943 ($C_{11}H_{14}O_3$ requires 194.0943).

4-Hydroxy-3-methoxy-2-nitrophenylacetic Acid (30). To a flask containing **29** (101 g, 397 mmol, 1.0 equiv) at 0 °C was added a solution of NaOH (63.5 g, 1.59 mol, 4.0 equiv) in H_2O (1.4 L). After 10 min, hydrogen peroxide (49.5 mL, 437 mmol, 1.1 equiv, 30% solution in water) was added dropwise. The deep purple solution slowly turned brown during the addition. The mixture was allowed to reach room temperature and stirred for 24 h. The reaction mixture was then acidified with concentrated HCl until pH ≈ 3, during which CO_2 was released and a fine yellow crystalline product precipitated. The mixture was filtered, washed with cold H_2O, and dried to yield 72.6 g (81%) of **30**. An analytical sample was recrystallized from H_2O to give bright yellow needles, mp 161−162 °C (when the reaction was carried out with 11.9 g of the phenylacetic acid, the yield was 93%).

^{1}H NMR (300 MHz) (acetone-d_6): δ TMS 2.83 (2H, br s, D_2O exch), 3.62 (2H,s), 3.91 (3H, s), 7.10 (2H, s). IR (KBr): 3488, 2958, 2641, 1668, 1533, 1399, 1344, 1296, 1225, 1051, 825 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 228 (M^+, 0.7), 227 (5.8), 166 (10.0), 106 (13.6), 44 (100). Microanal. Calcd for $C_9H_9NO_6$: C, 47.58; H, 3.99; N, 6.16. Found: C, 47.56; H, 4.06; N, 6.25.

1,3-Dihydro-6-hydroxy-7-methoxy-2*H*-indol-2-one (31). A mixture of **30** (23.0 g, 101 mmol, 1.0 equiv) in glacial acetic acid (100 mL) and Pd/C (10%, 1.5 g) was hydrogenated at 40 psi of H_2 in an oil bath (80 °C) for 5 h. The mixture was immediately filtered through a Celite plug and washed with a small amount of warm AcOH. The flask was kept under suction (cold) until a large quantity of white product had precipitated. This was filtered to collect the product, when an additional quantity of product precipitated under suction. This was collected, and the two crops of white flakes were combined and dried under reduced pressure to yield 17.2 g (95%) of **31**. An analytical sample was recrystallized from H_2O to give white crystals, mp 210−211 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 3.50 (2H, d, J = 1.0 Hz), 3.87 (3H, s), 5.49 (1H, s, D_2O exch), 6.60 (1H, d, J = 8.1 Hz), 6.86 (1H, d, J = 8.0 Hz), 7.94 (1H, s, D_2O exch). IR (KBr): 3287, 3014, 2953, 1686, 1633, 1504, 1466, 1315, 1163, 637 cm^{-1}. Microanal. Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.51; H, 5.05; N, 7.60.

1,3-Dihydro-7-methoxy-6-[(tolylsulfonyl)oxy]-2*H*-indol-2-one. To a stirred mixture of **31** (321.6 mg, 1.795 mmol, 1.0 equiv) in acetone (7 mL) at 0 °C under Ar were added K_2CO_3 (740.5 mg, 5.358 mmol, 2.98 equiv) and *p*-toluenesulfonyl chloride (376.4 mg, 1.974 mmol, 1.1 equiv). The mixture was stirred for 5 h at 0 °C and 1 h at room temperature. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed three times with 1 M NaOH and once with brine, dried over $MgSO_4$, and concentrated to dryness. The product, 572.3 mg (96%), was obtained as a rust-colored, amorphous solid.

^{1}H NMR (270 MHz) ($CDCl_3$): δ 2.47 (3H, s), 3.52 (2H, s), 3.81 (3H, s), 6.70 (1H, d, J = 8.2 Hz), 6.86 (1H, d, J = 8.1 Hz), 7.34 (2H, d, J = 8.1 Hz), 7.79 (2H, d, J = 8.3 Hz), 7.85 (1H, s, D_2O exch). IR (KBr): 3172 (br), 1709, 1616, 1496, 1458, 1371, 1338, 1175, 1093, 1050, 1000, 848, 815, 728, 662, 548, cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 333 (5.0), 269 (1.4), 178 (40), 91 (77), 28 (100).

1,3-Dihydro-7-hydroxy-6-[(tolylsulfonyl)oxy]-2*H*-indol-2-one. Boron tribromide (1.1 mL, 1.1 mmol, 2.0 equiv, 1 M/CH_2Cl_2) was added to a stirred mixture of 1,3-dihydro-7-methoxy-6-[(tolylsulfonyl)oxy]-2*H*-indol-2-one obtained above (181.5 mg, 0.54 mmol, 1.0 equiv) in CH_2Cl_2 (4.3 mL) under Ar, at −78 °C. The mixture was stirred for 8 h and stored at −20 °C for 12 h. The mixture was poured into ice/

water, stirred for 0.5 h, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to dryness to give 164.7 mg (95%) of a red solid.

^{1}H NMR (270 MHz) (acetone-d_6): δ TMS 2.45 (3H, s), 3.43 (2H, d, J = 0.8 Hz), 6.61 (1H, d, J = 8.1 Hz), 6.71 (1H, d, J = 8.1 Hz), 7.46 (2H, d, J = 8.6 Hz), 7.79 (2H, d, J = 8.3 Hz), 8.50 (1H, s, D_2O exch), 9.28 (1H, s, D_2O exch). IR (NaCl, neat): 3259 (br), 2921, 1698, 1365, 1175, 1142, 728 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 319 (3.4), 278 (6.0), 246 (6.7), 163 (49), 139 (73), 91 (100).

1,3-Dihydro-7-[(3-methyl-2-butenyl)oxy]-6-[(tolylsulfonyl)oxy]-2*H*-indol-2-oue (37). To a stirred solution of 1,3-dihydro-7-hydroxy-6-[(tolylsulfonyl)oxy]-2*H*-indol-2-one obtained above (159.4 mg, 0.49 mmol, 1.0 equiv) in DMF (1.5 mL) at 0 °C was added K_2CO_3 (103.5 mg, 0.75 mmol, 1.5 equiv) followed by prenyl bromide (0.09 mL, 0.75 mmol, 1.5 equiv). After 4 h the mixture was poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated to dryness. The product was purified by radial chromatography (eluted with 3:2 hexanes/EtOAc) to afford 71.9 mg (37%) of **37** as a red solid.

^{1}H NMR (270 MHz) ($CDCl_3$): δ TMS 1.58 (3H, s), 1.70 (3H, s), 2.45 (3H, s), 3.52 (2H, s), 4.47 (2H, d, J = 7.3 Hz), 5.35 (1H, t, J = 7.3 Hz), 6.74 (1H, d, J = 8.2 Hz), 6.87 (1H, d, J = 8.1 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.79 (2H, d, J = 8.3 Hz), 8.61 (1H, s, D_2O exch). IR (NaCl, neat): 3194 (br), 1714, 1627, 1464, 1376, 1196, 1175, 837, 728 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 387 (16), 319 (16), 164 (37), 91 (91), 67 (100).

1,3-Dihydro-6,7-dihydroxy-2*H*-indol-2-one (32). Boron tribromide (800 mL, 800 mmol, 2.5 equiv, 1M/CH_2Cl_2) was added dropwise to a stirred mixture of **31** (57.3 g, 320 mmol, 1.0 equiv)) in CH_2Cl_2 (640 mL) under N_2 at −78 °C. The reaction mixture was stirred at −78 °C for 8 h and was then poured into a large (4 L) beaker containing 1.5 L of ice/water, stirred for 10 min, and filtered to remove undissolved product. The remaining liquid was extracted with EtOAc, washed with brine, and dried over $MgSO_4$. The organic layer was evaporated to yield the pure product **32**, which was combined with the filter cake, total yield 52.3 g (99%). An analytical sample was recrystallized from H_2O (three times) to give a faint pink crystalline solid, mp 245 °C dec.

^{1}H NMR (300 MHz) (DMSO-d_6): δ TMS 3.32 (2H, s), 6.36 (1H, d, J = 7.9 Hz), 6.48 (1H, d, J = 2.9 Hz), 8.80 (2H, br s, D_2O exch), 10.0 (1H, br s, D_2O exch). IR (KBr): 3366−3123 (br), 1672, 1649, 1618, 1359, 1265, 1178, 786 cm^{-1}. Microanal. Calcd for $C_8H_7NO_3$: C, 58.18; N, 4.27; N, 8.48. Found: C, 58.34; H, 4.44; N, 8.25.

1,3-Dihydro-6-hydroxy-7-[(3-methyl-2-butenyl)oxy]-2*H*-indol-2-one (33). To a stirred solution of 6,7-dihydroxyoxindole (**32**) (19.0 g, 115 mmol, 1.0 equiv) in DMF (230 mL) at 0 °C under Ar was added K_2CO_3 (15.9 g, 115 mmol, 1.0 equiv). After 8 min prenyl bromide (14.8 mL, 127 mmol, 1.1 equiv) was added dropwise. The reaction mixture was stirred at 0 °C for 6.5 h, poured into a separatory funnel, diluted with H_2O, and extracted with ether. The ethereal solution was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The product was purified by column chromatography (eluted with 3:1 hexanes/EtOAc, then 1:1 hexanes/EtOAc) to yield 14.5 g (54%) of **33**. An analytical sample was recrystallized from toluene to give a red-white solid, mp 111 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.65 (3H, s), 1.80 (3H, s), 3.50 (2H, s), 4.47 (1H, d, J = 7.4 Hz), 5.50−5.55 (1H, m), 5.57 (1H, s, D_2O exch), 6.59 (1H, d, J = 8.1 Hz), 6.84 (1H, d, J = 8.0 Hz), 7.77 (1H, s, D_2O exch). IR (KBr): 3367, 3192, 2971, 1694, 1664, 1635, 1461, 1356, 1286, 1199, 1047 cm^{-1}. Microanal. Calcd for $C_{13}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.16; H, 6.52; N, 6.07.

(±)-1,3-Dihydro-7-[(3,3-dimethyloxiranyl)methoxy]-6-hydroxy-2*H*-indol-2-one. To a stirred solution of **33** (14.5 g, 62.1 mmol, 1.0 equiv) in CH_2Cl_2 (620 mL) were added $NaHCO_3$ (5.7 g, 68.3 mmol, 1.1 equiv) and *m*-CPBA (10.7 g, 62.1 mmol, 1.0 equiv). The mixture was stirred for 1 h, and an additional amount of each reagent was added, $NaHCO_3$ (5.7 g, 68.3 mmol, 1.1 equiv) and *m*-CPBA (10.7 g, 62.1 mmol, 1.0 equiv). The mixture was stirred for an additional 1 h, and a third portion each of $NaHCO_3$ (5.7 g, 68.3 mmol, 1.1 equiv) and *m*-CPBA (10.7 g, 62.1 mmol, 1.0 equiv) was added. The resulting mixture was stirred for 3 h, while the temperature was maintained at 0 °C. The reaction mixture was filtered into a flask containing 10% $Na_2S_2O_3$ and 10% $NaHCO_3$. The organic layer was isolated, diluted with CH_2Cl_2, and washed with 10% $Na_2S_2O_3$ and saturated $NaHCO_3$ and finally with brine. The organic layer was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and dried in vacuo to yield 17 g of the product, which was used directly for the next step. An analytical sample was recrystallized from toluene to give a white solid, mp 122−123 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.38 (3H, s), 1.42 (3H, s), 3.25 (1H, dd, J = 2.9, 8.5 Hz), 3.47−3.49 (2H, m), 3.80 (1H, dd, J = 8.5, 12.0 Hz), 4.54 (1H, dd, J = 2.9, 12.0 Hz), 6.25 (1H, s, D_2O exch), 6.58 (1H, d, J = 8.1 Hz), 6.84 (1H, d, J = 8.1 Hz), 8.44 (1H, s, D_2O exch). IR (KBr): 3495, 3146, 2982, 1717, 1694, 1635, 1501, 1466, 1321, 1187, 1047, 861 cm^{-1}. Microanal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.06; N, 5.62. Found: C, 62.70; H, 6.15; N, 5.66.

(±)-3,4,8,10-Tetrahydro-3-hydroxy-4,4-dimethyl-2*H*,9*H*-[1,4]dioxepino[2,3-*g*]indol-9-one (34). $SnCl_4$ (9.6 mL, 81.8 mmol, 1.2 equiv) was slowly added dropwise to a flame-dried flask, which had been flushed with Ar and charged with dry THF (960 mL). After 10 min a solution of (±)-1,3-dihydro-7-[(3,3-dimethyloxiranyl)methoxy]-6-hydroxy-2*H*-indol-2-one obtained above (17 g, 62 mmol, 1.0 equiv) in THF (73 mL) was added dropwise to the reaction vessel and stirred for 2 h. Approximately one-half of the solvent was removed under reduced pressure and the remaining solution poured into a separatory funnel containing saturated $NaHCO_3$ and H_2O (~50:50), which was then exhaustively extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give a dark crude product. The product was purified by column chromatography (eluted with 1:2 hexanes/EtOAc) to yield 10 g (64% for two steps) of **34**. An analytical sample was recrystallized from toluene to give a yellow crystalline solid, mp 194 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.24 (3H, s), 1.54 (3H, s), 2.94 (1H, d, J = 11.2 Hz, D_2O exch), 3.51 (2H, s), 3.63 (1H, ddd, J = 1.0, 4.0, 11.2 Hz), 4.12 (1H, dd, J = 1.0, 12.4 Hz), 4.24 (1H, dd, J = 4.0, 12.5 Hz), 6.64 (1H, d, J = 8.0 Hz), 6.83 (1H, d, J = 7.9 Hz), 7.64 (1H, s, D_2O exch). IR (KBr): 3460, 3320, 3169, 2982, 1711, 1682, 1461, 1327, 1216, 1047 cm^{-1}. Microanal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.08; N, 5.61. Found: C, 62.28; H, 6.21; N, 5.56.

(±)-3-Hydroxy-4,4-dimethyl-3,4,dihydro-2*H*,10*H*-[1,4]dioxepino-[2,3-*g*]indole (35). To a stirred solution of **34** (11.2 g, 44.8 mmol, 1.0 equiv) in THF (225 mL) under Ar at 0 °C was added BF_3·OEt_2 (19.3 mL, 157 mmol, 3.5 equiv). After 10 min, $NaBH_4$ (2.71 g, 71.8 mmol, 1.6 equiv) was added at once, and the mixture was stirred for 8 h at 0 °C and then at room temperature for 40 h. The reaction was completed by the slow addition of water (1 L) and was stirred for 0.5 h. HCl (concentrated) was added until pH = 1, and the mixture was stirred for an additional 0.5 h. The mixture was treated with 1 M NaOH until pH = 14 and stirred for 0.5 h. The mixture was poured into a separatory funnel and extracted with EtOAc/ether. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to leave 10 g of a crude solid. The product was purified by column chromatography (eluted with 2:1 hexanes/EtOAc) to yield 4.5 g (43%) of **35**. An analytical sample was recrystallized from benzene to afford a white crystalline solid, mp 202−205 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.22 (3H, s), 1.56 (3H, s), 3.03 (1H, d, J = 11.4 Hz, D_2O exch), 3.63 (1H, ddd, J = 4.0, 0.9, 11.3 Hz), 4.19 (1H, dd, J = 0.9, 12.3 Hz), 4.31 (1H, dd, J = 4.0, 12.3 Hz), 6.49 (1H, dd, J = 2.2, 3.1 Hz), 6.78 (1H, d, J = 8.4 Hz), 7.16−7.19 (2H, m), 8.29 (1H, s, D_2O exch). IR (KBr): 3340, 2984, 1580, 1504, 1444, 1338, 1224, 1133, 1057, 814, 753 cm^{-1}. Microanal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 67.16; H, 6.63; N, 5.79.

(±)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4,4-dimethyl-3,4-dihydro-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole. To a stirred solution of **35** (11.6 g, 49.7 mmol, 1.0 equiv) in DMF (124 mL) at room temperature under N_2 was added *tert*-butyldimethylsilyl chloride (15.0 g, 99.4 mmol, 2.0 equiv) immediately followed by imidazole (23.7 g, 348 mmol, 7.0 equiv). The solution was slowly heated to 40 °C, stirred overnight, poured into a separatory funnel, and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the crude solid purified by column chromatography (eluted with 5:1 hexanes/EtOAc) to yield 14.2 g (82%) of

the product. An analytical sample was recrystallized from cyclohexane to give a white solid, mp 118−119 °C.

^{1}H NMR (300 Hz) ($CDCl_3$): δ TMS 0.14 (6H, s), 0.89 (9H, s), 1.12 (3H, s), 1.48 (3H, s), 3.88 (1H, dd, J = 9.2, 11.5 Hz), 3.98 (1H, dd, J = 3.2, 9.2 Hz), 4,22 (1H, dd, J = 3.2, 11.5 Hz), 6.48 (1H, dd, J = 2.2, 3.1 Hz), 6.76 (1H, d, J = 8.4 Hz), 7.14 (2H, ddd, J = 2.4, 3.4, 3.5 Hz), 8.21 (1H, s, D_2O exch). IR (neat): 3412, 2936, 1500, 1438, 1234, 1093, 833 cm^{-1}. Microanal. Calcd for $C_{19}H_{29}NO_3Si$: C, 65.66; H, 8.41; N, 4.03. Found: C, 65.59; H, 8.20; N, 3.90.

(±)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4,4-dimethyl-8-[(*N*,*N*-dimethylamino)methyl]-3,4-dihydro-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]-indole (36). To a flask charged with acetic acid (136 mL) under Ar were added formaldehyde (3.4 mL, 45 mmol, 1.1 equiv, 37%/H_2O) and dimethylamine (20.5 mL, 163 mmol, 4.0 equiv, 40% solution in H_2O) followed by (±)-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4,4-dimethyl-3,4,dihydro-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole obtained above (14.2 g, 40.9 mmol, 1.0 equiv) over a 10 min period. The reaction mixture was stirred for 1 day when 10% K_2CO_3 was added until pH ≈ 8; then 2 M NaOH was added. The mixture was extracted with ether/EtOAc, washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, leaving 17.3 g (quantitative) of the pure product **36**. An analytical sample was recrystallized from toluene to give a white flaky solid, mp 152 °C.

^{1}H NMR (300 Hz) ($CDCl_3$): δ TMS 0.15 (6H, s), 0.90 (9H, s), 1.13 (3H, s), 1.48 (3H, s), 2.28 (6H, s), 3.58 (2H, s), 3.58 (2H, s), 3.88 (1H, dd, J = 9.2, 11.4 Hz), 3.98 (1H, dd, J = 3.2, 9.1 Hz), 4.21 (1H, dd, J = 3.2, 11.5 Hz), 6.76 (1H, d, J = 8.4 Hz), 8.44 (1H, s, D_2O exch). IR (NaCl, neat): 2932, 1502, 1458, 1360, 1251, 1218, 1093, 837, 777 cm^{-1}. Microanal. Calcd for $C_{22}H_{36}N_2O_3Si$: C, 65.31; H, 8.97; N, 6.92. Found: C, 65.09; H, 8.77; N, 6.73.

(±)-6(*R*)-(2*E*)-Methyl 3-[[3-[[(1,1-Dimethylethyl)dimethylsilyl]-oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indol-8-yl]methyl]-8a-[4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)methyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-carboxylate (39). To a stirred solution of **38** (23.0 mg, 0.043 mmol, 1.0 equiv) in CH_3CN (0.3 mL) and PBu_3 (5.4 μL, 0.022 mmol, 0.5 equiv) was added a solution of **36** (19.3 mg, 0.048 mmol, 1.1 equiv) in CH_3CN (0.3 mL). The mixture was refluxed for 5.5 h and stirred at room temperature overnight. The reaction mixture was then diluted with ether, washed with water, dilute HCl, and brine, and dried over $MgSO_4$. The solvent was removed and the crude oily solid purified by PTLC on silica gel (eluted with 1:4 EtOAc/hexanes) to yield 19.8 mg (51%) of **39**. An analytical sample was recrystallized from cyclohexane to give a white crystalline solid, mp 168−168.5 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) (a racemic mixture of two diastereomers): δ TMS 0.00 (6H, s), 0.01 (6H, s), 0.13 (6H, s), 0.14 (6H, s), 0.034−0.19 (2H, m), 0.43−0.52 (2H, m), 0.62−0.72 (2H, m), 0.84 (9H, s), 0.85 (9H, s), 0.86 (9H, s), 0.88 (9H, s), 1.05 (3H, s), 1.1 (3H, s), 1.45 (3H, s), 1.49 (3H, s), 1.537 (3H, s), 1.544 (3H, s), 1.33−1.67 (2H, m), 2.14−2.25 (2H, m), 2.52−2.60 (2H, m), 2.87−3.03 (2H, m), 3.27 (6H, s), 3.36−3.52 (2H, m), 3.66 (1H, $^1/_2$ ABq, J = 15.0 Hz), 3.66 (1H, $^1/_2$ ABq, J = 15.0 Hz), 3.75 (6H, s), 3.77−3.96 (12H, m), 4.14−4.20 (2H, m), 5.25−5.31 (2H, m); 5.48 (2H, $^1/_2$ ABq, J = 14.6 Hz), 6.70−6.89 (8H, m), 7.15−7.22 (6H, m), 8.29 (1H, s, D_2O exch), 8.32 (1H, s, D_2O exch). IR (NaCl, neat): 3303, 2954, 2856, 1752, 1660, 1512, 1447, 1251, 1098, 1049, 837, 777 cm^{-1}. Microanal. Calcd for $C_{48}H_{71}N_3O_9Si_2$: C, 64.76; H, 8.04; N, 4.72. Found: C, 64.95; H, 8.09; N, 4.53.

[(±)-[3α,8a*β*(*E*)]]-8-[[2-[(4-Methoxyphenyl)methyl]-8a-[4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)-dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole (40). **[(±)-[3*β*,8aα(*E*)]]-8-[[2-[(4-Methoxyphenyl)methyl]-8a-[4-[[(1,1-dimethylethyl) dimethylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]-methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole (41).** A dry flask containing **39** (24.4 mg, 0.027 mmol, 1.0 equiv) and lithium chloride (11.6 mg, 0.27 mmol, 10 equiv) under N_2 was charged with HMPA (0.21 mL) and water (1.5×10^{-3} mL, 0.082 mmol, 3.0 equiv). This mixture was heated to 100−105 °C for 2 h. The resulting solution was diluted with 1:1 EtOAc/hexanes and washed with water (5×) and brine. The organic layer was dried over $MgSO_4$ and concentrated to dryness. The product was purified by PTLC on silica gel (eluted with 1:3 EtOAc/hexanes) to yield 8.9 mg (39%) of **40** (oil) and 2.7 mg (12%) of **41** (oil). Total yield: 51%.

^{1}H NMR (300 MHz) ($CDCl_3$) (a racemic mixture of two diastereomers) (**40**): δ 0.036 (12H, s), 0.12 (6H, s), 0.13 (6H, s), 0.84 (9H, s), 0.87 (9H, s), 0.88 (9H, s), 0.882 (9H, s), 1.10 (3H, s), 1.11 (3H, s), 1.458 (9H, s), 1.463 (3H, s), 1.72−2.04 (10H, m), 2.12−2.23 (2H, m), 3.24−3.51 (8H, m), 3.72 (3H, s), 3.73 (3H, s), 3.79−3.82 (6H, m), 3.83 (2H, s), 3.86 (2H, s), 4.15−4.20 (4H, m), 5.15 (1H, $^1/_2$ ABq, J = 14.2 Hz), 5.20 (1H, $^1/_2$ ABq, J = 14.2 Hz), 5.28 (1H, m), 5.45 (1H, m), 6.67−6.71 (4H, m), 6.76 (2H, d, J = 8.5 Hz), 6.81−6.90 (6H, m), 7.16 (2H, d, J = 8.5 Hz), 8.12 (2H, s, D_2O exch). IR (*syn*) (NaCl, neat): 2920, 1655, 1508, 1449, 1250, 1220, 1091, 838 cm^{-1}.

^{1}H NMR (300 MHz) ($CDCl_3$) (a racemic mixture of two diastereomers) (**41**): δ −0.18 (12H, s), 0.12 (6H, s), 0.13 (6H, s), 0.26−0.41 (2H, m), 0.47−0.58 (2H, m), 0.62−0.72 (2H, m), 0.84 (18H, s), 0.87 (9H, s), 0.89 (9H, s), 1.06 (3H, s), 1.10 (3H, s), 1.44 (6H, s), 1.47 (3H, s), 1.48 (3H, s), 1.63−1.67 (2H, m), 2.10−2.17 (2H, m), 2.44−2.52 (2H, m), 2.89−3.05 (2H, m), 3.20−3.28 (2H, m), 3.40−3.52 (4H, m), 3.71−3.97 (16H, m), 4.08 (2H, br s), 4.14−4.21 (2H, m), 5.05 (2H, br s), 5.56 (1H, $^1/_2$ ABq, J = 14.2 Hz), 5.57 (1H, $^1/_2$ ABq, J = 14.5 Hz), 6.71 (1H, d, J = 8.6 Hz), 6.73 (1H, d, J = 8.6 Hz), 6.83−6.88 (6H, m), 7.14 (1H, d, J = 8.6 Hz), 7.18 (1H, d, J = 8.6 Hz), 7.22−7.23 (4H, m), 8.34 (2H, s, D_2O exch). IR (*anti*) (neat): 2932, 1649, 1508, 1455, 1250, 1220, 1103, 838 cm^{-1}. HRMS (EI) (*anti*): 831.46765 ($C_{46}H_{69}N_3O_7Si_2$ requires 831.4674).

[(±)-[3α,8aα(*E*)]]-1,1-Dimethylethyl 8-[[3-(Methoxycarbonyl)-2-[(4-methoxyphenyl)methyl]-8a-[4-[[(1,1-dimethylethyl)dimethylsilyl]-oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (42). To a stirred solution of **39** (260.0 mg, 0.292 mmol, 1.0 equiv) in CH_2Cl_2 (1.5 mL) at 0 °C under Ar were added DMAP (35.7 mg, 0.292 mmol, 1.0 equiv) and Et_3N (0.041 mL, 0.29 mmol, 1.0 equiv). After 5 min $(BOC)_2O$ (191.2 mg, 0.876 mmol, 3.0 equiv) was added in one portion. The resulting solution was stirred for 20 h, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude solid was purified by radial chromatography (eluted with 1:5 EtOAc/hexanes) to yield 260.4 mg (90%) of **42** as a white crystalline solid, mp 74−75 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ −0.01 (6H, s), 0.00 (6H, s), 0.113 (6H, s), 0.12 (6H, s), 0.58−0.68 (2H, m), 0.80−0.92 (38H, m), 1.06 (6H, s), 1.45−1.63 (2H, m), 1.47 (6H, s), 1.53 (6H, s), 1.60 (18H, s), 1.59−1.81 (2H, m), 2.22−2.34 (2H, m), 2.60 (2H, dd, J = 8.1, 15.0 Hz), 2.91−3.08 (2H, m), 3.26 (6H, s), 3.26−3.42 (2H, m), 3.56 (1H, $^1/_2$ ABq, J = 14.8 Hz), 3.59 (1H, $^1/_2$ ABq, J = 14.8 Hz), 3.71−3.80 (4H, m), 3.74 (6H, s), 3.83 (2H, s), 3.84 (2H, s), 3.90−3.97 (4H, m), 4.13−4.17 (2H, m), 3.32 (2H, m), 5.34 (1H, $^1/_2$ ABq, J = 14.8 Hz), 5.42 (1H, $^1/_2$ ABq, J = 14.8 Hz), 6.75−6.79 (4H, m), 6.88 (1H, d, J = 8.4 Hz), 6.89 (1H, d, J = 8.4 Hz), 7.03 (2H, s), 7.12−7.20 (6H, m). IR (NaCl, neat): 2943, 1752, 1660, 1507, 1496, 1464, 1463, 1404, 1365, 1251, 1153, 1109, 1082, 837, 772 cm^{-1}. HRMS (EI): 989.5249 ($C_{53}H_{79}N_3O_{11}Si_2$ requires 989.5253).

[(±)-[3*β*,8a*β*(*E*)]]-1,1-Dimethylethyl 8-[[8a-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)-methyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (*syn*-43). **[(±)-[3α,8a*β*(*E*)]]-1,1-Dimethylethyl 8-[[8a-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)methyl]-octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]-dioxepino[2,3-*g*]indole-10-carboxylate (anti-43).** A flask containing **42** (126.6 mg, 0.128 mmol, 1.0 equiv) and LiCl (27.1 mg, 0.64 mmol, 5.0 equiv) under N_2 was charged with HMPA (0.78 mL) and H_2O (3.4 × 10^{-3} mL, 1.9 × 10^{-4} mmol, 1.5 equiv). The solution was heated (100−105 °C) for 1.25 h and then poured into water and extracted with ether. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated, leaving a crude oily solid. The product

was purified by radial chromatography (eluted with 1:5 EtOAc/hexanes) to yield 79.2 mg (66%) of *syn*-**43** (an analytical sample was obtained by PTLC, eluted with 1:5 EtOAc/hexanes, to give an oil) and 3.1 mg (2.6%) of the *anti*-isomer (oil).

^{1}H NMR (300 MHz) ($CDCl_3$) (*syn*-**43**): δ 0.026 (6H, s), 0.32 (6H, s), 0.127 (6H, s), 0.14 (6H, s), 0.867 (9H, s), 0.873 (9H, s), 0.878 (9H, s), 0.883 (9H, s), 1.10 (6H, s), 1.48 (3H, s), 1.49 (3H, s), 1.55 (3H, s), 1.57 (3H, s), 1.610 (9H, s), 1.613 (9H, s), 1.83−1.96 (6H, s), 2.22−2.35 (4H, m), 2.46 (2H, dd, J = 6.0, 15.0 Hz), 3.11−3.21 (2H, m), 3.31−3.85 (2H, m), 3.37 (1H, ^{1}/2ABq, J = 14.5 Hz), 3.48 (1H, ^{1}/$_2$ ABq, J = 14.6 Hz), 3.71 (3H, s), 3.72 (3H, s), 3.76−3.98 (8H, m), 3.99 (2H, m), 4.02 (2H, s), 4.15−4.21 (4H, m), 5.17 (1H, ^{1}/$_2$ ABq, J = 14.5 Hz), 5.20 (1H, ^{1}/$_2$ ABq, J = 14.6 Hz), 5.35 (1H, m), 5.48 (1H, m), 6.62−6.70 (6H, m), 6.79 (2H, m), 6.91 (2H, d, J = 8.3 Hz), 7.14 (1H, d, J = 8.4 Hz), 7.16 (1H, d, J = 8.3 Hz), 7.22 (1H, s), 7.23 (1H, s). IR (NaCl, neat) (*syn*): 2932, 1755, 1661, 1455, 1367, 1250, 1156, 1114,1091, 838 cm^{-1}. HRMS (EI) (*syn*): 931.51955 ($C_{51}H_{77}N_3O_9Si_2$ requires 931.5198). Microanal. Calcd for $C_{51}H_{77}N_3O_9Si_2$: C, 65.70; H, 8.32; N, 4.51. Found: C, 65.37; H, 8.37; N, 4.54.

^{1}H NMR (300 MHz) ($CDCl_3$) (*anti*): δ −0.02 (6H, s), −0.01 (6H, s), 0.03−0.22 (2H, m), 0.12 (6H, s), 0.13 (6H, s), 0.146−0.62 (4H, m), 0.84 (9H, s), 0.85 (9H, s), 0.87 (18H, s), 1.05 (3H, s), 1.07 (3H, s), 1.43 (3H, s), 1.47 (3H, s), 1.49 (3H, s), 1.52 (3H, s), 1.55 (9H, s), 1.60 (9H, s), 1.80−1.91 (2H, m), 2.19−2.22 (2H, m), 2.50−2.61 (2H, m), 3.09−3.23 (2H, m), 3.29−3.52 (4H, m), 3.63−3.96 (18H, m), 4.13−4.20 (4H, m), 5.04−5.10 (1H, m), 5.28−5.32 (1H, m), 5.48 (1H, ^{1}/$_2$ ABq, J = 14.3 Hz), 5.52 (1H, ^{1}/$_2$ ABq, J = 14.3 Hz), 6.71−6.90 (6H, m), 7.04−7.22 (8H, m). IR (NaCl, neat) (*anti*: 3295 (br), 1753, 1657, 1510, 1447, 1249, 1152, 1090, 1034, 836, 773 cm^{-1}.

1,1-Dimethylethyl 8-[[8a-[4-Hydroxy-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)methyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-hydroxy-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (44). To a stirred solution of **43** (36.3 mg, 0.04 mmol, 1.0 equiv) under N_2 in THF (1.0 mL) was added n-Bu_4NF (0.12 mL, 0.12 mmol, 3.0 eq, 1.0M/THF). The solution was heated (~40 °C) for 3 h. At this time the solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine and dried over $MgSO_4$. The residue was purified by PTLC on silica gel (eluted with EtOAc) to yield 24.9 mg (79%) of **44**.

^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.19 (3H, s), 1.22 (3H, s), 1.52 (3H, s), 1.53 (3H, s), 1.56 (3H, s), 1.57 (3H, s), 1.59 (9H, s), 1.60 (9H, s), 1.72−2.21 (12H, m), 2.71 (2H, br s, D_2O exch), 3.18−3.49 (4H, m), 3.51 (2H, ^{1}/$_2$ ABq, J = 14.5 Hz), 3.56 (1H, s, D_2O exch), 3.61 (1H, s, D_2O exch), 3.72 (3H, s), 3.74 (3H, s), 3.75−3.94 (6H, s), 4.18−4.30 (4H, s), 4.26−4.27 (4H, m), 4.44 (2H, m), 5.25 (2H, ^{1}/$_2$ ABq, J = 14.5 Hz), 5.25 (2H, ^{1}/$_2$ ABq, J = 14.4 Hz), 6.70 (2H, d, J = 8.7 Hz), 6.77 (2H, d, J = 8.6 Hz), 6.83 (2H, d, J = 8.6 Hz), 6.927 (1H, d, J = 8.4 Hz), 6.932 (1H, d, J = 8.3 Hz), 7.03 (2H, d, J = 8.6 Hz), 7.12 (1H, d, J = 8.3 Hz), 7.15 (1H, d, J = 8.4 Hz), 7.21 (1H, s), 7.23 (1H, s). IR (NaCl, neat): 3422, 2976, 1753, 1649, 1513, 1496, 1457, 1371, 1333, 1251, 1153, 1033, 733 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 703 (M^+, 8), 604 (37), 603 (100). HRMS (EI): 703.3461 ($C_{39}H_{49}N_3O_9$ requires 703.3472).

1,1-Dimethylethyl 8-[[8a-[4-Chloro-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)methyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-hydroxy-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (45). To **44** (24.9 mg, 0.035 mmol, 1.0 equiv) in DMF (0.35 mL) at 0 °C under Ar were added dry LiCl (2.9 mg, 0.07 mmol, 1.9 equiv) and collidine (7 μL, 0.05 mmol, 1.5 equiv). After stirring for 10 min, methanesulfonyl chloride (4 μL, 0.05 mmol, 1.5 equiv) was added dropwise. The ice bath was removed and the mixture stirred at room temperature for 24 h. At this time additional collidine (2.5 equiv) and methanesulfonyl chloride (2.5 equiv) were added, and the mixture was stirred for 2 h. It was then diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to dryness. The product was purified by PTLC on silica gel (eluted with 2:1 EtOAc/hexanes) to yield 21.9 mg (86%) of **45** as an oil.

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.22 (3H, s), 1.23 (3H, s), 1.57 (3H, s), 1.58 (3H, s), 1.62 (9H, s), 1.63 (9H, s), 1.66 (3H, s), 1.73 (3H, s), 1.83−1.93 (8H, m), 2.05−2.37 (4H, m), 3.06 (2H, dd, J = 3.8, 11.4 Hz), 3.35−3.42 (6H, m, 1H, D_2O exch), 3.46−3.69 (4H, m), 3.75 (3H, s), 3.77 (3H, s), 3.86−3.94 (2H, m), 3.96 (2H, s), 4.02 (2H, s), 4.21−4.29 (6H, m), 5.20−5.29 (3H, m), 5.53 (1H, m), 6.69−6.81 (6H, m), 6.94−6.99 (4H, m), 7.18−7.21 (4H, m). IR (NaCl, neat): 3433, 2976, 1752, 1654, 1513, 1496, 1453, 1371, 1251, 1153 cm^{-1}.

1,1-Dimethylethyl 8-[[8a-[4-Hydroxy-3-methyl-2-butenyl]-2-[(4-methoxyphenyl)methyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (46). To a solution of **45** (28.2 mg, 0.04 mmol, 1.0 equiv) in CH_2Cl_2 (0.3 mL) at 0 °C under Ar was added *tert*-butyldimethylsilyl triflate (9.0 μL, 0.04 mmol, 1.2 equiv) followed immediately by 2,6-lutidine (6.0 μL, 0.047 mmol, 1.4 equiv). The mixture was stirred for 2 h, then diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The product was purified by radial chromatography (eluted with 1:1 EtOAc/hexanes) to yield 24.9 mg (76%) of **46** as an oil.

^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.12 (6H, s), 0.13 (6H, s), 0.87 (9H, s), 0.88 (9H, s), 1.08 (3H, s), 1.10 (3H, s), 1.48 (6H, s), 1.61 (9H, s), 1.63 (9H, s), 1.69 (3H, s), 1.79 (3H, s), 1.82−2.03 (8H, m), 2.16−2.24 (4H, m), 3.19 (2H, dd, J = 7.2, 8.5 Hz), 3.25−3.39 (4H, m), 3.49 (1H, ^{1}/$_2$ ABq, J = 14.5 Hz), 3.65 (1H, ^{1}/$_2$ ABq, J = 14.5 Hz), 3.72 (3H, s), 3.76 (3H, s), 3.79−3.99 (8H, m), 4.15−4.22 (4H, m), 5.19−5.28 (4H, m), 5.49 (2H, m), 6.67−6.81 (6H, m), 6.92 (4H, dd, J = 1.9, 8.4 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.14 (1H, d, J = 8.4 Hz), 7.20 (1H, s), 7.24 (1H, s). IR (NaCl, neat): 2932, 1752, 1654, 1512, 1491, 1447, 1365, 1251, 1153, 1088, 837 cm^{-1}.

[(±)-[3α,8aα,10(R*)]]-1,1-Dimethylethyl 8-[[Tetrahydro-2-[(4-methoxyphenyl)methyl]-10-(1-methylethenyl)-1,4-dioxo-6*H*-3,8a-ethanopyrrolo[1,2-*a*]pyrazin-3(4*H*)-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (47). To **46** (24.0 mg, 0.028 mmol, 1.0 equiv) in a flask equipped with a magnetic stir bar were added NaH (12.3 mg, 0.3 mmol, 10.8 equiv) and benzene (3.5 mL). The flask was fitted with a condenser and gently refluxed for 59 h (additional benzene (1.5 mL) was added during this time). The solution was stirred at room temperature for 8 days, after which NaI (10.8 mg, 0.072 mmol, 2.5 equiv) was added. The mixture was then stirred at reflux temperature for an additional 2 days. The resulting mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The product was purified by PTLC on silica gel (eluted with 1:1 hexanes/EtOAc) to afford 2.5 mg (11% or 19% based on recovered **46**) of **47** as an amorphous yellow solid.

^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.12 (6H, s), 0.14 (6H, s), 0.882 (9H, s), 0.885 (9H, s), 1.10 (3H, s), 1.13 (3H, s), 1.48 (3H, s), 1.49 (3H, s), 1.55 (3H, s), 1.56 (3H, s), 1.59 (18H, s), 1.80 (2H, dd, J = 5.7, 13.3 Hz), 1.90 (2H, dd, J = 13.2 Hz), 2.03−2.08 (4H, m), 2.22 (2H, dd, J = 10.4, 13.4 Hz), 2.85−2.98 (4H, m), 3.08 (2H, ^{1}/$_2$ ABq, J = 17.1 Hz), 3.29 (2H, ^{1}/$_2$ ABq, J = 17.6 Hz), 3.56−3.62 (4H, m), 3.72 (3H, s), 3.73 (3H, s), 3.74−3.83 (2H, dd, J = 9.4, 12.5 Hz), 3.91−3.96 (2H, m), 4.18 (2H, dd, J = 3.6, 12.2 Hz), 4.28 (1H, ^{1}/$_2$ ABq, J = 15.9 Hz), 4.37 (1H, ^{1}/$_2$ ABq, J = 15.9 Hz), 4.54−4.74 (6H, m), 6.62−6.75 (8H, m), 6.89−6.94 (2H, m), 6.99−7.04 (2H, m), 7.25 (1H, s), 7.28 (1H, s). IR (NaCl, neat): 2932, 1687, 1365, 1251, 1158, 1088 cm^{-1}. HRMS (EI): 799.4252 ($C_{45}H_{61}N_3O_8Si$ requires 799.4228).

(*R*)-(*E*)-8a-[3-Methyl-4-oxo-2-buten-yl]hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (49). To a stirred solution of **48** (17.25 g, 48.45 mmol, 1.0 equiv) in a 2:1 solution of CH_3CN (343 mL) and H_2O (171 mL) was added, in one portion, CAN (93 g, 170 mmol, 3.8 equiv). After stirring for 2 h, the orange solution was poured into a large separatory funnel and exhaustively extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified by column chromatography (eluted with 95:4:1 CH_2Cl_2/MeOH/AcOH) to yield 9.0 g (79%) of **49** as a yellow oil. An analytical sample was obtained by PTLC (silica gel, eluted with 1:1 hexanes/EtOAc).

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 1.76 (3H, s), 1.99−2.10 (2H, br s), 2.17−2.26 (2H, m), 2.78 (1H, dd, J = 7.3, 14.5 Hz), 2.90 (1H,

dd, J = 8.0, 14.8 Hz), 3.54−3.63 (1H, m), 3.84 (1H, dt, J = 12.3, 8.4 Hz), 3.95 (1H, d $^1/_2$ ABq, J = 3.4, 17.6 Hz), 4.10 (1H, $^1/_2$ ABq, J = 17.6 Hz), 6.55 (1H, t, J = 7.2 Hz), 7.96 (1H, br s, D_2O exch), 9.45 (1H, s). IR (NaCl, neat): 3246, 1684, 1448, 1326, 1107 cm^{-1}. $[\alpha]^{25}_D$ = $-1.51/1.92 \times 10^{-2})° = -78.4°$ (CH_2Cl_2, c = 0.164). Microanal. Calcd: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.88; H, 6.66; N, 11.71. HRMS (EI): 236.1155 ($C_{12}H_{16}N_2O_3$ requires 236.11609).

(*R*)-(*E*)-8a-[4-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]hexahydro-2*H*-pyrrolo[1,2-*a*]pyrazine-1,4-dione (50). To a stirred solution of **49** (9.0 g, 37 mmol, 1.0 equiv) in absolute ethanol (742 mL) at room temperature was added $NaBH_4$ (2.85 g, 75.5 mmol, 2.0 equiv). After 2 h the excess hydride was quenched with water (500 mL) and the pH adjusted to 3−4 by the slow addition of 1 M HCl. Fifteen minutes later, the water and ethanol were removed under reduced pressure and the crude residue was dried in vacuo overnight. The resulting mass (10.87 g) was triturated (1:4 CH_3OH/CH_2Cl_2) and filtered to remove the salts. The remaining solution was concentrated to yield 9.1 g of the crude allylic alcohol, which was immediately utilized for the next step without additional purification. The crude allylic alcohol (9.1 g, 38 mmol, 1.0 equiv) was dissolved in DMF (191 mL) under Ar, and to this mixture was added imidazole (11.9 g, 175.3 mmol, 4.6 equiv) followed by *tert*-butyldiphenylsilyl chloride (12.9 mL, 49.5 mmol, 1.3 equiv). After 2 days the reaction mixture was diluted with water (1 L) and extracted with a 1:1 solution of hexanes and EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The crude solid was recrystallized (ethyl acetate, two crops) to give 10.5 g of the product. The remaining mother liquor was chromatographed (eluted with EtOAc) to give 3.0 g of the pure product. Total yield of **50**: 13.5 g (75% from the enone, two steps). An analytical sample was recrystallized from acetone to provide a white crystalline solid, mp 132 °C.

^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.03 (9H, s), 1.54 (3H, s), 1.92−2.19 (4H, m), 2.49 (1H, dd, J = 8.6, 14.1 Hz), 2.58 (1H, dd, J = 7.5, 14.1 Hz), 3.44−3.53 (1H, m), 3.73 (1H, d $^1/_2$ ABq, J = 4.1, 16.9 Hz), 3.78−3.85 (1H, m), 4.01 (2H, s), 4.06 (1H, $^1/_2$ ABq, J = 16.9 Hz), 5.56−5.62 (1H, m), 6.38 (1H, d, J = 3.7 Hz, D_2O exch), 7.32−7.43 (6H, m), 7.62 (4H, dd, J = 1.8, 7.6 Hz). IR (NaCl, neat): 3232 (br), 2930, 2857, 1664, 1446, 1435, 1113, 822, 733, 702 cm^{-1}. $[\alpha]^{25}_D$ = −63.3° ($CDCl_3$, c = 0.0822). Microanal. Calcd for $C_{28}H_{36}N_2O_3Si$: C,70.55; H, 7.61; N, 5.88. Found C, 70.60; H, 7.56; N, 5.91.

[(R)-[3α*β*,8a*β*(*E*)]]-Methyl 8a-[4-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-2-(methoxycarbonyl)-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-carboxylate (51). To a stirred solution of **50** (8.12 g, 17.0 mmol, 1.0 equiv) in THF (208 mL) at −78 °C, was added a solution of *n*-BuLi (10.65 mL, 17.03 mmol, 1.0 equiv, 1.6 M/hexanes) dropwise. After 25 min methyl chloroformate (1.45 mL, 18.7 mmol, 1.1 equiv) was added dropwise to the reaction mixture and stirred for 25 min. The solution was then transferred via cannula to a cold (−100 °C) flask charged with $LiN[Si(CH_3)_3]_2$ (37.47 mL, 37.47 mmol, 2.2 equiv, 1.0 M/THF) and methyl chloroformate (1.45 mL, 18.7 mmol, 1.1 equiv). The resulting solution was stirred for 45 min, diluted with EtOAc, and washed with saturated aqueous NH_4Cl and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluted with 2:1 hexanes/EtOAc) to yield 9.4 g (93%) of **51** (as a mixture of two diastereomers, *anti*/*syn*). An analytical sample (oil) was obtained by PTLC (eluted with 2:1 hexanes/EtOAc).

^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.04 (9H, s), 1.40 (3H, s), 1.86−2.03 (2H, m), 2.12−2.31 (2H, m), 2.55 (1H, d, J = 7.4 Hz), 3.43−3.52 (2H, m), 3.74−3.82 (1H, m), 3.83 (3H, s), 3.88 (3H, s), 4.03 (2H, br s), 5.48−5.53 (2H, m), 7.34−7.41 (6H, m), 7.57−7.66 (4H, m). IR (NaCl, neat): 2960, 1790, 1740, 1681, 1430, 1366, 1272, 1223, 1109, 735, 705 cm^{-1}. Microanal. Calcd for $C_{32}H_{40}N_2O_7Si$: C, 68.06; H, 7.14; N, 4.96. Found: C, 67.87; H, 7.27; N, 4.77.

[3*β*,8a*β*(*E*)]-Methyl 3-[[3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indol-8-yl]methyl]-8a-[4-[[(1,1-dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazine-3-carboxylate (52). To a flask containing **51** (5.89 g, 14.56 mmol, 1.0 equiv) and **36** (8.64 g, 14.56 mmol, 1.1 equiv) were added CH_3CN (291 mL) and tributylphosphine (1.82 mL, 7.28 mmol, 0.5 equiv). The resulting mixture was gently refluxed for 3.5 h and then stirred at room temperature overnight. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (eluted with 1:2 EtOAc/hexanes) to yield 9.56 g (73%) of **52**. An analytical sample was purified by PTLC on silica gel (eluted with 1:2 EtOAc/hexanes) to give a white crystalline solid, mp 106−108 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.10 (6H, s), 0.115 (3H, s), 0.12 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 1.02 (18H, s), 1.096 (3H, s), 1.10 (3H, s), 1.45 (3H, s), 1.46 (3H, s), 1.54 (6H, s), 1.60−1.88 (6H, m), 2.02−2.11 (2H, m): 2.92 (2H, dd, J = 7.1, 14.4 Hz), 2.44 (2H, dd, J = 8.1, 14.5 Hz), 3.32−3.44 (4H, m), 3.60 (3H, s), 3.62 (3H, s), 3.72−3.93 (8H, m), 3.98 (4H, br s), 4.18 (2H, dd, J = 2.9, 8.4 Hz), 5.43 (2H, m), 6.38 (1H, s, D_2O exch), 6.41 (1H, s, D_2O exch), 6.74 (1H, d, J = 8.5 Hz), 6.75 (1H, d, J = 8.5 Hz), 6.89 (1H, d, J = 2.3 Hz), 6.92 (1H, d, J = 2.3 Hz), 7.08 (2H, d, J = 8.5 Hz), 7.33−7.41 (12H, m), 7.61−7.63 (8H, m), 8.43 (1H, d, J = 2.9 Hz, D_2O exch), 8.64 (1H, d, J = 1.9 Hz, D_2O exch.). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 4.8, 4.2, 9.5, 17.9, 19.2, 19.3, 19.5, 20.3, 25.7, 26.8, 28.0, 28.3, 29.7, 33.7, 35.6, 46.1, 46.2, 53.3, 66.9, 68.0, 71.6, 76.3, 80.7, 80.8, 108.2, 112.9, 117.1, 117.9, 118.0, 123.5, 123.6, 125.5, 127.6, 129.1, 129.2, 129.6, 133.6, 135.5, 138.8, 141.6, 141.8, 161.4, 169.7, 170.5, 170.6. IR (NaCl, neat): 3281 (br), 2954, 2932, 2856, 1747, 1670, 1665, 1649, 1431, 1251, 1224, 1109, 1088, 733, 706 cm^{-1}. HRMS (EI): 893.4457 ($C_{50}H_{67}N_3O_8Si_2$ requires 893.4467). Microanal. Calcd for $C_{50}H_{67}N_3O_8Si_2$: C, 67.16; H, 7.55; N, 4.70. Found: C, 66.93; H, 7.36; N, 4.51.

[3*β*,8a*β*(*E*)]-8-[[8a-[4-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole (53). [3α,8a*β*(*E*)] 8-[[8a-[4-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-a]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-g]indole (54). A flask containing **52** (9.56 g, 10.7 mmol, 1.0 equiv) and LiCl (2.26 g, 53.45 mmol, 5.0 equiv) under Ar was charged with HMPA (82 mL) and water (0.29 mL, 16.0 mmol, 1.5 equiv). This mixture was gently heated (100−105 °C) for 9 h and then diluted with 1:1 hexanes/EtOAc. The resulting solution was washed with water. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography (eluted with 1:2 EtOAc/hexanes) to yield 5.90 g (66%) of **53** (two diastereomers; an analytical sample was recrystallized from CCl_4, mp (*syn*) 167−168 °C) and 2.10 g (23%) of **54** (two diastereomers); an analytical sample was obtained by PTLC on silica gel (eluted with 1:2 EtOAc/hexanes, mp (*anti*) 95−99 °C, white crystalline solid). Total combined yield: 8.00 g (89%).

^{1}H NMR (300 MHz) ($CDCl_3$) (**53**, mixture of two diastereomers): δ TMS 0.12 (6H, s), 0.13 (6H, s), 0.90 (18H, s), 1.0 (18H, s), 1.126 (3H, s), 1.13 (3H, s), 1.48 (6H, s), 1.64 (6H, s), 1.94−2.06 (6H, m), 2.20−2.24 (2H, m), 2.36−2.46 (2H, m), 2.60−2.72 (2H, m), 2.98 (2H, dd, J = 11.6, 14.1 Hz), 3.44−3.57 (4H, m), 3.88 (2H, dd, J = 6.7, 9.2 Hz), 3.97 (2H, dd, J = 3.1, 9.1 Hz), 4.02−4.06 (2H, m), 4.10 (4H, s), 4.17−4.25 (4H, m), 5.58 (2H, m), 5.68 (2H, br s, D_2O exch), 6.75 (2H, d, J = 8.5 Hz), 6.86 (1H, d, J = 2.2 Hz), 6.88 (1H, J = 2.2 Hz), 7.14 (2H, d, J = 8.4 Hz), 7.26−7.44 (12H, m), 7.60−7.64 (8H, m), 8.04 (1H, s, D_2O exch), 8.06 (1H, s, D_2O exch).

The analytical samples of the *syn*-diastereomers were separable by PTLC.

^{1}H NMR (300 MHz) ($CDCl_3$) (**53a**, less polar): δ TMS 0.12 (3H, s), 0.13 (3H, s), 0.88 (9H, s), 1.03 (9H, s), 1.11 (3H, s), 1.46 (3H, s), 1.63 (3H, s), 1.92−2.04 (3H, m), 2.18−2.23 (1H, m), 2.39 (1H, dd, J = 7.2, 14.2 Hz), 2.64 (1H, dd, J = 8.7, 14.2 Hz), 2.99 (1H, dd, J = 11.4, 14.2 Hz), 3.42−3.46 (1H, m), 3.51 (1H, dd, J = 2.7, 14.2 Hz), 3.85 (1H, dd, J = 9.2, 11.3 Hz), 3.94 (1H, dd, J = 3.0, 9 Hz), 3.99−4.06 (1H, m), 4.08 (2H, s), 4.11−4.15 (1H, m), 4.19 (1H, dd, J = 3.0, 11.3 Hz), 5.58 (1H, t, J = 7.8 Hz), 5.76 (1H, d, J = 2.7 Hz, D_2O exch), 6.73 (1H, d, J = 8.4 Hz), 6.85 (1H, d, J = 2.1 Hz), 7.11 (1H, d, J = 8.5 Hz), 7.26−7.42 (6H, m), 7.57−7.63 (4H, m), 8.15 (1H, s, D_2O exch).

^{1}H NMR (300 MHz) ($CDCl_3$) (**53b**, more polar): δ TMS 0.12 (3H, s), 0.14 (3H, s), 0.88 (9H, s), 1.03 (9H, s), 1.11 (3H, s), 1.46 (3H, s), 1.62 (3H, s), 1.91−2.04 (3H, m), 2.18−2.22 (1H, m), 2.36 (1H, dd, J

= 7.3, 14.2 Hz), 2.60 (1H, dd, J = 8.6, 14.3 Hz), 2.97 (1H, dd, J = 11.3, 14.2 Hz), 3.41−3.44 (1H, m), 3.50 (1H, dd, J = 3.1, 14.2 Hz), 3.86 (1H, dd, J = 9.3, 11.3 Hz), 3.95 (1H, dd, J = 3.0, 9.1 Hz), 3.99−4.03 (1H, m), 4.08 (2H, s), 4.14−4.16 (1H, m), 4.20 (1H, dd, J = 2.9, 11.6 Hz), 5.56 (1H, t, J =7.5 Hz), 5.72 (1H, d, J = 2.6 Hz, D_2O exch), 6.73 (1H, d, J = 8.4 Hz), 6.84 (1H, d, J = 2.1 Hz), 7.11 (1H, d, J = 8.4 Hz), 7.26−7.42 (6H, m), 7.57−7.62 (4H, m), 8.07 (1H, s, D_2O exch). IR (NaCl, neat) (*syn*): 3274 (br), 2929, 2858, 1666, 1651, 1453, 1428, 1250, 1224, 1112, 1052, 858, 838, 777 cm^{-1}. Microanal. Calcd for $C_{49}H_{65}N_3O_6Si_2$ (*syn*): C, 68.94; H, 7.84; N, 5.02. Found: C, 69.06; H, 7.76; N, 5.03.

^{1}H NMR (300 MHz) ($CDCl_3$) (**54**, mixture of two diastereomers): δ TMS 0.14 (6H, s), 0.16 (6H, s), 0.90 (18H, s), 1.04 (9H, s), 1.045 (9H, s), 1.09 (3H, s), 1.13 (3H, s), 1.47 (6H, s), 1.53 (3H, m), 1.54 (3H, m), 1.97−2.17 (8H, m), 2.47−2.62 (4H, m), 2.78−2.88 (2H, m), 3.54−3.65 (4H, m), 3.82−3.99 (6H, m), 4.02 (4H, s), 4.21 (2H, dd, J = 3.1, 11.0 Hz), 4.35−4.39 (2H, m), 5.52−5.54 (2H, m), 5.69 (2H, br s, D_2O exch), 6.60 (2H, d, J = 8.4 Hz), 6.63 (2H, d, J = 8.4 Hz), 6.89 (2H, d, J = 2.1 Hz), 6.98 (2H, d, J = 8.4 Hz), 7.36−7.42 (10H, m), 7.62−7.69 (8H, m), 8.08 (2H, br s, D_2O exch). IR (NaCl, neat) (*anti*): 3289 (br), 2929, 2855, 1666, 1444, 1428, 1254, 1222, 1111, 857, 836, 704 cm^{-1}. Mass spectrum (EI) (*anti*): *m/e* (relative intensity) 833 (M^+, 0.1), 512 (6.4), 361 (26), 360 (100), 199 (47). Microanal. Calcd for $C_{48}H_{65}N_3O_6Si_2$ (*anti*): C, 68.94; H, 7.84; N, 5.02. Found: C, 68.76; H, 7.60; N, 4.82.

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[2-[(1,1-Dimethylethoxy)carbonyl]-8a-[4-[[(1,1-dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (58). To a stirred solution of **53** (310 mg, 0.37 mmol, 1.0 equiv) at 0 °C under Ar in CH_2Cl_2 (7.4 mL) were added Et_3N (0.1 mL, 0.74 mmol, 2.0 equiv) and DMAP (90.7 mg, 0.74 mmol, 2.0 equiv). After 5 min, $(BOC)_2O$ (486.2 mg, 2.2 mmol, 6.0 equiv) was added in one portion. The resulting solution was stirred for 8.5 h, poured into water, and extracted with EtOAc. The organic layer was washed with 10% $CuSO_4$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:2 EtOAc/hexanes) to yield 375 mg (97%) of **58** as an amorphous solid.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.12 (6H, s), 0.13 (6H, s), 0.879 (9H, s), 0.880 (9H, s), 1.01 (18H, s), 1.05 (3H, s), 1.07 (3H, s), 1.14 (9H, s), 1.18 (9H, s), 1.55 (6H, s), 1.47 (6H, s), 1.57 (18H, s), 1.88−2.16 (6H, m), 2.17−2.26 (2H, m), 2.28−2.36 (2H, m), 2.50 (2H, dd, J = 8.1, 14.5 Hz), 3.22 (2H, m), 3.32−3.45 (4H, m), 3.71−3.81 (2H, m), 3.84−3.96 (4H, m), 4.00 (4H, br s), 4.13−4.18 (2H, m), 5.02−5.07 (2H, m), 5.42 (1H, t, J = 7.3 Hz), 5.53 (1H, t, J = 7.5 Hz), 6.91 (2H, d, J = 8.3 Hz), 7.16 (1H, d, J = 8.0 Hz), 7.19 (1H, d, J = 8.2 Hz),7.22 (1H, s), 7.24 (1H, s), 7.30−7.40 (12H, m), 7.57−7.61 (8H, m). IR (NaCl, neat): 2932, 1752, 1730, 1660, 1371, 1251, 1153, 1109, 1088, 706 cm^{-1}. HRMS (EI): 1035.5481 ($C_{58}H_{81}N_3O_{10}Si_2$ requires 1035.5461).

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[2-[(1,1-Dimethylethoxy)carbonyl]-8a-[4-hydroxy-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-4,4-dimethyl-3-hydroxy-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate. To a stirred solution of **53** (511 mg, 0.61 mmol, 1.0 equiv) at 0 °C under Ar in CH_2Cl_2 (12.2 mL) were added DMAP (149.4 mg, 1.2 mmol, 2.0 equiv) and Et_3N (0.17 mL, 1.2 mmol, 2.0 equiv). After 5 min, $(BOC)_2O$ (801.0 mg, 3.67 mmol, 6.0 equiv) was added in one portion. The resulting solution was stirred for 2.7 h, and reaction was found to be complete by TLC analysis; during this period, the reaction temperature slowly reached 15 °C. The reaction flask was then charged with THF (12 mL) and the CH_2Cl_2 removed by evaporation (until the volume of the flask was approximately 12 mL). The solution was stirred at room temperature and n-Bu_4NF (1.96 mL, 1.96 mmol, 3.2 eq, 1.0 M/THF) added quickly. After 22 h, additional n-Bu_4NF (1.0 mL, 1.0 mmol, 1.6 equiv, 1.0 M/THF) was added to the reaction flask and stirred for 24 h. The reaction was complete by TLC and was poured into water and extracted with EtOAc. The organic layer was washed with 10% $CuSO_4$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with EtOAc) to yield 369 mg (89%) of the diol (obtained as a pale yellow, amorphous solid).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.21 (3H, s), 1.24 (3H, s), 1.29 ((9H, s), 1.35 (9H, s), 1.47 (6H, s), 1.52 (6H, s), 1.56 (18H, s), 1.63−2.21 (14H, m), 3.21−3.38 (8H, m), 3.54 (1H, br s, D_2O exch), 3.58 (1H, br s, D_2O exch), 3.81−3.87 (6H, m, 2H D_2O exch), 4.22 (4H, d, J = 8.0 Hz), 4.62 (1H, t, J = 8.4 Hz), 4.96−5.01 (2H, m), 5.07 (1H, t, J = 7.2 Hz), 6.90 (1H, d, J = 8.4 Hz), 6.91 (1H, d, J = 8.4 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.18 (1H, d, J = 8.4 Hz), 7.22 (1H, s), 7.23 (1H, s). IR (NaCl, neat): 3436, 2978, 1755, 1649, 1367, 1249, 1149, 732 cm^{-1}.

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[2-[(1,1-Dimethylethoxy)carbonyl]-8a-[4-chloro-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-4,4-dimethyl-3-hydroxy-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate. To a stirred solution of the diol obtained above (50.0 mg, 0.0725 mmol, 1.0 equiv) in DMF (0.73 mL) at 0 °C under Ar were added collidine (0.014 mL, 0.11 mmol, 1.5 equiv) and LiCl (5.27 mg, 0.12 mmol, 1.7 equiv). After 15 min, MsCl (8.4 μL, 0.11 mmol, 1.5 equiv) was added and the reaction mixture allowed to reach room temperature in the course of 16 h. At this time an additional amount (1.0 equiv) of each reagent was added in the same manner as above. After 8.5 h there was little change by TLC, so a large excess of MsCl (0.06 mL, 0.775 mmol, 10.7 equiv) was added at 0 °C and stirred for ~12 h until only the desired product was apparent by TLC. The solution was diluted with 1:1 hexanes/EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated, under reduced pressure. The residue was purified by radial chromatography, 1:1 EtOAc/hexanes, to yield 45.5 mg (91%) of the product allylic chloride (obtained as a foamy glass).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.18 (3H, s), 1.20 (3H, s), 1.24 (9H, s), 1.30 (9H, s), 1.51 (3H, s), 1.54 (3H, s), 1.58 (18H, s), 1.64 (3H, s), 1.66 (3H, s), 1.74−2.18 (10H, m), 2.27 (2H, dd, J = 8.1, 15.0 Hz), 3.02 (2H, br s, D_2O exch), 3.19 (2H, dd, J = 7.2, 14.8 Hz), 3.27−3.44 (4H, m), 3.56 (2H, br s), 3.81−3.89 (2H, m), 3.91 (2H, s), 3.94 (2H, s), 4.18−4.30 (4H, m), 4.99−5.06 (2H, m), 5.21 (1H, t, J = 8.3 Hz), 5.38−5.43 (1H, m), 6.93 (2H, d, J = 8.3 Hz), 7.17 (1H, d, J = 8.3 Hz), 7.20 (1H, d, J = 8.3 Hz), 7.21 (1H, s), 7.24 (1H, s). IR (NaCl, neat): 3384, 2920, 1750, 1736, 1657, 1367, 1250, 1149 cm^{-1}.

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[2-[(1,1-Dimethylethoxy)carbonyl]-8a-[4-chloro-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (55). To a stirred solution of the allylic chloride obtained above (96.2 mg, 0.37 mmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL) under Ar were added 2,6-lutidine (0.016 mL, 0.14 mmol, 0.38 equiv) and *tert*-butyldimethylsilyl triflate (0.03 mL, 0.14 mmol, 0.38 equiv). After 1 h an additional amount (0.5 equiv) of the two reagents was added. The mixture was stirred for 1 h, and another portion (0.5 equiv) of each reagent was added. The solution was stirred for 75 min and was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:2 EtOAc/hexanes) to yield 106.5 mg (99%) of **55** as a white crystalline solid, mp 70−73 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.10 (3H, s), 0.11 (6H, s), 0.12 (3H, s), 0.877 (18H, s), 1.04 (3H, s), 1.06 (3H, s), 1.22 (9H, s), 1.29 (9H, s), 1.44 (3H, s), 1.46 (3H, s), 1.58 (18H, s), 1.62 (3H, s), 1.65 (3H, s), 1.76−2.13 (10H, m), 2.22 (2H, dd, J = 8.4, 14.8 Hz), 3.19 (2H, dd, J = 7.1, 14.7 Hz), 3.26−3.42 (4H, m), 3.68−3.78 (2H, m), 3.81−3.87 (4H, m), 3.90 (2H, s), 3.94 (2H, s), 4.10−4.17 (2H, m), 5.00−5.05 (2H, m), 5.22 (1H, t, J = 7.6 Hz), 5.41 (1H, t, J = 7.6 Hz), 6.91 (2H, d, J = 8.3 Hz), 7.14 (1H, d, J = 8.3 Hz), 7.16 (1H, d, J = 8.3 Hz), 7.21 (1H, s), 7.24 (1H, s). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ −5.0, −4.1, −4.0, 14.3, 17.8, 18.3, 19.7, 19.8, 25.6 27.3, 27.4, 27.9, 28.5, 29.6, 30.1, 34.5, 34.7, 36.1, 45.2, 45.32, 51.3, 51.4, 60.5, 68.1, 68.2, 70.9, 70.9, 75.7, 80.2, 83.1, 84.2, 84.2, 113.6, 113.8, 114.1, 114.2, 120.0, 120.1, 122.6, 122.7, 126.9, 127.1, 127.8, 127.9, 129.0, 135.6, 135.8, 140.43, 146.3, 146.4, 148.3, 148.4, 150.3, 150.5, 164.4, 164.5, 168.6, 168.7. IR (NaCl, neat): 2936, 1754, 1729, 1663, 1496, 1456, 1370,

1248, 1152, 1086, 838 cm^{-1}. HRMS (EI): 815.3973 ($C_{42}H_{62}N_3O_9SiCl$ requires 815.3944).

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[8a-[4-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]octahydro-1,4-dioxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (59). To a flask fitted with a reflux condenser was added **58** (799 mg, 0.771 mmol, 1.0 equiv) followed by CH_3CN (15.4 mL) and dimethylamine (0.53 mL, 3.85 mmol, 5.0 equiv, 40% solution in water). The resulting solution was refluxed for 2 h and 20 min. The solvent was removed under reduced pressure and the residue purified by radial chromatography (eluted with 1:2 EtOAc/hexanes) to yield 657 mg (92%) of **59**. An analytical sample was obtained by PTLC, on silica gel (eluted with 1:2 EtOAc/hexanes) (foamy oil).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.14 (6H, s), 0.23 (6H, s), 0.88 (18H, s), 1.01 (18H, s), 1.10 (6H, s), 1.48 (6H, d), 1.59 (18H, s), 1.62 (6H, s), 1.98−2.05 (6H, m), 2.07−2.19 (2H, m), 2.37−2.47 (2H, m), 2.64−2.75 (2H, m), 2.94 (2H, dd, J = 11.6, 14.1 Hz), 3.41−3.47 (4H, m), 3.82 (2H, dd, J = 9.6, 12.2 Hz), 3.93−4.03 (4H, m), 4.07 (4H, br s), 4.10−4.15 (2H, m), 4.20 (2H, dd, J = 2.7, 12.4 Hz), 5.56−5.61 (2H, m), 5.78 (1H, d, J = 3.0 Hz, D_2O exch), 5.81 (1H, d, J = 2.8 Hz, D_2O exch), 6.877 (1H, d, J = 8.4 Hz), 6.884 (1H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.4 Hz), 7.20−7.40 (14H, m), 7.56 − 7.61 (8H, m). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ −5.0, −4.1, 13.7, 14.0, 17.8, 18.6, 18.8, 19.1, 19.6, 22.5, 25.7, 26.7, 28.0, 28.4, 28.4, 31.4, 31.6, 31.7, 34.9, 35.8, 44.81, 57.5, 67.5, 68.2, 71.0, 75.8, 76.6, 77.0, 77.4, 80.3, 83.3, 83.1, 113.3, 114.6, 116.6, 120.1, 126.3, 126.3, 127.5 127.6, 128.1, 128.2, 128.4, 128.4, 128.6, 133.1, 133.2, 135.4, 139.2, 140.5, 140.6, 146.4, 146.5, 148.4, 164.4, 169.6, 169.7. IR (NaCl, neat): 3246, 2960 2861, 1750, 1676, 1662, 1430, 1366, 1252, 1159, 1109, 1090 cm^{-1}. HRMS (EI): 935.48955 ($C_{53}H_{73}N_3O_8Si_2$ requires 935.4936). Microanal. Calcd for $C_{53}H_{73}N_3O_8Si_2$: C, 67.57; H, 7.96; N, 4.54. Found: C, 67.62; H, 7.94; N, 4.32.

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-[4-[[(1,1-dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (60). To a stirred solution of **53** (3.87 g, 4.63 mmol, 1.0 equiv) in CH_2Cl_2 (46 mL) under Ar at 0 °C was added Na_2CO_3 (9.8 g, 92.6 mmol, 20.0 equiv). After 10 min, Me_3OBF_4 (3.42 g, 23.15 mmol, 5.0 equiv) was added in one portion. The mixture was stirred for 4.0 h at room temperature, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (eluted with 1:2 hexanes/EtOAc; then 1:1 hexanes/EtOAc) to yield 3.20 g (81%) of **60**. An analytical sample was obtained by PTLC on silica gel (eluted with EtOAc) (isolated as a white solid, mp 74−76 °C).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.120 (12H, s), 0.875 (18H, s), 1.02 (18H, s), 1.06 (3H, s), 1.07 (3H, s), 1.45 (12H, s), 1.65−2.08 (14H, m), 3.07−3.15 (2H, m), 3.26 (2H, dd, J = 6.2, 12.6 Hz), 3.32−3.40 (2H, m), 3.61 (6H, s), 3.70−3.86 (2H, m), 3.91−3.95 (4H, m), 3.99 (2H, s), 4.15 (2H, dd, J = 3.6, 11.7 Hz), 4.36−4.40 (2H, m), 5.37−5.44 (2H, br m), 6.69 (2H, d, J = 8.4 Hz), 7.01 (2H, d, J = 1.7 Hz), 7.15 (2H, d, J = 8.4 Hz), 7.26−7.41 (12H, m), 7.58−7.62 (8H, m), 8.06 (2H, s, D_2O exch). IR (NaCl, neat): 3292, 2932, 1687, 1643, 1447, 1251, 1218, 1109, 837 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 849 (M^+, 8.9), 361 (26), 360 (95), 167 (100). Microanal. Calcd for $C_{49}H_{67}N_3O_6Si_2$: C, 69.02; H, 7.94; N, 4.94. Found: C, 69.02; H, 7.88; N, 4.79.

[3α,8aβ(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-[4-[[(1,1-dimethylethyl)diphenylsilyl]oxy]-3-methyl-2-butenyl]-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (61). To a stirred solution of **54** (8.47 g,10.13 mmol, 1.0 equiv) in CH_2Cl_2 (101 mL) at 0 °C under Ar was added Na_2CO_3 (21.26 g, 202.6 mmol, 20.0 equiv). After 15 min Me_3OBF_4 (7.49 g, 50.64 mmol, 5.0 equiv) was added in one portion. The mixture was stirred for 5 min, the ice bath was removed, and the reaction mixture was stirred for 4.5 h. The mixture was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (eluted with 1:2 EtOAc/hexanes) to yield 5.30 g (62%) of **61**. [The yield of **61** was 365 mg (71%) from 508 mg of **54**.] An analytical sample was obtained by PTLC on silica gel (eluted with 1:2 EtOAc/hexanes and obtained as a white crystalline solid, mp 54−58 °C).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.13 (3H, s), 0.14 (9H, s), 0.89 (18H, s), 1.03 (9H, s), 1.04 (9H, s), 1.087 (3H, s), 1.093 (3H, s), 1.28−1.43 (4H, m), 1.48 (6H, s), 1.50 (6H, s), 1.79−1.89 (4H, m), 2.24−2.38 (4H, m), 3.22−3.42 (6H, m), 3.60 (3H, s), 3.62 (3H, s), 3.68−3.76 (2H, m), 3.79−3.87 (2H, m), 3.94 (2H, d, J = 3.4 Hz), 3.97 (4H, br s), 4.15−4.20 (2H, m), 4.26−4.32 (2H, m), 5.41 (2H, t, J = 7.8 Hz), 6.701 (1H, d, J = 8.5 Hz), 6.703 (1H, d, J = 8.4 Hz), 6.96 (1H, d, J = 2.6 Hz), 6.97 (1H, d, J = 2.6 Hz), 7.28 (2H, d, J = 8.5 Hz), 7.32−7.44 (12H, m), 7.60−7.64 (8H, m), 7.97 (2H, br s, D_2O exch). IR (NaCl, neat): 3304, 2930, 1695, 1645, 1447, 1249, 1221, 836 cm^{-1}. HRMS (EI): 849.4550 ($C_{49}H_{67}N_3O_6Si_2$ requires 849.4568). Microanal. Calcd for $C_{49}H_{67}N_3O_6Si_2$: C, 69.22; H, 7.94; N, 4.94. Found: C, 59.06; H, 8.04; N, 4.89.

[3β,8aβ(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-hydroxy-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-3-hydroxy-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (62). To stirred solution of **60** (5.45 g, 6.41 mmol, 1.0 equiv) in CH_2Cl_2 (32 mL) under Ar at 0 °C were added Et_3N (0.89 mL, 6.41 mmol, 1.0 equiv) and DMAP (783.1 mg, 6.41 mmol, 1.0 equiv). After 10 min $(BOC)_2O$ (4.20 g, 19.2 mmol, 3.0 equiv) was added in one portion. The reaction mixture was stirred for 6 h and diluted with THF (45 mL). The remaining CH_2Cl_2 was removed by evaporation under reduced pressure (until the volume in the flask was 45 mL). The flask was charged with *n*-Bu_4NF (19.2 mL, 19.2 mmol, 3.0 equiv, 1.0 M/THF), and the mixture was stirred at room temperature for approximately 12 h. The solution was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (eluted with 1:2 EtOAc/hexanes; then 2:1 EtOAc/hexanes) to yield 3.45 g (90%) of **62**. [The yield of **62** was 243 mg (97%) from 355 mg of **60**.] An analytical sample was obtained by PTLC on silica gel (eluted with 2:1 EtOAc/hexanes) to afford a white solid, mp 72−85 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.18 (6H, s), 1.52 (3H, s), 1.53 (3H, s), 1.56 (3H, s), 1.57 (21H, s), 1.61−2.07 (10H, m), 2.14 (2H, dd, J = 8.6, 14.5 Hz), 2.85 (2H, br s, D_2O exch), 2.92−3.01 (2H, m), 3.18−3.35 (6H, m), 3.56 (2H, br s, D_2O exch), 3.62 (3H, s), 3.64 (3H, s), 3.88 (4H, br s), 3.91−4.00 (2H, m), 4.25 (4H, br s), 4.30−4.39 (2H, m), 4.98−5.01 (2H, m), 6.87 (1H, d, J = 8.3 Hz), 6.88 (1H, d, J = 8.3 Hz), 7.16 (1H, d, J = 8.3 Hz), 7.17 (1H, d, J = 8.3 Hz), 7.34 (1H, s), 7.35 (1H, s). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 13.4, 19.5, 19.7, 23.5, 23.6, 25.1, 25.3, 27.9, 30.3, 30.5, 34.4, 34.8, 35.1, 35.3, 43.4, 43.6, 52.6, 52.7, 62.0, 62.4, 65.3, 65.4, 67.7, 67.8, 70.6, 75.4, 82.6, 82.6, 114.5, 114.7, 116.8, 116.9, 118.2, 118.3 119.0, 119.1, 126.3, 128.0, 128.1, 129.9 130.0, 138.6, 138.7, 140.7, 146.2, 148.5, 161.32, 161.5, 168.5, 168.7 IR (NaCl, neat): 3390 (br), 2976, 1752, 1692, 1632, 1491, 1453, 1371, 1251, 1158, 733 cm^{-1}. Microanal. Calcd for $C_{32}H_{43}N_3O_8$: C, 64.30; H, 7.25; N, 7.03. Found: C, 64.12; H, 7.41; N, 6.88. HRMS (EI): m/e 597.3065 ($C_{32}H_{43}N_3O_8$ requires 597.3050).

[3α,8aβ(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-hydroxy-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-3-hydroxy-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (63). To a stirred solution of **61** (5.30 g, 5.65 mmol, 1.0 equiv) under Ar in CH_2Cl_2 (1.5 mL) at 0 °C were added Et_3N (0.79 mL, 5.65 mmol, 1.0 equiv) and DMAP (689.7 mg, 5.65 mmol, 1.0 equiv). After 5 min $(BOC)_2O$ (3.70 g, 16.94 mmol, 3.0 equiv) was added in one portion. The reaction mixture was stirred for 4.5 h and diluted with THF (40 mL). The remaining CH_2Cl_2 was removed under reduced pressure (until the reaction volume was 40 mL). The flask was charged with *n*-Bu_4NF (17.0 mL, 17.0 mmol, 3.0 equiv, 1.0 M/THF), and the mixture was stirred at room temperature for ~12 h. The solution was diluted with water and extracted with EtOAc.

The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography (eluted with EtOAc) to yield 3.16 g (85%) of **63** as a white, amorphous solid, mp 72−80 °C [The yield of **63** was 179 mg (98%) with 260 mg of **61**].

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.16 (3H, s), 1.18 (3H, s), 1.51 (3H, s), 1.52 (3H, s), 1.55 (6H, s), 1.57 (18H, s), 1.60−2.14 (10H, m, 2H D_2O exch), 2.22−2.37 (4H, m), 3.06−3.18 (3H, m, 1H D_2O exch), 3.26−3.36 (5H, m, 1H D_2O exch), 3.55 (3H, s), 3.56 (2H, br s), 3.60 (3H, s), 3.63−3.72 (2H, m), 3.89 (4H, m), 4.18−4.23 (2H, m), 4.25 (4H, br s), 5.21−5.27 (2H, m), 6.857 (1H, d, J = 8.3 Hz), 6.861 (1H, d, J = 8.3 Hz), 7.22 (2H, d, J = 8.3 Hz), 7.24 (2H, s). IR (NaCl, neat): 3401 (br), 2976, 1747, 1692, 1632, 1496, 1436, 1371, 1251, 1158, 733 cm^{-1}. HRMS (EI): 597.3050 ($C_{32}H_{43}N_3O_8$ requires 597.3050).

[3*β*,8a*β*(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-chloro-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-3-hydroxy-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (64). Dimethyl sulfide (0.67 mL, 9.13 mmol, 8.0 equiv) was added dropwise to a stirred solution of NCS (1.22 g, 9.13 mmol, 8.0 equiv) in CH_2Cl_2 (51 mL) at 0 °C under Ar. The resulting mixture was stirred for 10 min and then cooled to −23 °C. After 10 min, **62** (682.4 mg, 1.14 mmol, 1.0 equiv) was added to the flask in one portion and stirring continued for 6 h. At this time the reaction flask was placed in a freezer (−35 °C) for 16 h, followed by an additional 10 h of stirring at −23 °C. The mixture was then diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:2 hexanes/EtOAc) to yield 565.8 mg (81%) of **64** as a white amorphous solid. [The yield of **64** was 2.12 g (37% or 74% based on recovered **62**) with 5.60 g of **62**.] An analytical sample was obtained by PTLC on silica gel (eluted with 2:1 EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.17 (6H, s), 1.52 (6H, s), 1.57 (18H, s), 1.65 (6H, s), 1.73−2.20 (10H, m), 2.84 (2H, dd, J = 9.0, 14.4 Hz), 3.06 (1H, br s, D_2O exch), 3.10 (1H, br s, D_2O exch), 3.26−3.36 (4H, m), 3.55−3.58 (4H, m), 3.62 (3H, s), 3.63 (3H, s), 3.91 (4H, s), 3.95−4.05 (2H, m), 4.24−4.25 (4H, m), 4.30−4.36 (2H, m), 5.28 (2H, m), 6.88 (2H, d, J = 8.3 Hz), 7.14 (1H, d, J = 8.3 Hz), 7.15 (1H, d, J = 8.3 Hz), 7.376 (1H, s), 7.384 (1H, s). IR (NaCl, neat): 3403, 2979, 1750, 1716, 1642, 1348, 1154 cm^{-1}. HRMS (EI): 615.2709 ($C_{32}H_{42}N_3O_7Cl$ requires 615.2711). Microanal. Calcd for $C_{32}H_{42}N_3O_7Cl$: C, 62.38; H, 6.87; N, 6.82. Found: C, 62.53; H, 6.86; N, 6.67.

[3*α*,8a*β*(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-chloro-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3,4-dihydro-3-hydroxy-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]-indole-10-carboxylate (65). To a stirred solution of NCS (5.67 g, 42.4 mmol, 8.0 equiv) at 0 °C under Ar in CH_2Cl_2 (206 mL) was added dimethyl sulfide (3.12 mL, 42.4 mmol, 8.0 equiv) dropwise. After 0.5 h the mixture was cooled (−23 °C) and stirred for an additional 0.5 h. At this time the lactim ether−diol **63** (3.17 g, 5.30 mmol, 1.0 equiv) was added [approximately 3 g was added as a solid; the remaining amount was added as a solution in CH_2Cl_2 (30 mL) via cannula]. The white mixture was stirred for 12 h, diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluted with 2:1 hexanes/EtOAc; then 1:1 hexanes/EtOAc) to afford 2.80 g (86%) of **65** as a glass.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.17 (3H, s), 1.18 (3H, s), 1.52 (3H, s), 1.54 (3H, s), 1.57 (18H, s), 1.65 (6H, s), 1.71−1.92 (8H, m), 2.24−2.39 (4H, m), 3.03−3.19 (4H, m, 2H D_2O exch), 3.28−3.37 (4H, m), 3.56 (3H, s), 3.60 (3H, s), 3.59−3.75 (4H, m), 3.89 (4H, s), 4.21−4.29 (6H, m), 5.35 (2H, t, J = 7.5 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.87 (1H, d, J = 8.3 Hz), 7.23 (2H, d, J = 8.3 Hz), 7.22 (1H, s), 7.27 (1H, s). IR (NaCl, neat): 3412 (br), 2976, 1752, 1698, 1638, 1365, 1251, 1158 cm^{-1}. HRMS (EI): 615.2714 ($C_{32}H_{42}N_3O_7Cl$ requires 615.2711).

[3*β*,8a*β*(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-chloro-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (66). To a stirred solution of **64** (3.55 g, 5.76 mmol, 1.0 equiv) in CH_2Cl_2 (23 mL) at 0 °C under Ar was added 2,6-lutidine (0.74 mL, 6.34 mmol, 1.1 equiv) followed by *tert*-butyldimethylsilyl triflate (1.08 mL, 6.34 mmol, 1.1 equiv). After 3 h an additional amount (1.1 equiv) of each reagent was added to the reaction flask; after stirring for 2 h, an additional amount (1.1 equiv) of each reagent was added. The mixture was stirred for 1 h, diluted with EtOAc, washed four times with water and once with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (eluted with 1:1 hexanes/EtOAc) to yield 3.23 g (77%) of **66** as an amorphous, white solid. An analytical sample was obtained by PTLC on silica gel (eluted with 1:1 hexanes/EtOAc).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.12 (6H, s), 0.13 (6H, s), 0.88 (18H, s), 1.06 (6H, s), 1.47 (6H, s), 1.59 (18H, s), 1.65 (6H, s), 1.78−1.98 (8H, s), 2.02−2.12 (2H, m), 2.86 (2H, dd, J = 9.0, 14.6 Hz), 3.31−3.34 (2H, m), 3.33 (2H, dd, J = 4.0, 13.6 Hz), 3.62 (3H, s), 3.64 (3H, s), 3.71−3.79 (2H, m), 3.73 (1H, dd, J = 4.2, 9.8 Hz), 3.77 (1H, dd, J = 4.4, 9.7 Hz), 3.92 (4H, s), 3.94−4.01 (4H, m), 4.15 (2H, dd, J = 3.8, 12.4 Hz), 4.32−4.37 (2H, m), 5.28−5.30 (2H, m), 6.87 (2H, d, J = 8.3 Hz), 7.12 (1H, d, J = 8.3 Hz), 7.13 (1H, d, J = 8.3 Hz), 7.38 (2H, s). IR (NaCl, neat): 2930, 1750, 1691, 1652, 1494, 1424, 1366, 1248, 1159, 1088 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 729 (M^+, 4.2), 731 (M + 2, 2.1), 629 (9.4), 361 (24.1), 360 (100), 167 (94.8), 57.2 (63). Microanal. Calcd for $C_{38}H_{56}N_3O_7SiCl$: C, 62.49; H, 7.73; N, 5.75. Found: C, 62.57; H, 7.71; N, 5.55.

[3*α*,8a*β*(*E*)]-1,1-Dimethylethyl 8-[[3,4,6,7,8,8a-Hexahydro-8a-(4-chloro-3-methyl-2-butenyl)-1-methoxy-4-oxopyrrolo[1,2-*a*]pyrazin-3-yl]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (67). To a stirred solution of **65** (2.73 g, 4.43 mmol, 1.0 equiv) under Ar at 0 °C in CH_2Cl_2 (18 mL) was added 2,6-lutidine (0.57 mL, 4.87 mmol, 1.1 equiv) followed by *tert*-butyldimethylsilyl triflate (0.87 mL, 4.87 mmol, 1.1 equiv). After 1 h, 1.1 equiv of each reagent was added and stirred for 3 h. The solution was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluted with 1:2 EtOAc/hexanes) to yield 2.76 g (85%) of **67** as a white amorphous solid. An analytical sample was obtained by PTLC on silica gel (eluted with 1:2 EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.12 (6H, s), 0.13 (6H, s), 0.87 (18H, s), 1.05 (3H, s), 1.06 (3H, s), 1.47 (6H, s), 1.50−1.53 (2H, m), 1.58 (18H, s), 1.65 (6H, s), 1.72−1.91 (6H, m), 2.21−2.37 (4H, m), 3.06−3.19 (2H, m), 3.28−3.36 (4H, m), 3.56 (3H, s), 3.60 (3H, s), 3.63−3.87 (4H, m): 3.89 (4H, s), 3.93 (2H, dd, J = 3.9, 9.8 Hz), 4.13−4.18 (2H, m), 4.22−4.35 (2H, m), 5.30−5.40 (2H, m), 6.85 (1H, d, J = 8.3 Hz), 6.86 (1H, d, J = 8.3 Hz), 7.19−7.26 (4H, m). IR (NaCl, neat): 2949, 1751, 1693, 1652, 1493, 1424, 1369, 1250, 1156, 1086 cm^{-1}. Microanal. Calcd for $C_{38}H_{56}N_3O_7SiCl$: C, 62.49; H, 7.73; N, 5.75. Found: C, 62.29; H, 7.61; N, 5.76. HRMS (EI): 729.3555 ($C_{38}H_{56}N_3O_7SiCl$ requires 729.3576).

1,1-Dimethylethyl 8-[[7,8-Dihydro-1-methoxy-10-(1-methylethenyl)-4-oxo-6*H*-3,8a-ethanopyrrolo[1,2-*a*]pyrazin-3(4*H*)-yl]]methyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3,4-dihydro-4,4-dimethyl-2*H*,10*H*-[1,4]dioxepino[2,3-*g*]indole-10-carboxylate (68). To a stirred solution of **66** (1.43 g, 1.96 mmol, 1.0 equiv) in benzene (300 mL) was added NaH (939 mg, 39.16 mmol, 20.0 equiv, freshly washed in pentane). This mixture was gently stirred at reflux temperature for 8.25 h, diluted with EtOAc, and washed with water and dilute HCl. The organic layer was isolated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:3 EtOAc/hexanes) to yield 1.26 g of **68** (93%). [The yield of **68** was 2.52 g (86%) from 3.10 g of **66**.]

To a stirred solution of **67** (1.60 g, 2.19 mmol, 1.0 equiv) in benzene (313 mL) was added NaH (1.05 g, 43.8 mmol, 20.0 equiv, freshly washed in pentane). This mixture was gently stirred at reflux temperature for 5.5 h and stirred at room temperature overnight. At this time, a small sample was removed, washed with water, and extracted with EtOAc. A crude proton NMR (in $CDCl_3$) indicated that the reaction was complete. The remaining mixture was diluted with EtOAc and washed with water. The organic layer was washed with

brine, dried over Na_2SO_4, and concentrated under reduced pressure. The two samples were combined and purified by radial chromatography (eluted with 1:3 EtOAc/hexanes) to yield 1.29 g of **68** (85%). An analytical sample was obtained by PTLC on silica gel (eluted with 1:3 EtOAc/hexanes); the product was obtained as a white solid, mp 105−108 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.12 (6H, s), 0.13 (6H, s), 0.872 (9H, s), 0.875 (9H, s), 1.06 (3H, s), 1.07 (3H, s), 1.46 (6H, s), 1.58 (18H, s), 1.61 (3H, s), 1.64 (3H, s), 1.72−2.03 (8H, m), 2.25−2.42 (2H, m), 2.47 (2H, dd, J = 5.1, 9.7 Hz), 2.54 (2H, dd, J = 5.8, 9.7 Hz), 3.05 (1H, $^1/_2$ ABq, J = 15.0 Hz), 3.07 (1H, $^1/_2$ ABq, J = 15.0 Hz), 3.31−3.53 (6H, m), 3.57 (3H, s), 3.64 (3H, s), 3.73−3.89 (2H, m), 3.94 (2H, dd, J = 3.7, 9.7 Hz), 4.17 (2H, dd, J = 3.1, 11.6 Hz), 4.62 (1H, s), 4.75 (1H, s), 4.78 (1H, s), 4.85 (1H, s), 6.82 (2H, d, J = 8.4 Hz), 7.31 (1H, d, J = 8.4 Hz), 7.38 (1H, d, J = 8.4 Hz), 7.44 (1H, s), 7.52 (1H, s). IR (NaCl, neat): 2935, 1752, 1684, 1637, 1496, 1418, 1365, 1350, 1250, 1220, 1156, 1083 cm^{-1}. HRMS (EI): *m/e* 693.3834 ($C_{38}H_{55}N_3O_7Si$ requires 693.3809). Microanal. Calcd for $C_{38}H_{55}N_3O_7Si$: C, 65.77; H, 7.99; N, 6.05. Found: C, 65.85; H, 7.99; N, 5.91.

1,1-Dimethylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,4,8,-12,13,14,14a,15-octahydro-4,4,15,15-tetramethyl-9,17-dioxo-11*H*,16*H*-8a,13a-(iminomethano)-2*H*,9*H*-[1,4]dioxepino[2,3-*a*]indolizino[6,7-*h*] carbazole-16-carboxylate (69). To a flask charged with $PdCl_2$ (827.9 mg, 4.67 mmol, 3.0 equiv) and $AgBF_4$ (605.3 mg, 3.11 mmol, 2.0 equiv) was added dry CH_3CN (50 mL). The mixture was stirred for 6.5 h, when a solution of **68** (1.08 g, 1.56 mmol, 1.0 equiv) in CH_3CN (5.0 mL) was syringed into the flask. The reaction mixture was stirred for 48 h, and EtOH (55 mL) was added, followed by small portions of $NaBH_4$ (590 mg, 15.6 mmol, 10.0 equiv) at 0 °C. The addition was complete in 0.5 h, and the mixture was stirred for an additional 0.5 h. The black mixture was filtered to remove palladium and the solvent evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with dilute aqueous HCl (0.01 M) and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 25:25:1 CH_2Cl_2/Et_2O/MeOH) to afford 676.3 mg (63%) of **69** as a white amorphous solid. An analytical sample was obtained by PTLC on silica gel (eluted with 25:25:1 CH_2Cl_2/Et_2O/MeOH).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.081 (6H, s), 0.11 (6H, s), 0.87 (9H, s), 0.88 (9H, s), 1.08 (3H, s), 1.17 (3H, s), 1.26 (3H, s), 1.27 (3H, s), 1.34 (3H, s), 1.35 (3H, s), 1.44 (3H, s), 1.46 (3H, s), 1.56 (9H, s), 1.58 (9H, s), 1.81−1.90 (2H, m), 1.96−2.06 (6H, m), 2.20 (2H, dd, J = 10.3, 13.5 Hz), 2.52−2.60 (4H, m), 2.78 (2H, dt, J = 6.5, 12.9 Hz), 3.36−3.49 (2H, m), 3.51−3.57 (2H, m), 3.63−3.84 (4H, m), 3.88−3.92 (2H, m), 4.04−4.16 (2H, m), 6.24 (1H, s, D_2O exch), 6.26 (1H, s, D_2O exch), 6.78 (1H, d, J = 8.3 Hz), 6.80 (1H, d, J = 8.5 Hz), 6.98 (1H, d, J = 8.2 Hz), 6.99 (1H, d, J = 8.4 Hz). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ −5.2, −5.1, −5.0, −4.5, −4.3, 17.6. 18.7, 19.3, 19.7, 19.9, 24.3, 25.5, 25.6, 26.9, 26.2, 27.2, 27.8, 27.9, 28.3, 28.5, 29.1, 31.1, 36.2, 43.8, 50.5, 50.6, 53.3, 54.8, 55.7, 59.4, 60.2, 60.2, 66.3, 67.6, 71.1, 72.7, 75.9, 78.0, 80.5, 84.1, 84.3, 108.3, 112.4, 112.5, 113.6, 117.9, 118.5, 124.6, 124.9, 128.7, 128.9, 129.4, 137.7, 138.3, 139.4, 139.6, 143.0, 143.2, 152.9, 153.0, 168.3, 174.1. IR (neat): 3214, 2928, 2856, 1745, 1556, 1496, 1443, 1368, 1252, 1233, 1154, 1141, 1091, 1052, 994, 859, 838, 777, 733. Microanal. Calcd for $C_{37}H_{53}N_3O_7Si$: C, 65.36; H, 7.86; N, 6.18. Found: C, 65.18; H, 7.77; N, 6.18. MS (EI): *m/e* (relative intensity) 679 (M^+, 0.3), 580 (20.4), 579 (51), 73 (100). HRMS (EI): *m/e* 679.3661 ($C_{37}H_{53}N_3O_7Si$ requires 679.3653).

1,1-Dimethylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,4,8,-12,13,14,14a,15-octahydro-4,4,15,15-tetramethyl-17-methoxy-9-oxo-11*H*,16*H*-8a,13a-(iminomethano)-2*H*,9*H*-[1,4]dioxepino[2,3-*a*]indolizino[6,7-*h*]carbazole-16-carboxylate (71). To a stirred solution of **69** (26.1 mg, 0.38 mmol, 1.0 equiv) in CH_2Cl_2 (1 mL) under Ar at 0 °C was added Na_2CO_3 (81.0 mg, 0.76 mmol, 20.0 equiv). After 10 min Me_3OBF_4 (28.3 mg, 0.191 mmol, 5.0 equiv) was added in one portion. The mixture was stirred for 4 h at room temperature, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by PTLC on silica gel (eluted with 1:2 hexanes/EtOAc) to afford 19.6 mg (74%) of **71** as a white amorphous solid.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ TMS 0.10−0.15 (12H, m), 0.89 (9H, s), 0.90 (9H, s), 1.09 (6H, s), 1.26 (3H, s), 1.29 (3H, s), 1.33 (3H, s), 1.36 (3H, s), 1.46 (3H, s), 1.48 (3H, s), 1.58 (9H, s), 1.60 (9H, s), 1.76−2.51 (10H, m), 2.23−2.31 (2H, m), 2.60−2.70 (2H, m), 3.027 (1H, $^1/_2$ ABq, J = 16.4 Hz), 3.032 (1H, $^1/_2$ ABq, J = 16.4 Hz), 3.31−3.41 (2H, m), 3.46−3.54 (2H, m), 3.68 (2H, dd, J = 9.1, 12.1 Hz), 3.77 (6H, s), 3.87−3.94 (2H, m), 3.90 (2H, $^1/_2$ ABq, J = 16.3 Hz), 4.08 (2H, dd, J = 3.5, 11.9 Hz), 6.79 (1H, d, J = 8.3 Hz), 6.80 (1H, d, J = 8.3 Hz), 7.063 (1H, d, J = 8.3 Hz), 7.061 (1H, d, J = 8.3 Hz). IR (NaCl, neat): 2952, 2886, 1745, 1683, 1640, 1496, 1412, 1355, 1252, 1232, 1156, 1140, 1111, 1090, 1052, 992, 838, 770 cm^{-1}. HRMS (EI): *m/e* 693.3810 ($C_{38}H_{55}N_3O_7Si$ requires 693.3810).

3-(Hydroxy)-3,4,8,12,13,14,14a,15-octahydro-4,4,15,15-tetramethyl-9,17-dioxo-11*H*,16*H*-8a,13a-(iminomethano)-2*H*,9*H*-[1,4]dioxepino-[2,3-*a*]indolizino[6,7-*h*]carbazole (76). To a stirred solution of **69** (150 mg, 0.22 mmol, 1.0 equiv) in CH_2Cl_2 (4.4 mL) under N_2 at 0 °C was added TFA (1.4 mL, 17.8 mmol, 80 equiv) dropwise. The reaction mixture was allowed to reach room temperature overnight. The solution was concentrated and the residue taken up in EtOAc. The resulting solution was washed with 10% Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was purified by radial chromatography (eluted with EtOAc) to yield 102 mg (95%) of **76**. An analytical sample was obtained by PTLC on silica gel (eluted with 1:1 EtOAc/hexanes) as a white amorphous solid.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.06 (3H, s), 1.08 (3H, s), 1.18 (3H, s), 1.20 (3H, s), 1.23 (3H, s), 1.29 (3H, s), 1.49 (3H, s), 1.55 (3H, s), 1.79−2.04 (8H, m), 2.17 (2H, td, J = 5.1, 11.9 Hz), 2.43 (1H, m), 2.43 (1H, $^1/_2$ ABq, J = 15.5 Hz), 2.51 (1H, dd, J = 4.8, 10.2 Hz), 2.59 (1H, $^1/_2$ ABq, J = 15.5 Hz), 2.78 (2H, dt, J = 6.5, 12.9 Hz), 3.21 (1H, br s, D_2O exch), 3.33−3.41 (3H, m), 3.41−3.56 (3H, m), 3.60 (1H, br s, D_2O exch), 3.70 (1H, $^1/_2$ ABq, J = 15.4 Hz), 3.78 (1H, $^1/_2$ ABq, J = 15.4 Hz), 4.12 (2H, dd, J = 8.4, 12.0 Hz), 4.25 (2H, td, J = 4.0, 12.2 Hz), 6.65 (2H, s, D_2O exch), 6.72 (1H, d, J = 8.3 Hz), 6.73 (1H, d, J = 8.3 Hz), 7.02 (1H, d, J = 7.9 Hz), 7.05 (1H, d, J = 8.1 Hz), 7.98 (1H, s, D_2O exch), 8.10 (1H, s, D_2O exch). IR (NaCl, neat): 3308, 1684, 1679, 1402, 1367, 1232, 1044, 733 cm^{-1}. HRMS (EI): *m/e* 465.2248 ($C_{26}H_{31}N_3O_5$ requires 465.2264).

14-Deoxy-29-demethyl-24,25-dihydro-25-hydroxy-12-oxo-17-nor-paraherquamide (79). To a stirred mixture of **76** (16.5 mg, 0.035 mmol, 1.0 equiv) in CH_2Cl_2 (0.7 mL) at 0 °C under N_2 was added Et_3N (4.6 μL, 0.04 mmol, 1.1 equiv) followed by *t*-BuOCl (5.4 μL, 0.04 mmol, 1.1 equiv). After 0.5 h, the resulting clear, yellow solution was concentrated to dryness (the flask being kept cold). The residue was immediately subjected to a solution of MeOH/H_2O/AcOH (40:20:1) and stirred under N_2 at room temperature for 0.5 h. The solution was diluted with saturated $NaHCO_3$, and the organic layer was washed three times with saturated $NaHCO_3$, washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by PTLC on silica gel (eluted with 20:1 CH_2Cl_2/MeOH) to yield 5.0 mg (29%) of **79** as an amorphous solid.

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.46 (3H, s), 0.48 (3H, s), 0.93 (6H, s), 1.22 (3H, s), 1.23 (3H, s), 1.45 (3H, s), 1.51 (3H, s), 1.65−2.09 (14H, m), 2.71−2.79 (2H, m), 2.87 (2H, td, J = 3.2, 9.3 Hz), 3.40−4.99 (2H, m), 3.56−3.66 (6H, m, 2H D_2O exch), 4.08−4.26 (4H, m), 6.56 (1H, d, J = 8.1 Hz), 6.61 (1H, d, J = 8.1 Hz), 6.80 (1H, d, J = 7.7 Hz), 6.82 (1H, d, J = 7.8 Hz), 6.96 (1H, s, D_2O exch), 7.09 (1H, s, D_2O exch), 8.03 (1H, s, D_2O exch), 8.11 (1H, s, D_2O exch). IR (NaCl, neat): 3411, 3237, 1698, 1632, 1496, 1404, 1333, 1213, 728 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 481 (M^+, 23.9), 412 (15.2), 249 (12.7), 220 (100), 149 (60.6). HRMS (EI): *m/e* 481.2194 ($C_{26}H_{31}N_3O_6$ requires 481.2213).

1,1-Dimethylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,4,8,-12,13,14,14a,15-octahydro-4,4,15,15-tetramethyl-17-oxo-11*H*,16*H*-8a,13a-(iminomethano)-2*H*,9*H*-[1,4]dioxepino[2,3-*a*]indolizino[6,7-*h*]carbazole-16-carboxylate (70). To a stirred solution of **69** (164 mg, 0.24 mmol, 1.0 equiv) in THF (4.9 mL) at −78 °C under Ar was added Et_3Al (0.14 mL, 0.26 mmol, 1.1 equiv, 1.9 M in toluene)

dropwise. After 10 min the solution was warmed to 0 °C and AlH_3·DMEA (6.0 mL, 1.20 mmol, 5.0 equiv, 0.2 M in toluene) was added dropwise. The ice bath was removed and the solution stirred for 1 h and 20 min at room temperature. At this time MeOH (4.7 mL) and AcOH (0.31 mL) were syringed into the flask, followed by $NaCNBH_3$ (179 mg, 2.85 mmol, 11.9 equiv). This mixture was stirred for 10 min, and the solvent was removed under reduced pressure and replaced with ethyl acetate. The resulting solution was washed with $NaHCO_3$ (saturated) and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:1 hexanes/EtOAc) to yield 102 mg (65%) of **70** as a white amorphous solid. An analytical sample was obtained by PTLC on silica gel (eluted with 1:1 EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.085 (6H, s), 0.11 (6H, s), 0.87 (9H, s), 0.88 (9H, s), 1.12 (3H, s), 1.15 (3H, s), 1.23 (3H, s), 1.24 (3H, s), 1.36 (3H, s), 1.37 (3H, s), 1.45 (6H, s), 1.59 (9H, s), 1.61 (9H, s), 1.88−1.92 (6H, s), 1.97−2.10 (2H, m), 2.17−2.26 (2H, m), 2.54−2.63 (2H, m), 2.70 (2H, $^1/_2$ ABq, J = 15.5 Hz), 2.829 (1H, $^1/_2$ ABq, J =15.4 Hz), 2.835 (1H, $^1/_2$ ABq, J = 15.6 Hz), 3.06−3.09 (2H, m), 3.45−3.49 (4H, m), 3.67−3.85 (4H, m), 3.90 (2H, dd, J = 3.4, 8.7 Hz), 4.09−4.18 (4H, m), 6.03 (2H, s, D_2O exch), 6.78 (1H, d, J = 8.3 Hz), 6.79 (1H, d, J = 8.3 Hz), 6.89 (2H, d, J = 8.3 Hz). IR (NaCl, neat): 3227, 2928, 1746, 1683, 1597, 1371, 1254, 1233, 1154, 1138, 1090, 836 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 665 (M^+, 0.3), 565 (30.6), 521 (40.1), 164 (100). Microanal. Calcd for $C_{37}H_{55}N_3O_6Si$: C, 66.73; H, 8.32; N, 6.31. Found: C, 66.50; H, 8.18; N, 6.33. HRMS (EI): *m/e* 665.38365 ($C_{37}H_{55}N_3O_6Si$ requires 665.3860).

1,1-Dimethylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,4,8,-12,13,14,14a,15-octahydro-4,4,15,15,18-pentamethyl-17-oxo-11*H*,-16*H*-8a,13*a*-(iminomethano)-2*H*,9*H*-[1,4]dioxepino[2,3-*a*]indolizino-[6,7-*h*]carbazole-16-carboxylate (72). To a stirred solution of **70** (147.5 mg, 0.22 mmol, 1.0 equiv) in DMF (2.2 mL) under Ar at 0 °C was added NaH (13.3 mg, 0.55 mmol, 2.5 equiv). After 5 min, MeI (27.6 μL, 0.44 mmol, 2.0 equiv) was syringed in dropwise. The mixture was stirred for 4 h, when a small amount of water and mercaptoethanol (21.6 μL) were added. After a few minutes, the mixture was diluted with water and extracted with 1:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with 1:2 hexanes/EtOAc) to yield 146.9 mg (98%) of **72** as a white amorphous solid. An analytical sample was obtained by PTLC on silica gel (eluted with 1:1 EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 0.089 (6H, s), 0.11 (6H, s), 0.87 (9H, s), 0.88 (9H, s), 1.13 (3H, s), 1.15 (3H, s), 1.25 (6H, s), 1.36 (3H, s), 1.37 (3H, s), 1.46 (6H, s), 1.59 (9H, s), 1.61 (9H, s), 1.86−2.06 (10H, m), 2.09−2.20 (6H, m), 2.61−2.70 (2H, m), 2.747 (1H, $^1/_2$ ABq, J = 15.4 Hz), 2.754 (1H, $^1/_2$ ABq, J = 15.4 Hz), 2.30−3.05 (2H, m), 3.05 (6H, s), 3.14 (2H, $^1/_2$ ABq, J = 15.4 Hz), 3.39 (2H, d, J = 10.5 Hz), 3.74−3.85 (2H, m), 3.89−3.93 (2H, m), 4.07−4.18 (2H, m), 6.797 (1H, d, J = 8.3 Hz), 6.804 (1H, d, J = 8.3 Hz), 6.93 (2H, d, J = 8.3 Hz). IR (NaCl, neat): 2921, 1747, 1665, 1496, 1371, 1251, 1235, 1158, 1142, 1108, 1093, 837, 755 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 679 (M^+, 2.1), 579 (4.2), 520 (4.2), 178 (100). Microanal. Calcd for $C_{38}H_{57}N_3O_6Si$: C, 67.12; H, 8.45; N, 6.18. Found: C, 67.33; H, 8.27; N, 6.44. HRMS (EI): *m/e* 679.4008 ($C_{38}H_{57}N_3O_6Si$ requires 679.4017).

3-Hydroxy-3,4,8,12,13,14,14a,15-octahydro-4,4,15,15,18-pentamethyl-17-oxo-11*H*,16*H*-8a,13a-(iminomethano)-2*H*,9*H*-[1,4]dioxepino[2,3-*a*]indolizino[6,7-*h*]carbazole (73). To a stirred solution of **72** (294.7 mg, 0.43 mmol, 1.0 equiv) in CH_2Cl_2 (8.7 mL) at 0 °C under Ar was added TFA (2.77 mL, 34.7 mmol, 80.0 equiv) dropwise. The solution was stirred for 15 h, the temperature being maintained at 15 °C. At this time the solution was concentrated under reduced pressure, diluted with EtOAc, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by radial chromatography (eluted with EtOAc) to yield 194.8 mg (96%) of **73** as a white amorphous solid. An analytical sample was obtained by PTLC on silica gel (eluted with 1:1 EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 1.21 (3H, s), 1.23 (3H, s), 1.29 (3H, s), 1.32 (3H, s), 1.42 (3H, s), 1.45 (3H, s), 1.54 (6H, s), 1.88−2.00 (10H, m), 2.07−2.22 (6H, m), 2.63−2.72 (2H, m), 2.79 (1H, $^1/_2$ ABq, J = 15.1 Hz), 2.80 (1H, $^1/_2$ ABq, J = 15.1 Hz), 3.01−3.07 (4H, m, 2H D_2O exch), 3.07 (6H, s), 3.17 (1H, $^1/_2$ ABq, J = 15.1 Hz), 3.19 (1H, $^1/_2$ ABq, J = 15.4 Hz), 3.37−3.43 (2H, m), 3.62 (2H, br s), 4.20 (2H, dd, J = 4.4, 12.3 Hz), 4.29 (1H, dd, J = 4.0, 12.3 Hz), 4.31 (1H, dd, J = 4.0, 12.3 Hz), 6.750 (1H, d, J = 8.4 Hz), 6.753 (1H, d, J = 8.3 Hz), 7.01 (2H, d, J = 8.4 Hz), 8.01 (2H, s, D_2O exch). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ 14.0, 20.8, 22.6, 23.9, 24.4, 24.5, 24.7, 25.1, 27.7, 27.9, 30.2, 30.3, 31.3, 34.4, 45.9, 54.3, 57.4, 60.0, 60.2, 64.0, 71.0, 75.5, 76.6, 77.0, 77.4, 79.5, 104.6, 112.2, 116.17, 116.22, 125.0, 129.2, 137.2, 140.4, 141.6, 171.0, 174.3. IR (NaCl, neat): 3324, 2954, 1654, 1507, 1474, 1365, 1235, 1071, 1049, 908, 733 cm^{-1}. Microanal. Calcd for $C_{27}H_{35}N_3O_4Si$: C, 69.65; H, 7.58; N, 9.02. Found: C, 69.54; H, 7.66; N, 8.89. Mass spectrum (EI): *m/e* (relative intensity) 465 (M^+, 9.7), 406 (14.5), 287 (11.8), 178 (100). HRMS (EI): *m/e* 465.2625 ($C_{27}H_{35}N_3O_4Si$ requires 465.2628).

14-Deoxy-24,25-dihydro-25-hydroxy-17-norparaherquamide (80). To a stirred solution of **73** (99 mg, 0.21 mmol, 1.0 equiv) in pyridine (4 mL) at −15 °C under Ar was added *t*-BuOCl (37 μL, 0.32 mmol, 1.5 equiv). After 2 h the solvent was removed under reduced pressure to give 106 mg (quantitative) of the crude chloroindolenines (**74**/**75** as a mixture of epimers). The majority of the crude chloroindolenines, **74**/**75** (71 mg, 0.14 mmol, 1.0 equiv), was dissolved in THF (10 mL) and water (1 mL), and *p*-toluenesulfonic acid monohydrate (135 mg, 0.41 mmol, 15 equiv) was added. The resulting yellow solution was stirred at reflux temperature for 20 min and diluted with EtOAc and aqueous K_2CO_3. The organic layer was isolated, washed with brine, dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was purified by PTLC on silica gel (eluted with 20:1 CH_2Cl_2/MeOH) to yield (from the chloroindolenines) 52 mg (76%) of **80** and 2.7 mg (4%) of **81**.

^{1}H NMR (300 MHz) ($CDCl_3$) (**80** mixture of two diastereomers): δ TMS 0.80 (3H, s), 0.83 (3H, s), 1.08 (3H, s), 1.10 (3H, s), 1.22 (3H, s), 1.26 (3H, s), 1.50 (3H, s): 1.52 (3H, s), 1.40−1.60 (8H, m), 1.77−1.93 (8H, m), 2.05−2.21 (2H, m), 2.55−2.71 (4H, m), 3.02−3.10 (4H, m), 3.06 (6H, s), 3.63 (4H, br s, 2H D_2O exch), 4.05−4.24 (4H, m), 6.60 (1H, d, J = 8.1 Hz), 6.62 (1H, d, J = 8.2 Hz), 6.78 (1H, d, J = 8.1 Hz), 6.79 (1H, d, J = 8.2 Hz), 7.42 (1H, s, D_2O exch), 7.45 (1H, s, D_2O exch). IR (NaCl, neat): 3333, 2974, 2933, 1703, 1651, 1646, 1631, 1456, 1395, 1323, 1200, 1046, 903, 728 cm^{-1}. Mass spectrum (EI): *m/e* (relative intensity) 481 (M^+, 0.7), 422 (20.7), 421 (15), 135 (48), 133 (100). HRMS (CI): *m/e* 481 ($C_{27}H_{35}N_3O_5$ requires 481.2578), [M + H] 482.2645 ($C_{27}H_{36}N_3O_5$ requires 482.2655).

^{1}H NMR (500 MHz) ($CDCl_3$) (**81** mixture of two diastereomers): δ TMS 0.53 (3H, s), 0.56 (3H, s), 0.84 (3H, s), 0.86 (3H, s), 1.22 (3H, s), 1.25 (3H, s), 1.50 (3H, s), 1.52 (3H, s), 1.41−1.73 (8H, m), 1.83−1.90 (8H, m), 2.09−2.13 (2H, m), 2.28−2.41 (6H, m), 2.51−2.58 (2H, m), 3.00 (3H, s), 3.01 (3H, s), 3.63 (2H, br s), 3.78 (1H, D_2O exch), 3.81 (1H, s D_2O exch), 4.05−4.24 (4H, m), 6.60 (2H, d, J = 8.0 Hz), 6.62 (2H, d, J = 7.4 Hz), 7.42 (2H, s, D_2O exch). IR (NaCl, neat): 3271, 2924, 2854, 1714, 1644, 1496, 1464, 1393, 1375, 1211, 1142, 1066 cm^{-1}.

(+)-Paraherquamide B (12). To a stirred solution of **80** (22.5 mg, 0.047 mmol, 1.0 equiv) in DMPU (500 μL) under Ar at room temperature was added MTPI (90 mg, 0.20 mmol, 4.0 equiv). After 16 h KOH (10 mL, 1 M) was added, and the mixture was stirred for an additional 10 min. The pH was adjusted to 2 (addition of HCl) and the mixture extracted with EtOAc. The mixture was diluted with 1:1 hexanes/EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by PTLC on silica gel (eluted with 20:1 CH_2Cl_2/MeOH) to afford 17.1 mg (79%) of (+)-paraherquamide B (**12**) as a white, amorphous solid. This material proved to be identical to an authentic sample of natural (−)-paraherquamide B by ^{1}H NMR, ^{13}C NMR, TLC mobility, IR, mass spectrum, and UV (see text for CD spectrum, Figure 7).

^{1}H NMR (300 MHz) ($CDCl_3$): δ TMS 0.82 (3H, s), 1.09 (3H, s), 1.40 (3H, s), 1.41 (3H, s), 1.64 (1H, dd, J = 9.7, 12.4 Hz), 1.73−1.92 (4H, m), 1.82 (1H, $^1/_2$ ABq, J = 15.5 Hz), 2.16 (1H, dd, J = 8.6, 17.8 Hz), 2.54−2.59 (1H, m), 2.61 (1H, $^1/_2$ ABq, J = 11.1 Hz), 2.66 (1H, $^1/_2$ ABq, J = 15.5 Hz), 3.03−3.10 (2H, m), 3.05 (3H, s), 3.60 (1H, $^1/_2$

ABq, J = 11.1 Hz), 4.87 (1H, d, J = 7.7 Hz), 6.30 (1H, d, J = 7.7 Hz), 6.64 (1H, d, J = 8.2 Hz), 6.78 (1H, d, J = 8.2 Hz), 8.5 (1H, br s, D_2O exch). ^{13}C NMR (75.5 MHz) ($CDCl_3$): δ 20.7 (q), 23.8 (q), 26.2 (q), 28.2 (q), 28.8 (t), 29.8 (t), 29.9 (q), 37.2 (t), 46.1 (s), 52.8 (d), 53.8 (t), 59.5 (t), 63.0 (s), 65.2 (s), 67.4 (s), 79.7 (s), 115.0 (d), 117.2 (d), 120.3 (d), 125.3 (s), 132.5 (s), 135.3 (s), 139.0 (d), 146.0 (s), 172.9 (s), 183.1 (s). IR (NaCl, neat): 3190, 2974, 2933, 1703, 1697, 1651, 1631, 1503, 1456, 1328, 1195, 1046 728 cm^{-1}. UV: λ_{max} 226 nm (ϵ = 30 200). $[\alpha]^{25}_D = (+0.4/7.75 \times 10^{-3})^\circ = +51.6^\circ$ ($CHCl_3$, c = 0.008). Mass spectrum (EI): *m/e* (relative intensity) 463 (M^+, 0.5), 404 (15.6), 135 (41.5), 133 (100). HRMS (EI): *m/e* 463.2456 ($C_{27}H_{33}N_3O_4$ requires 463.2471).

Spiro Product 56. ^1H NMR (300 MHz) (acetone-d_6) (mixture of two diastereomers): δ TMS 0.21 (12H, s), 0.93 (18H, s), 1.13 (6H, s), 1.41 (18H, s), 1.48 (6H, s), 1.62 (18H, s), 1.82 (6H, s), 1.88−2.15 (6H, m), 2.54 (2H, t, J = 11.3 Hz), 2.81−2.83 (4H, m), 3.02−3.06 (4H, m), 3.36−3.42 (2H, m), 3.62−3.64 (2H, m), 3.88 (2H, dd, J = 9.3, 12.2 Hz), 3.99 (2H, dd, J = 3.5, 9.3 Hz), 4.21 (2H, dd, J = 3.5, 12.2 Hz), 4.61−4.83 (4H, m), 4.96 (2H, br s), 5.07 (2H, br s), 5.94 (2H, d, J = 8.5 Hz, D_2O exch), 6.92 (2H, d, J = 8.3 Hz), 7.25 (2H, d, J = 8.3 Hz), 7.41 (2H, s). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ −4.9 (q), −4.0 (q), 16.5 (q), 17.9 (s), 18.4 (q), 24.1 (t), 25.7 (q), 28.0 (q), 28.3 (q), 28.6 (q), 29.4 (t), 29.7 (d), 35.6 (t), 36.6 (t), 47.9 (t), 51.7 (d), 66.6 (s), 70.9 (t), 75.9 (d), 76.6 (s), 79.8 (d), 80.4 (s), 83.1 (s), 113.7 (d), 113.9 (t), 114.6 (s), 120.3 (d), 126.4 (d), 127.9 (s), 129.3 (s), 140.4 (s), 141.8 (s), 146.6 (s), 148.5 (s), 155.0 (s), 169.7 (s), 176.6 (s). IR (NaCl, neat): 2932, 1780, 1752, 1714, 1649, 1496, 1425, 1365, 1251, 1229, 1158, 1088 cm^{-1}.

Spiro Product 57. ^1H NMR (300 MHz) (acetone-d_6) (mixture of two diastereomers): δ TMS 0.21 (12H, s), 0.94 (18H, s), 1.14 (6H, s), 1.41 (18H, s), 1.47 (6H, s), 1.62 (18H, s), 1.80 (6H, s), 1.96−2.07 (6H, m), 2.58 (2H, t, J = 11.3 Hz), 2.84 (4H, br s), 2.98−3.13 (4H, m), 3.48−3.50 (2H, m), 3.51−3.52 (2H, m), 3.88 (2H, dd, J = 9.3, 12.1 Hz), 4.00 (2H, dd, J = 3.4, 9.1 Hz), 4.22 (2H, dd, J = 3.4, 12.2 Hz), 4.72 (2H, dd, J = 6.6, 15.0 Hz), 4.84 (2H, dd, J = 6.3, 10.7 Hz), 4.96 (2H, br s), 5.08 (2H, br s), 5.95 (2H, d, J = 8.6 Hz, D_2O exch), 6.91 (2H, d, J = 8.3 Hz), 7.23 (2H, d, J = 8.3 Hz), 7.38 (2H, s). ^{13}C NMR (75.5 MHz) ($CDCl_3$) (mixture of two diastereomers): δ −4.9 (q), −4.0 (q), 16.5 (q), 17.9 (s), 18.8 (q), 24.1 (t), 25.7 (q), 28.0 (q), 28.3 (q), 29.0 (t), 29.7 (d), 35.6 (t), 36.7 (t), 48.0 (t), 52.4 (d), 66.7 (s), 71.0 (t), 75.8 (d), 76.6 (s), 79.8 (d), 80.4 (s), 83.1 (s), 113.6 (d), 113.8 (t), 114.7 (s), 120.0 (d), 126.1 (d), 127.8 (s), 129.3 (s), 140.4 (s), 141.7 (s), 146.4 (s), 148.6 (s), 155.0 (s), 169.8 (s), 175.7 (s). IR (neat): 2926, 1783, 1754, 1715, 1652, 1494, 1457, 1367, 1250, 1160, 1087 cm^{-1}.

Acknowledgment. This work was supported in part by the National Institutes of Health (Grant CA 43969) and the National Science Foundation (CHE 9320010). Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and The Colorado State University Agricultural Experiment Station (USDA SAES Western Project W-122) for partial support of this work. Mass spectra were obtained on instruments supported by the National Institutes of Health Shared Instrumentation Grant GM49631. We would like to thank Dr. Chris Rithner of Colorado State University for technical assistance with several 2D NMR experiments. Narashima Sreerama and Prof. Robert W. Woody (Department of Biochemistry and Molecular Biology, Colorado State University) are gratefully acknowledged for help in obtaining CD spectra. We would also like to acknowledge Dr. Dusan Stanojevic and Prof. Gregory L. Verdine (Department of Chemistry, Harvard University) for their assistance in obtaining CD spectra of paraherquamide B. We are grateful to Dr. John Ondeyka of Merck & Co. for furnishing NMR spectra of natural paraherquamide B. J.F.S.-C. thanks the Conselleria de Educacio i Ciencia de la Generalitat Valenciana (Spain) for a fellowship. We also wish to acknowledge Renee Gallegos (Colorado State University), Felix Sancenon (University of Valencia), and M. Eugenia Martinez (University of Valencia) for valuable assistance in isolating natural (−)-paraherquamide B from *P. fellutanum*. Mr. Dan Bond is gratefully acknowledged for providing synthetic chemical technical assistance.

JA952666C

JOC Article

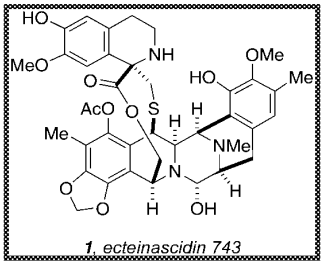
Synthetic Studies on Et-743. Assembly of the Pentacyclic Core and a Formal Total Synthesis

Dan Fishlock[†] and Robert M. Williams*[,†,‡]

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, and University of Colorado Cancer Center, Aurora, Colorado 80045

rmw@lamar.colostate.edu

Received May 30, 2008



A formal total synthesis of the potent anticancer agent Et-743 is described. The tetrahydroisoquinoline core is stereoselectively constructed using a novel radical cyclization of a glyoxalimine. Further elaboration of this core rapidly accessed the pentacyclic core of Et-743, but a mixture of regiosisomers was obtained in the key Pictet−Spengler ring closure. A known advanced intermediate in the synthesis of Et-743 was intercepted, constituting a formal synthesis of the molecule.

Introduction

Members of the tetrahydroisoquinoline family of alkaloids display a wide range of biological properties such as antitumor and antimicrobial activities.[1] Of particular significance within this family is Ecteinascidin 743 (Et-743, **1**, Figure 1,) which has been demonstrated to possess extremely potent cytotoxic activity with in vitro IC_{50} values in the 0.1−1 ng/mL range in several cell lines (as a measure of RNA, DNA, and protein synthesis inhibition).[2] Et-743 is currently in phase II/III clinical trials for the treatment of ovarian, endometrial, and breast cancers and several sarcoma lines.[3]The scarcity of the natural product from marine sources renders Et-743 an important target for synthesis. Corey and co-workers reported the first total synthesis of Et-743 in 36 steps with an overall yield of 0.72%.[4a]

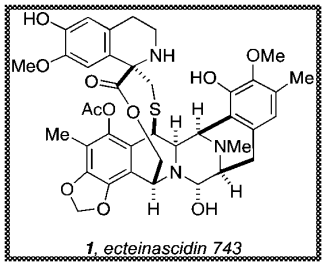


FIGURE 1. Ecteinascidin 743 (**1**).

A second-generation synthesis improved the overall yield to 2.04%, but still required 36 steps.[4b] Fukuyama and co-workers achieved a total synthesis of Et-743 in 50 steps and 0.56% overall yield.[5] More recently, Zhu and co-workers reported a 31 step synthesis in 1.7% overall yield.[6] Most recently, Danishefsky and co-workers reported a formal total synthesis[7]via a pentacyclic compound that intercepted a late-stage intermediate of Fukuyama's route.[5] Despite the advancements in the state-of-the-art in total synthetic approaches to Et-743, the clinical supply of this complex drug is semisynthetically derived from natural cyanosafracin B, obtained by fermentation as reported by PharmaMar.[8]

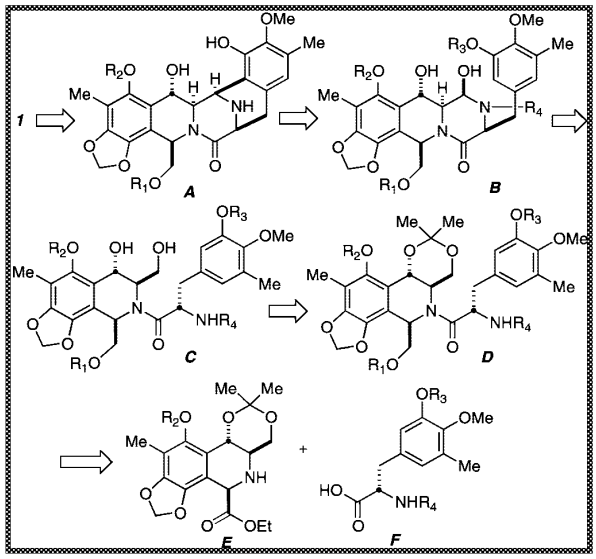
Our laboratory has been developing methodology for the assembly of tetrahydroisoquinoline natural products and has

[†] Colorado State University.
[‡] University of Colorado Cancer Center.

(1) Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669–1730.
(2) (a) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Keifer, P. A.; Wilson, G. R.; Perun, T. J.; Sakai, R.; Thompson, A. G.; Stroh, J. G.; Shield, L. S.; Seigler, D. S. *J. Nat. Prod.* **1990**, *53*, 771–792. (b) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512–4515. (c) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. P.; Koehn, F. E.; McConnell, O. J. *J. Org. Chem.* **1990**, *55*, 4508–4512. (d) Guan, Y.; Sakai, R.; Rinehart, K. L.; Wang, A. H.-J. *J. Biomol. Struct. Dyn.* **1993**, *10*, 793–818. (e) Aune, G. J.; Furuta, T.; Pommier, Y. *Anti-Cancer Drugs* **2002**, *13*, 545–555. (f) Rinehart, K. L. *Med. Drug Rev.* **2000**, 1–27.

10.1021/jo801159k CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/08/2008

SCHEME 1. Synthetic Plan



reported syntheses of D,L-quinocarcinamide,[9] (−)-tetrazomine,[10] (−)-renieramycin G,[11] (−)-jorumycin,[11] and cribrostatin 4 (renieramycin H).[12] As a part of this program, we have targeted Et-743 by a convergent route that envisioned coupling of a suitably functionalized tyrosine derivative[13] with the complete tetrahydroisoquinoline core (Scheme 1.) We have successfully deployed this strategy, with the present objective of construction of pentacycle **A**, in the synthesis of (−)-renieramycin G and (−)-jorumycin.[11,12]

We have previously reported a concise and highly diastereoselective synthesis of the tetrahydroisoquinoline core of Et-743 (**E**).[14] This was achieved via an intramolecular 6-*endo* radical closure on a glyoxalimine, and the desired 1,3-*cis*-diastereomer was obtained exclusively. The synthesis of a tetrahydroisoquinoline such as **E** can be problematic because of the acid sensitivity of the benzylic hydroxyl, particularly because it is *ortho* to the phenolic hydroxyl of the aromatic ring and thus has a high propensity for *ortho*-quinonemethide formation. Herein, we report a formal total synthesis of Et-743 as part of our ongoing efforts to devise a practical and scalable synthesis of this potent antitumor antibiotic that would be amenable to the construction of analogues with anticipated potent cytotoxic activity.

Results and Discussion

The synthesis began with Borchardt's catechol **3**[15] that was regioselectively brominated to generate **4** (92% yield) (Scheme 2.) Conversion of catechol **4** to the methylenedioxy aldehyde **5** was accomplished using bromochloromethane in a sealed vessel (69% yield). Baeyer−Villiger oxidation using *m*-CPBA provided bromophenol **6** as an off-white solid following hydrolysis of the resulting formate intermediate (73% yield). Stereoselective aldol condensation of the titanium phenolate of **6** with (*R*)-Garner's aldehyde (**7**)[16] using a modification of Casiraghi's method[17] provided the *anti*-product **8** followed by allyl protection of the phenolic oxygen delivering **9** (65% yield, two steps). Subsequent hydrolysis of the oxazolidine and formation of the *trans*-acetonide (84% yield, two steps) provided **10** as an oil that cleanly underwent *N*-Boc deprotection using Ohfune's protocol[18] (76% yield) to afford free amine **11** as a stable crystalline solid. From **11**, the glyoxalimine intermediate **13** (see Scheme 3) was readily obtained by condensation with ethyl glyoxalate. Following isolation by filtration through Celite and concentration, the radical ring closure commenced with slow addition of Bu_3SnH and AIBN via syringe pump to a refluxing dilute solution of the glyoxalimine (**13**). Concentration and KF/silica chromatography[19] of the crude reaction mixture provided solid **12** as a single diastereomer (58% yield, two steps). The relative stereochemistry of **12** was secured ^{1}H NMR data and corroborated by X-ray crystallography. Examination of the crude ^{1}H NMR revealed the formation of a single diastereomer in the radical closure and exclusive 6-*endo* regioselectivity. In addition to **12** and tin impurities visible in the ^{1}H NMR spectrum, an aromatic proton arising from hydride quenching of the aryl radical revealed a ~6.6:1 ratio of **12** to reduced substrate. Slower addition rates (over 18 or 36 h) did not improve the isolated yield of **12**.

(3) (a) Zelek, L.; Yovine, A.; Etienne, B.; Jimeno, J.; Taamma, A.; Martín, C.; Spielmann, M.; Cvitkovic, E.; Misset, J. L. *Ecteinacidin-743 in Taxane/Antracycline Pretreated Advanced/Metastatic Breast Cancer Patients: Preliminary Results with the 24 h Continuous Infusion Q3 Week Schedule*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 592. (b) Delaloge, S.; Yovine, A.; Taamma, A.; Cottu, P.; Riofrio, M.; Raymond, E.; Brain, E.; Marty, M.; Jimeno, J.; Cvitkovic, E.; Misset, J. L. *Preliminary Evidence of Activity Of Ecteinacidin-743 (ET-743) in Heavily Pretreated Patients with Bone and Soft Tissue Sarcomas*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 2181. (c) Le Cesne, A.; Judson, I.; Blay, J. Y.; Radford, J.; an Oosterom, A.; Lorigan, P.; Rodenhuis, E.; Donato Di Paoula, E.; Van Glabbeke, M.; Jimeno, J.; Verweij, J. *Phase II of ET-743 in Advance Soft Tissue Sarcoma in Adult:A STBSG-EORTC Trial*; 36th Annual Meeting of the American Society of Clinical Oncology, New Orleans, May 20-23, 2000; Abstract number 2182. (d) Aune, G. J.; Furuta, T.; Pommier, Y. *Anti-Cancer Drugs* **2002**, *13*, 545–555.

(4) (a) Corey, E. J.; Gin, D. Y.; Kania, R. S. *J. Am. Chem. Soc.* **1996**, *118*, 9202–9203. (b) Martinez, E. J.; Corey, E. J. *Org. Lett.* **2000**, *2*, 993–996.

(5) Endo, A.; Yanagisawa, A.; Abe, M.; Tohma, S.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2002**, *124*, 6552–6554.

(6) Chen, J.; Chen, X.; Bois-Choussy, M.; Zhu, J. *J. Am. Chem. Soc.* **2006**, *128*, 87–89.

(7) Zheng, S.; Chan, C.; Furuuchi, T.; Wright, B. J. D.; Zhou, B.; Guo, J.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 1754–1759.

(8) Cuevas, C.; Pérez, M.; Martín, M. J.; Chicharro, J. L.; Fernández-Rivas, C.; Flores, M.; Francesch, A.; Gallego, P.; Zarzuelo, M.; de la Calle, F.; Garcia, J.; Polanco, C.; Rodríguez, I.; Manzanares, I. *Org. Lett.* **2000**, *2*, 2545–2548.

(9) Flanagan, M. E.; Williams, R. M. *J. Org. Chem.* **1995**, *60*, 6791–6797.

(10) (a) Scott, J. D.; Williams, R. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1463–1465. (b) Scott, J. D.; Williams, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 2951–2956.

(11) (a) Lane, J. W.; Chen, Y.; Williams, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 12684–12690. (b) Lane, J. W.; Estevez, A.; Mortara, K.; Callan, O.; Spencer, J. R.; Williams, R. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3180–3183. (c) Vincent, G.; Lane, J. W.; Williams, R. M. *Tetrahedron Lett.* **2007**, *48*, 3719–3722. (d) Jin, W.; Metobo, S.; Williams, R. M. *Org. Lett.* **2003**, *5*, 2095–2098.

(12) (a) Vincent, G.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1517–1520. (b) Vincent, G.; Chen, Y.; Lane, J. W.; Williams, R. M. *Heterocycles* **2007**, *72*, 385–398.

(13) Jin, W.; Williams, R. M. *Tetrahedron Lett.* **2003**, *44*, 4635–4639.

(14) Fishlock, D.; Williams, R. M. *Org. Lett.* **2006**, *8*, 3299–3301.

(15) Prep a red from 2,3-dimethoxytoluene according to: Shinhababu, A. K.; Ghosh, A. K.; Borchardt, R. T. *J. Med. Chem.* **1985**, *28*, 1273–1279.

(16) (*R*)-Garner's aldehyde was synthesized from D-serine according to: Garner, P.; Park, J. M. *Org. Synth.* **1992**, *70*, 18–28.

(17) Casiraghi, G.; Cornia, M.; Rassu, G. *J. Org. Chem.* **1988**, *53*, 4919–4922. In our case, sonication was not required as described in the original paper.

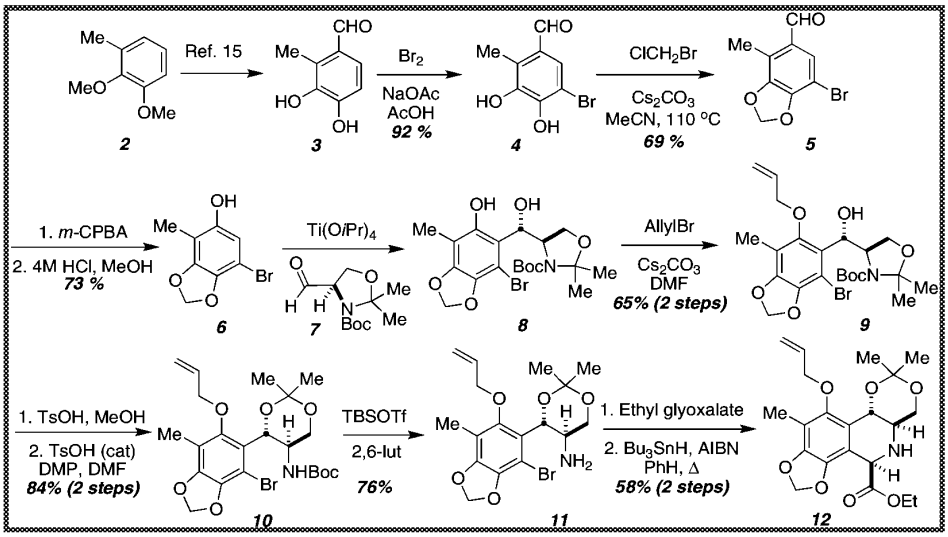
(18) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870–876.

(19) Effective removal of tin impurities from Bu_3SnH-mediated reactions: Harrowven, D. C.; Guy, I. L. *Chem. Commun.* **2004**, 1968–1969.

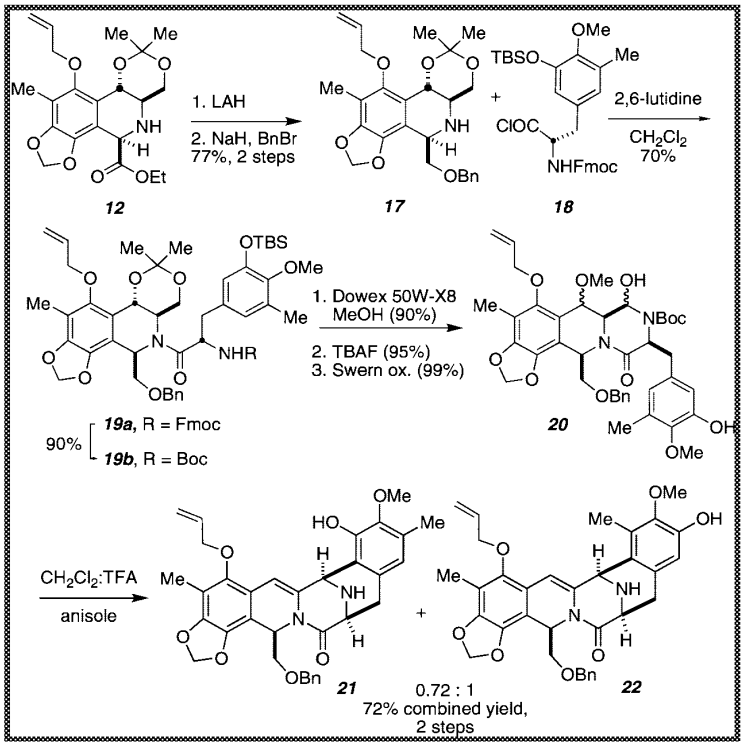
(20) (a) De Paolis, M.; Chiaroni, A.; Zhu, J. *Chem. Commun.* **2003**, 2896–2897. (b) Chen, X.; Chen, J.; De Paolis, M.; Zhu, J. *J. Org. Chem.* **2005**, *70*, 4397–4408.

(21) (a) Herberich, B.; Kinugawa, M.; Vazquez, A.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 543–546. (b) Jin, W; Metobo, S.; Williams, R. M. *Org. Lett.* **2003**, *5*, 2095–2098.

SCHEME 2. Tetrahydroisoquinoline Core of Et-743



SCHEME 3. Pentacycle Construction



The diastereoselectivity of this reaction stands apart from numerous Pictet–Spengler cyclizations on related substrates that provide tetrahydroisoquinolines exclusively as the 1,3-*trans*-diastereomers.[11,20,21] We qualitatively rationalize the *cis*-diastereoselectivity of this radical process using the Beckwith–Houk chairlike transition state model for intramolecular radical ring closures (Figure 2).[22] The lowest-energy chair conformation (**A**) adopted by the *trans*-acetonide of the substrate (**13**) results in both the glyoxalimine and aryl substituent being in an equatorial disposition. In this conformation, 1,3-diaxial steric effects and allylic strain interactions are minimized in the ring-forming transition state. To further examine the stereocontrol imparted by the acetonide ring, the *cis*-acetonide substrate **14** was prepared (using Casiraghi's method from the magnesium phenolate of **6**).[17] Substrate **14** resulted in a 1:1 mixture of 1,3-

(22) (a) Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron* **1985**, *41*, 3925–3941. (b) Spellmeyer, D. C.; Houk, K. N. *J. Org. Chem.* **1987**, *52*, 959–974.

(23) Chen, X.; Zhu, J. *Angew. Chem., Int. Ed* **2007**, *46*, 3962–3965.

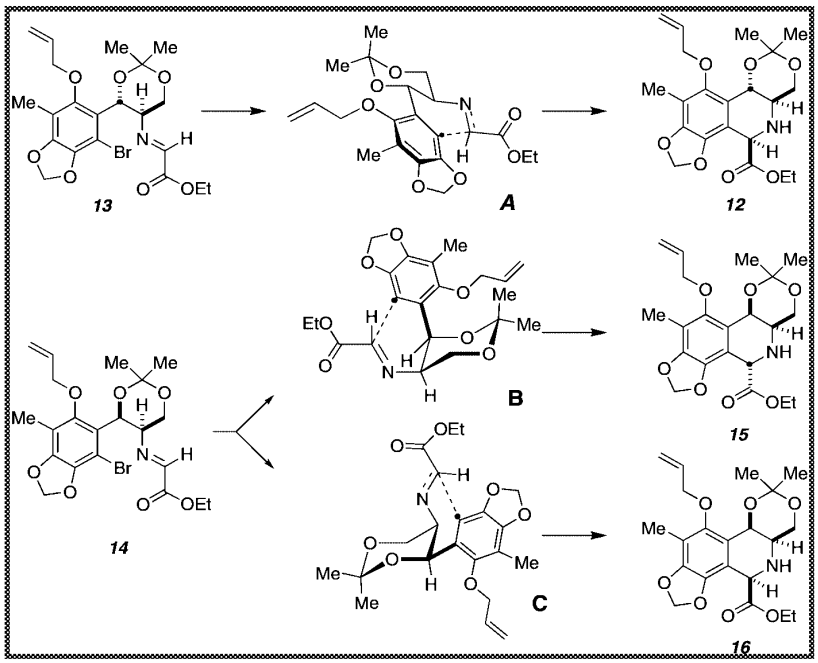


FIGURE 2. Transition state models to rationalize the observed 1,3 relative stereochemistry in the tetrahydroisoquinoline radical ring closure.

trans- and 1,3-*cis*-tetrahydroisoquinolines (**15** and **16**, both are known compounds),[20] which suggests the energy difference between transition state conformations **B** and **C** (axial aryl group versus axial glyoxalimine) is negligible.

As shown in Scheme 3, reduction of the tetrahydroisoquinoline ester (**12**)[14] with LAH, followed by immediate protection as the benzyl ether (**17**), proceeded cleanly in 77% yield over two steps. The substituted tyrosine amino acid component (**18**) has been previously reported by us, utilizing the oxazinone template technology developed in our laboratory that was benzylated with the advanced aromatic side chain.[13] Thus, acylation of the tetrahydroisoquinoline (**17**) was achieved via the *N*-Fmoc-protected amino acid chloride (**18**) to give amide **19a** without epimerization. The use of the *N*-Boc free acid with a variety of coupling agents (DCC, HOBt, HATU) all resulted in very sluggish reactions with poor isolated yields, as did the attempted use of the *N*-Boc acid fluoride.

Treatment of **19a** with diethylamine provided the free amine, which was not isolated in favor of immediate evaporation of excess base and solvent and subsequent Boc protection of the crude material. Isolation following chromatography provided compound **19b** in 90% yield. Removal of the acetonide from **19b** was accomplished using the extremely mild, albeit slow, method of stirring with Dowex 50W-X8 cationic resin in methanol. Complete deprotection took 8−12 h, but the yield was quantitative following simple filtration and concentration. Instead of providing the usual diol product, this substrate incorporated methanol at the benzylic position thus providing the methyl ether as a ~1:1 mixture of diastereomers. Not unexpectedly, the benzylic stereogenic center loses stereochemical integrity since the methanol is incorporated via the incipient *ortho*-quinonemethide species arising from the acidic deprotection conditions.

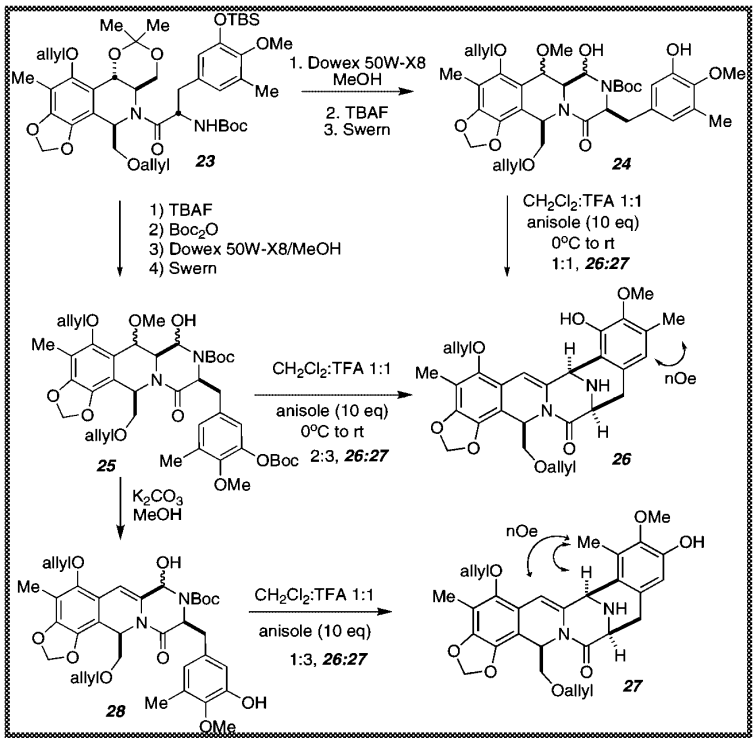
Alternatively, we found that the use of water/dichloromethane with cationic resin on **19b** could provide the corresponding free diol, but oxidation of the primary alcohol (in the presence of the free benzylic alcohol) could not, in our hands, be cleanly accomplished. The methyl ether was thus a fortuitous selective protection of the benzylic alcohol, ultimately simplifying the subsequent manipulations.

Facile deprotection of the *O*-TBS-protected phenol using TBAF was followed by oxidation of the primary alcohol using Swern conditions in high yield. This oxidation product (**20**) existed as an equilibrium mixture of the aldehyde and the corresponding hemiaminal species (illustrated) as observed by ^{1}H NMR, which was otherwise additionally complicated by amide and carbamate rotamers. The attempted oxidation using either Dess−Martin periodinane or TPAP/NMO both failed, leading to extensive decomposition. Following filtration of crude **20** through a plug of silica gel, this substance was immediately subjected to the Pictet−Spengler conditions.

The objective at this stage was to achieve the Pictet−Spengler reaction via *N*-Boc deprotection, iminium ion formation, and electrophilic aromatic substitution to provide the desired pentacyclic core of Et-743. This meant that the aromatic substitution must occur *ortho* to the free phenol, and the benzylic methyl ether must survive these conditions. Unfortunately, it had already been demonstrated above that the electron-rich aromatic ring of the tetrahydroisoquinoline component was highly sensitive to protic conditions, leading to *ortho*-quinonemethide formation.

Indeed, when substrate **20** was treated with trifluoroacetic acid in methylene chloride, it cleanly underwent the expected pentacycle formation furnishing **21** + **22** as a ~0.72:1 *ortho:para* mixture of regioisomers in 72% combined yield. As anticipated, the benzylic methoxy group was eliminated presumably via the incipient *ortho*-quinonemethide species that forms under these conditions. In a fruitless effort to circumvent the vexing olefin formation, pentacycle formation with TFA in dry methanol resulted in extensive decomposition of the substrate.

SCHEME 4. Pictet–Spengler Regioselectivity



As part of these synthetic investigations, the intermediate **23** was prepared (in parallel with the *O*-benzyl-protected synthesis) bearing an *O*-allyl-protected hydroxymethyl at C1 of the THIQ core. This substrate was used to examine the regioselectivity of the pentacycle-forming ring closure and was utilized to acquire detailed ^{1}H NMR data, while the *O*-benzyl material **21** was carried forward in the synthesis. One interesting observation was the behavior of compound **25** containing the *O*-Boc carbonate-protected phenolic oxygen. Treatment of **25** under the same reaction conditions provided the pentacycles **26** + **27** in a 2:3 ratio of *ortho*:*para* regioisomers. The *O*-Boc carbonate would presumably be deprotected quickly under these conditions to reveal the free phenol-containing reactive species, thus resulting in a comparable regioselectivity as observed with substrate **20** (beginning with a free phenol on the aryl nucleophile moiety). Notably, however, when substrate **25** was treated with K_2CO_3/MeOH, the *O*-Boc carbonate was selectively removed (**28**) with apparent olefin formation prior to the Pictet–Spengler reaction and pentacycle formation. Treatment of **28** with TFA in dichloromethane produced the pentacycles **26** + **27** in a 1:3 ratio of *ortho*:*para* regioisomers, supporting the hypothesis that some regioselectivity in the closure might arise from an intramolecular H bond with a heteroatom at the benzylic position.[11c]

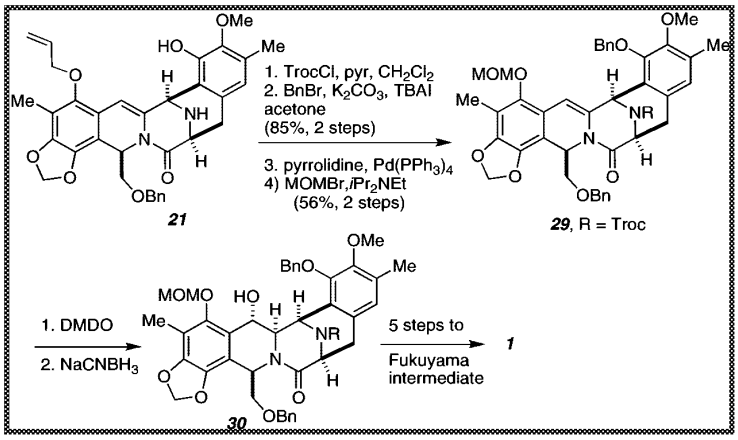
In their synthesis of renieramycin H, the Zhu group has interestingly reported control of Pictet–Spengler regioselectivity in a related system by variation of acid concentration (Scheme 4).[23] It was found in that case that lowering the concentration of methanesulfonic acid to 0.01% in CH_2Cl_2 could invert the *ortho*:*para* selectivity from 3.4:1 to 2:3. Furthermore, the use of acetonitrile as the solvent instead of dichloromethane favored the undesired isomer, giving *ortho*:*para* selectivity of 1:10. Our attempt to reproduce the Zhu conditions on substrate **24** using 0.01% methanesulfonic acid in CH_2Cl_2 did not affect the regioselectivity of this reaction. The substrate was consumed to provide some material that appeared to still contain the *N*-Boc protecting group, but the ^{1}H NMR of the crude product was prohibitively complex. Subsequent treatment of this reaction crude with a TFA/anisole/CH_2Cl_2 mixture provided the pentacycles **26** + **27** with ~1:1 regioselectivity. The same ratio is obtained if the TFA/anisole conditions are used directly on substrate **24**.

In order to redeem the synthetic utility of the olefinic products (**21** or **26**), our attention was captured by the recent formal synthesis of Et-743 reported by the Danishefsky group[7] in which the olefin (**29**, Scheme 5) underwent facile oxidation using DMDO and immediate hydride reduction delivering the benzylic alcohol **30**. With the availability of this methodology in the literature, our efforts were briefly redirected to convert our synthetic pentacycle **21** into compound **29** which would constitute a formal total synthesis of Et-743 by relay through the Danishefsky[7] and then Fukuyama[5] syntheses, respectively.

In the event, the desired pentacycle **21** (Scheme 3) was *N*-protected as the trichloroethyl carbamate (Troc), and the phenolic residue was protected as the corresponding *O*-benzyl ether in 85% yield for the two steps (Scheme 5). Removal of the *O*-allyl group under standard conditions followed by reprotection as the corresponding MOM ether provided compound **29** (56% yield for the two steps). Compound **29** perfectly matched Danishefsky's substrate by ^{1}H, ^{13}C NMR, and optical rotation, confirming the structure of compound **29**.

Since Danishefsky has previously converted[7] compound **29** into a late-stage intermediate in Fukuyama's total synthesis[5] (namely, compound **30**, Scheme 5), this two-stage relay of our

SCHEME 5. Formal Synthesis of Et-743 via 21 to 29 and Danishefsky to Fukuyama Relay



synthetic **21** thus constitutes a formal total synthesis of Et-743 and provides firm structural corroboration of our synthetic material and methods.

While the present formal synthesis reveals that our glyoxal-imine radical cyclization technology[14] holds considerable potential for the efficient total synthesis of Et-743 and congeners, we are currently endeavoring to improve the regioselectivity of the key pentacycle formation (**20** to **21**) as well as refining the overall synthetic efficiency of our approach. These objectives are currently under study in our laboratory and will be reported in due course.

Experimental Section

For general methods and considerations, see Supporting Information.

Compound 19. The Fmoc-amino acid (410 mg, 0.727 mmol, 1.2 equiv) was dissolved in dry toluene and concentrated (×2), and then dried under high vacuum. This oil was dissolved in dry CH_2Cl_2 (4 mL) to which was added oxalyl chloride (1 mL) at room temperature, followed by dry DMF (20 μL). After stirring for 20 min, the solution was concentrated and reconcentrated from dry toluene (×2) and then dried under high vacuum. This acid chloride **18** was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 °C. THIQ(OBn) **17** (275 mg, 0.61 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (2 mL) and 2,6-lutidine (77 μL 0.67 mmol, 1.1 equiv). This solution was transferred into the acid chloride solution slowly dropwise, and the resulting mixture was warmed to rt and stirred 7 h (TLC showed consumption of the THIQ(OBn) starting material). The reaction was quenched with saturated NH_4Cl (aq) and then extracted to EtOAc (×3). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated to provide a crude orange oil. Purification by flash chromatography (hexanes:EtOAc 5:1, silica gel) gave the peptide **19a** as a pale yellow oil (426 mg, 70%); R_f = 0.34 (3:1 hexanes:EtOAc, UV, CAM); $[\alpha]^{25}_D$ −22.8 (*c* 1.14, CH_2Cl_2); IR (thin film) 3289, 2929, 2858, 1717, 1634 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to amide and carbamate rotamers. See the rt ($CDCl_3$) and 373 K (DMSO-d_6) ^1H spectra and rt ($CDCl_3$) ^{13}C spectra in the Supporting Information: HRMS(ESI/APCI+) *m/z* calcd for $C_{58}H_{68}N_2O_{11}NaSi$ $(M + Na)^+$ 1019.4485, found 1019.4499.

Compound 19b. Fmoc (OBn) peptide **19a** (146 mg, 0.146 mmol) was dissolved in a 20% v/v solution of Et_2NH in CH_2Cl_2 [CH_2Cl_2 (2.5 mL) and diethylamine (0.6 mL)]. After stirring for 6 h, the solution was concentrated and then reconcentrated from toluene and dried under high vacuum. The crude material was dissolved in EtOH:CH_2Cl_2 (2:0.5 mL) to which was added Boc_2O (370 mg, 10 equiv). After stirring for 12 h, the reaction was concentrated and immediately purified by flash chromatography (9:1 to 5:1 hexanes: EtOAc, silica gel) to provide **19b** as a clear colorless oil (115 mg, 90% over 2 steps): R_f = 0.43 (3:1 hexanes:EtOAc, UV, CAM); $[\alpha]^{25}_D$ −26.6 (*c* 1.0, CH_2Cl_2); IR (thin film) 3319, 2930, 2858, 1711, 1646 cm^{-1}; ^1H and ^{13}C NMR spectra are extremely complex due to amide and carbamate rotamers; see the ^1H spectra ($CDCl_3$, rt) and (DMSO-d_6, 373 K) and ^{13}C spectrum ($CDCl_3$, rt) in the Supporting Information: HRMS(ESI/APCI+) *m/z* calcd for $C_{48}H_{66}N_2O_{11}NaSi$ $(M + Na)^+$ 897.4328, found 897.4310.

Compounds 21 and 22. Boc (OBn) peptide **19b** (115 mg, 0.132 mmol) was dissolved in dry MeOH (5 mL), and Dowex 50W-X8 cationic resin (100 mg) was added (the resin was first rinsed with dry methanol and dried under a stream of argon). After 65 h, the reaction was complete by TLC and a single streak was observed (during the course of the reaction, two streaks initially arise due to a mixture of diol and methyl ether/alcohol products). The reaction was filtered through a plug of Celite, eluting with dry MeOH, and the filtrate was combined to provide the methyl ether as clear, colorless oil (100 mg, 90% yield): R_f = 0 to 0.35 streak (3:1 hexanes:EtOAc, UV, CAM); HRMS(FAB+) *m/z* calcd for $C_{46}H_{65}N_2O_{11}Si$ $(M + H)^+$ 849.4358, found 849.4354. The methyl ether (100 mg) was dissolved in THF (3 mL), and TBAF (1 M in THF, 125 μL, 1.06 equiv) was added in one portion. After 20 min, the reaction was concentrated by rotary evaporation and passed through a silica plug (eluting with 3:1 to 1:1 hexanes:EtOAc) to provide the free phenol as a clear, colorless oil (82 mg, 95% yield): R_f = 0 to 0.43 streak (3:1 hexanes:EtOAc, UV, CAM); HRMS-(FAB+) *m/z* calcd for $C_{40}H_{51}N_2O_{11}$ $(M + H)^+$ 735.3493, found 735.3490. Oxalyl chloride (15 μL 1.5 equiv) was added carefully to a solution of DMSO (25 μL, 3.2 equiv) in CH_2Cl_2 (1 mL) previously cooled to −78 °C. A solution of the above alcohol (82 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) was added dropwise, and the resulting mixture was stirred at −78 °C for 40 min. The reaction was quenched with Et_3N (125 μL, 8 equiv) and then allowed to warm to rt. The reaction was diluted with CH_2Cl_2 and washed with brine, and then the combined organic fractions were dried (Na_2SO_4), filtered, and concentrated. The crude material was passed through a silica gel plug (eluting with hexanes:EtOAc 1:1) to provide a yellow oil/foam (82 mg, quant.) of hemiaminal **20** which was used without further purification: R_f = 0.5 (hexanes:EtOAc 1:1, UV, CAM). Hemiaminal **20** (232 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (3 mL) to which were added TFA (3 mL) and anisole (0.350 mL) at rt. The reaction was stirred for 14 h and then concentrated to remove TFA, then redissolved in CH_2Cl_2 and washed with saturated aq $NaHCO_3$. The organic fraction was dried (Na_2SO_4), filtered, and concentrated. Crude ^1H NMR shows 0.72:1 *ortho* (**21**) to *para* (**22**) regioisomers. Purification by PTLC (2% MeOH in

EtOAc) provided the *ortho* (63 mg) and *para* products (69 mg) for a combined yield of 72%. Data for **21**: R_f = 0.61 (EtOAc:MeOH 95:5, UV, CAM); $[\alpha]^{25}_D$ −18.0 (*c* 1.0, CH_2Cl_2); IR (thin film) 3295, 2932, 1672, 1632, 1455, 1428 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.14−7.30 (m, 3H), 6.98 (s, 1H), 6.97 (s, 1H), 6.24 (s, 1H), 6.19 (s, 1H), 6.12 (dddd, *J* = 16.0, 11.0, 5.4, 5.4 Hz, 1H), 6.05 (dd, *J* = 7.2, 5.0 Hz, 1H), 5.85 (br s, 1H), 5.82 (br s, 1H), 5.78 (v br s, 1H), 5.45 (app dd, *J* = 17.1, 1.1 Hz, 1H), 5.29 (app dd, *J* = 10.3, 0.8 Hz, 1H), 4.9 (s, 1H), 4.30 (app d of AB quartet, *J* = 12.3, 5.4 Hz, 2H), 4.03 (d, *J* = 6.1 Hz, 1H), 3.87 (AB quartet, *J* = 12.1 Hz, 2H), 3.63 (s, 3H), 2.95−3.2 (m, 5H), 2.11 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 147.6, 145.6 (×2), 143.4, 139.7, 138.7, 134.5, 133.9, 129.4, 128.8, 128.0 (×2), 127.1, 126.8 (×2), 122.5, 119.3, 117.7, 117.5, 113.0, 108.7, 101.5, 100.2, 75.3, 72.6, 70.0, 60.8, 54.4, 50.0, 46.9, 33.4, 15.9, 9.4. HRMS(ESI/APCI+) *m/z* calcd for $C_{34}H_{35}N_2O_7$ $(M + H)^+$ 583.2439, found 583.2441. Data for **22**: R_f = 0.5 (EtOAc:MeOH 95:5, UV, CAM); $[\alpha]^{25}_D$ +47.8 (*c* 1.45, CH_2Cl_2); IR (thin film) 3298, 2931, 1671, 1631, 1430, 1409 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.11−7.22 (m, 3H), 6.94 (s, 1H), 6.92 (s, 1H), 6.41 (s, 1H), 6.11 (dddd, *J* = 16.1, 10.6, 5.5, 5.5 Hz, 1H), 6.08 (s, 1H), 6.03 (dd, *J* = 6.6, 5.1 Hz, 1H), 5.86 (br s, 1H), 5.83 (br s, 1H), 5.45 (app dd, *J* = 17.1, 1.1 Hz, 1H), 5.30 (app dd, *J* = 10.4, 0.8 Hz, 1H), 4.65 (s, 1H), 4.36 (app d of A of AB quartet, *J* = 12.5, 5.5 Hz, 1H), 4.24 (app d of B of AB quartet, *J* = 12.5, 5.5 Hz, 2H), 4.01 (d, *J* = 6.0 Hz, 1H), 3.91 (AB quartet, *J* = 12.2 Hz, 1H), 3.56 (s, 3H), 2.95−3.24 (m, 5H), 2.27 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 148.0, 147.6, 145.9, 144.3, 139.7, 138.4, 134.6, 133.7, 129.6, 128.7, 128.1 (×2), 127.2, 126.9 (×2), 124.9, 117.8, 117.1, 113.8, 113.0, 108.8, 101.5, 100.5, 75.4, 72.8, 70.1, 61.0, 54.3, 52.6, 46.8, 35.4, 12.0, 9.4; HRMS (ESI/APCI+) *m/z* calcd for $C_{34}H_{35}N_2O_7$ $(M + H)^+$ 583.2439, found 583.2429.

Preparation of Compound 29. The desired *ortho*-regioisomer **21** (55 mg, 0.095 mmol) was dissolved in CH_2Cl_2 (2 mL) and pyridine (11 μL, 0.14 mmol, 1.5 equiv) at 0 °C. TrocCl (13.5 μL, 0.1 mmol, 1.0 equiv) was added and the reaction maintained at 0 °C for 2 h, and then diluted with CH_2Cl_2 and washed with saturated aq NH_4Cl. The organic layer was dried (Na_2SO_4), filtered, and then concentrated. The crude oil was passed through a plug of silica gel eluting with EtOAc, and then concentrated and dried under vacuum. The resulting oil was dissolved in CH_2Cl_2 (600 μL), and MeOH (200 μL) and K_2CO_3 (52 mg, 0.38 mmol, 4 equiv) were added followed by benzyl bromide (22 μL, 0.19 mmol, 2 equiv) and a catalytic amount of tetrabutylammonium iodide. The resulting mixture was stirred at rt for 13.5 h then filtered through a pad of Celite, rinsing with CH_2Cl_2. Flash chromatography (5:1 hexanes:EtOAc) provided the *N*-Troc/*O*-benzyl product as a pale yellow oil (68 mg, 85% over 2 steps): R_f = 0.46 (hexanes:EtOAc 3:1, UV, CAM); $[\alpha]^{25}_D$ +58.1 (*c* 1.7, CH_2Cl_2); IR (thin film) 2927, 1724, 1681, 1434, 1371 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, mixture of carbamate rotamers) δ 7.30−7.56 (m, 3H), 7.14−7.25 (m, 4H), 6.92−7.00 (m, 2H), 6.45 (d, *J* = 9.9 Hz, 1H), 6.22 (d, *J* = 4.1 Hz, 1H), 6.12 (*J* = 16.1 Hz, 1H), 6.01−6.08 (m, 1H), 5.79−5.90 (m, 3H), 4.98−5.29 (m, 5H), 4.85 (d, *J* = 12.0, 2.8 Hz, 1H), 4.60 (d, *J* = 11.9, 6.5 Hz, 1H), 3.97−4.11 (m, 3H), 2.85 (app d, *J* = 12.1 Hz, 1H), 3.70 (s, 3H), 3.04−3.30 (m, 4H), 2.10 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of carbamate rotamers) δ 166.1/166.0, 151.5/151.4, 149.9, 148.9, 148.4, 148.3/148.2, 146.2, 139.6, 138.5, 137.6/137.5, 133.8/133.7, 132.6/132.5, 131.3/131.2, 128.9 (×2), 128.3, 128.1 (×2), 128.0, 127.2, 126.8, 126.6/126.5, 125.4/125.0, 117.6, 117.3, 116.9, 113.3/113.3, 108.6/108.4, 103.3, 102.9, 101.6, 95.3/95.2, 75.4/75.3, 75.2/75.0, 74.6/74.4, 72.6, 69.9/69.8, 60.5, 54.4/53.7, 50.9/50.1, 47.3/47.2, 32.8/32.4, 16.0, 9.5; HRMS(ESI/APCI+) *m/z* calcd for $C_{44}H_{42}N_2O_9Cl_3$ $(M + H)^+$ 847.1950, found 847.1949.

The allyl-protected pentacycle obtained above (20 mg, 0.024 mmol) was dissolved in CH_2Cl_2 (400 μL), and pyrrolidine (6 μL, 3 eq) was added, followed by $Pd(PPh_3)_4$ (2 mg, 0.002 mmol) under Ar. After 16 h, the reaction was still not complete, so additional portions of pyrrolidine and palladium catalyst were added. After stirring an additional 4 h (20 h total), the dark green reaction was applied directly to flash chromatography (silica gel, hexanes:EtOAc 3:1). The pure fractions were combined to provide the phenol as yellow oil (11 mg 56%), used without characterization: R_f = 0.26 (hexanes:EtOAc 3:1, UV, CAM). Phenol (11 mg, 0.014 mmol) was dissolved in CH_2Cl_2 (200 μL) to which were added iPr$_2$NEt (12 μL, 0.07 mmol, 5 equiv) and MOMBr (3.3 μL, 0.042 mmol, 3 equiv). The mixture was stirred for 30 min at rt and then quenched with water and extracted with CH_2Cl_2 (×3). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (hexanes:EtOAc 3:1) provided the protected pentacycle **29** (11.5 mg, quant.): R_f = 0.41 (hexanes:EtOAc 3:1, UV, CAM); $[\alpha]^{25}_D$ +45.4 (*c* 0.8, $CHCl_3$) [lit. +50 (*c* 1.0, $CHCl_3$)]; IR (thin film) 2932, 1723, 1681, 1654, 1432, 1371 cm^{-1}. ^1H and ^{13}C NMR spectra perfectly match the data provided by the Danishefsky group for this intermediate in their formal synthesis (copies of their spectra included in the Supporting Information):[7] ^1H NMR (400 MHz, $CDCl_3$, mixture of carbamate rotamers) δ 7.56−7.31 (m, 5H), 7.13−7.23 (m, 3H), 6.96 (app br d, *J* = 6.9 Hz, 2H), 6.46 (d, *J* = 9.4 Hz, 1H), 6.01−6.15 (m, 3H), 5.86 (app d, *J* = 3.0 Hz, 2H), 5.82 (br s, 1H), 4.97−5.19 (m, 4H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.79 (A of AB quart, *J* = 12.0 Hz, 1H), 4.68 (B of AB quart, *J* = 11.9 Hz, 1H), 4.49−4.60 (m, 2H), 4.43 (app d, *J* = 6.1 Hz, 1H), 4.01 (d of A of AB quart, *J* = 11.8, 4.4 Hz, 1H), 3.85 (B of AB quart, *J* = 12.1 Hz, 1H), 3.71 (app d, *J* = 10.6 Hz, 3H), 3.38 (rotomeric s, 3H), 3.03−3.29 (m, 5H), 2.11 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of carbamate rotamers) δ 166.0/165.9, 151.6/151.4, 149.9, 148.7/148.2, 147.3, 146.2/146.1, 139.8, 138.4/138.4, 137.8/137.7, 132.6/132.5, 131.1/131.1, 128.8, 128.2, 127.9, 127.2, 126.8, 126.6/126.5, 125.2/125.0, 117.0/116.8, 113.7/113.6, 108.5/108.4, 103.3/102.7, 101.6, 100.4/100.4, 95.3/95.2, 75.4/75.3, 74.4/74.0, 72.6/72.6, 69.9/69.9, 60.4, 57.6/57.5, 54.4/53.7, 50.8/50.1, 47.4/47.3, 32.7/32.3, 16.0, 9.9; HRMS(ESI/APPI+) *m/z* calcd for $C_{43}H_{42}N_2O_{10}Cl_3$ $(M + H)^+$ 851.1900, found 851.1897.

Acknowledgment. This paper is fondly dedicated to the memory of the late Professor A.I. Meyers. We gratefully acknowledge financial support from the National Institutes of Health (Grant CA85419). We are grateful to Prof. Samuel J. Danishefsky of the Memorial Sloan-Kettering Cancer Research Institute for kindly providing characterization data for compound **29**.

Note Added after ASAP Publication. Reference 6 contained an incorrect publication date and the description of the conditions used by Zhu et al. (below Scheme 4) was erroneous in the version published ASAP August 8, 2008; the corrected version was published ASAP September 17, 2008.

Supporting Information Available: Complete experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

JO801159K

ORGANIC LETTERS
2005
Vol. 7, No. 8
1489–1491

A Pauson–Khand Approach to the Synthesis of Ingenol

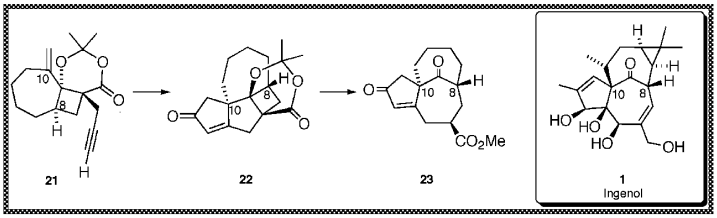
Jeffrey D. Winkler,* Esther C. Y. Lee, and LaToya I. Nevels

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

winkler@sas.upenn.edu

Received January 18, 2005

ABSTRACT

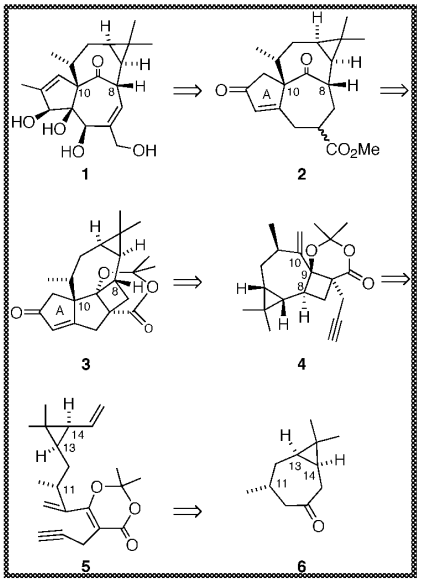


Pauson–Khand cyclization of dioxanone photoadduct 21 leads to the formation of a single product in good yield. However, retro-aldol fragmentation of the pentacyclic cyclopentenone 22 leads to the formation of 23, with cis C-8/C-10 intrabridgehead stereochemistry, unlike the target compound ingenol 1, which possesses C-8/C-10 trans intrabridgehead stereochemistry.

The therapeutic importance of C-3 esters of ingenol **1** and the dearth of exploration of structure–activity relationship data for this class of compounds make the development of efficient pathways for the synthesis of ingenol and analogues an important goal. Of particular note in the synthesis of ingenol is the establishment of the C-8/C-10 trans intrabridgehead stereochemistry, which is critical for the biological activity of **1**. In 2002, we reported the first total synthesis of racemic **1**, in which the trans intrabridgehead stereochemistry was established via intramolecular dioxenone photoaddition. The total synthesis proceeded in 42 steps from commercially available starting materials in an overall yield of 0.042%.[1] Since that time, two other total syntheses have appeared by: Tanino and Kuwajima (2003) and Wood (2004), which proceeded in ca. 45 and 38 steps, respectively.[2]

In an effort to develop a more efficient approach to the synthesis of ingenol, we have examined the strategy outlined in Scheme 1 for the synthesis of **1**, in which the C-8/C-10 intrabridgehead stereochemical relationship is established via

Scheme 1

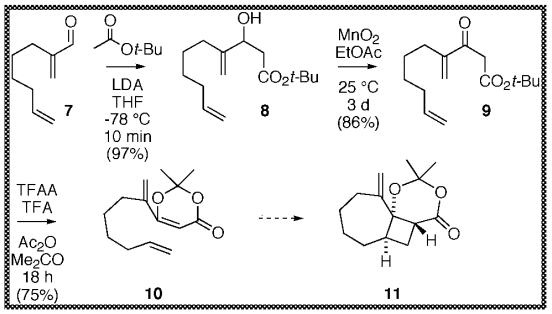


(1) Winkler, J. D.; Rouse, M. B.; Greaney, M. F.; Harrison, S. J.; Jeon, Y. T. *J. Am. Chem. Soc.* **2002**, *124*, 9726–9728.

(2) a) Tanino, K.; Onuki, K.; Miyashita, M.; Nakamura, T.; Takahashi, Y.; Kuwajima, I. *J. Am. Chem. Soc.* **2003**, *125*, 1498–1500. (b) Nickel, A.; Maruyama, T.; Tang, H.; Murphy, P.; Greene, B.; Yusuff, N.; Wood, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 16300–16301.

10.1021/ol050103s CCC: $30.25
Published on Web 03/17/2005

Scheme 2

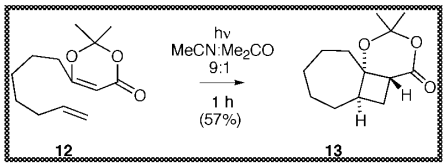


Pauson−Khand cyclization of **4** to give **3**. The A-ring cyclopentenone moiety in retroaldol product **2** would then be used to complete the synthesis of **1**. The Pauson−Khand substrate **4** should be available by the intramolecular dioxenone photocycloaddition of **5**. We envisioned that the C-11 methyl group (ingenol numbering) and the *gem*-dimethylcyclopropane in **5** would be derived from **6**, the preparation of which has been described from (+)-carene.[3] We report herein the results of our model study for this new reaction sequence.

To determine the viability of the route outlined in Scheme 1, we examined the irradiation of **10** (Scheme 2) as a model system for the photocycloaddition of methylene dioxenone **5** (Scheme 1). The synthesis of **10** is outlined in Scheme 2. Unsaturated aldehyde **7** was prepared in a one-pot procedure by Swern oxidation of 7-octen-1-ol followed by reaction of the intermediate aldehyde with Eschenmoser's salt.[4] Reaction of **7** with the conjugate base of *tert*-butyl acetate then gave **8**, which on MnO_2 oxidation afforded ketoester **9**. Exposure of **9** to dioxenone-forming conditions (TFAA, TFA, Ac_2O, Me_2CO) led to the formation of the dioxenone photosubstrate **10** in 75% yield. However, irradiation of **10** (3.0 mM in 10% Me_2CO/MeCN, 450 W Hanovia mercury lamp, 3 h) resulted only in the recovery of unreacted **10** without formation of the desired photoadduct **11**.

While we have shown that irradiation of **12** leads to the formation of **13** in good yield (Scheme 3),[5] irradiation of a

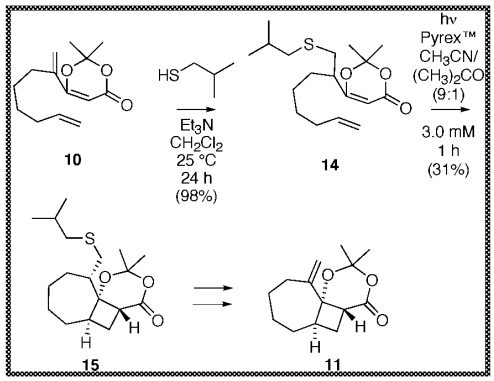
Scheme 3



1:1 mixture of **10** and **12** led to the formation of none of the desired photoadduct **13**, a result that is consistent with quenching of the dioxenone triplet (of both **10** and **12**) by the diene moiety present in **10**.

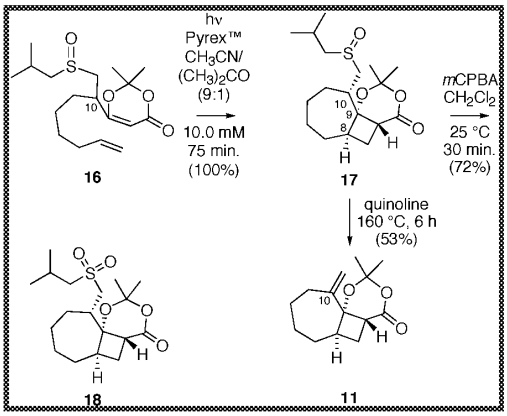
We therefore turned our attention to sulfide **14** as a protecting group for the offending diene functionality in **10** (Scheme 4). Oxidative elimination of **15**, the photoadduct obtained from **14**, would then lead to the formation of **11**. Conjugate addition of isobutylthiol to **10** gave **14**). While

Scheme 4



irradiation of **14** does lead to the formation of the desired photoadduct **15**, the irradiation of the corresponding sulfoxide **16** (Scheme 5), obtained by reaction of **14** with *m*-CPBA

Scheme 5



(−78 °C, 97% yield, as a ca. 1:1 ratio of sulfoxide diastereomers), gave a cleaner reaction and higher yields.

Irradiation of **16** led to the formation of a ca. 1:1 mixture of diastereomeric photoadducts **17**. Oxidation of the mixture of diastereomeric products to a single sulfone (*m*-CPBA, 72% yield) confirmed that the photocycloaddition of **16** proceeded with a unique sense of induction from the C-10 stereocenter.

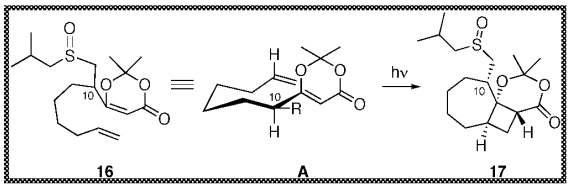
(3) Satoh, T.; Kaneko, Y.; Okuda, T.; Uwaya, S.; Yamakawa, K. *Chem. Pharm Bull.* **1984**, *32*, 3452−3460.

(4) Takano, S.; Inomata, K.; Samizu, K.; Tomita, S.; Yanase, M.; Suzuki, M.; Iwabuchi, Y.; Sugihara, T.; Ogasawara, K. *Chem. Lett.* **1989**, 1283−1284.

(5) Winkler, J. D.; Hey, J. P.; Hannon, F. J. *Heterocycles* **1987**, *25*, 55−60.

The stereochemical outcome of the photocycloaddition of **16** can be attributed to allylic strain effects. Selective formation of **17** is consistent with reaction via the conformation shown in **A** [Scheme 6; R = $CH_2S(O)i$-Bu], in which

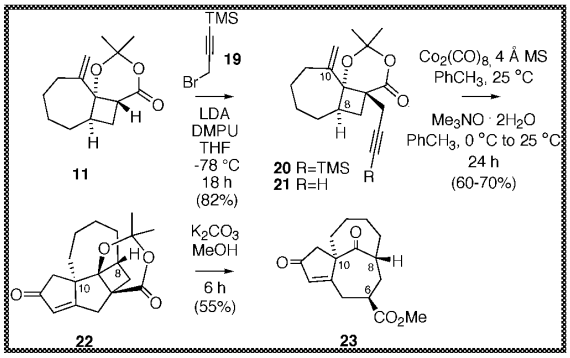
Scheme 6



the C-10 hydrogen eclipses the dioxenone ring. The structure of **18**, the sulfone derived from **17**, was confirmed by X-ray crystallographic analysis. Heating sulfoxide photoadduct **17** to 160 °C in quinoline led to the formation of the desired methylene photoadduct **11**, the formal product of [2 + 2] cycloaddition of **10** (Scheme 4) in good yield.

The Pauson–Khand substrate **21** was then prepared via alkylation of the conjugate base of **11** (LDA, THF, DMPU, −78 °C) with 3-trimethylsilylpropargyl bromide **19** to give **20**, followed by desilylation with TBAF (THF, 100%) to give **21** (Scheme 7). Reaction of **21** with $Co_2(CO)_8$ and 4 Å

Scheme 7



molecular sieves in toluene at room temperature for 2 h followed by slow addition of a suspension of trimethylamine *N*-oxide dihydrate in toluene at 0 °C led to the formation of **22** as a single diastereomer in 60–70% yield.[6] It is noteworthy that the Pauson–Khand reaction of **21** in the presence of the trimethylamine *N*-oxide *dihydrate* was considerably more efficient than the reaction using anhydrous trimethylamine *N*-oxide. This pronounced difference could be attributed to the attenuation of the nucleophilicity of the hydrated amine oxide ligand, which could retard decomplexation of the initially formed cobalt–alkyne complex.[7]

The structure and stereochemistry of **22** was confirmed by X-ray crystallographic analysis, which revealed that it did not contain the requisite C-8/C-10 relative stereochemistry for the synthesis of ingenol. Retro-aldol fragmentation of **22** led to the formation of **23**, with cis intrabridghead stereochemistry, which was verified by X-ray crystallographic analysis. While the fragmentation product was initially formed as a single C-6 epimer (C-6β ester as shown in **23**), prolonged exposure of **23** to the basic reaction conditions (K_2CO_3, MeOH) led to the formation of a mixture of C-6 epimeric products.

While the C-8/C-10 intrabridghead stereochemical relationship in **22** is established in the Pauson–Khand reaction of **21**, that relationship is indirectly established in **21**, since the propargyl moiety in **21** can only approach the C-10 exocyclic methylene from the β-face as shown to give **22**.

In the retrosynthetic plan outlined in Scheme 1, the C-8/C-9 ring fusion stereochemistry in **4** is trans, which forces the approach of the propargyl moiety in **4** to the α-face of the C-10 methylene, thereby generating the requisite C-8/C-10 trans intrabridgehead stereochemistry shown in **3**. However, irradiation of **16** led to the exclusive formation of the cis-fused bicyclo[5.2.0]nonane moiety as shown in **17** (Scheme 5). The successful implementation of the retrosynthetic plan in Scheme 1 therefore depends on the preparation of a trans-fused photoadduct or its equivalent from **16**. Studies directed toward the construction of the requisite trans-fused photoadduct are currently in progress, and our results will be reported in due course.

Acknowledgment. We would like to thank the National Institutes of Health (CA40250), GlaxoSmithKline, Amgen, and Merck for their generous support of this program.

Supporting Information Available: Spectral data and experimental procedures for **8**–**11**, **14**–**18**, and **20**–**23** and X-ray data for **18**, **22**, and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL050103S

(6) Perez-Serrano, L.; Casarrubios, L.; Dominguez, G.; Perez-Castells, J. *Org. Lett.* **1999**, *1*, 1187–1188.

(7) (a) Shen, J.; Shi, Y.; Gao, Y.; Shi, Q.; Basolo, F. *J. Am. Chem. Soc.* **1988**, *110*, 2414–2418. (b) Shojaie, A.; Atwood, J. D. *Organometallics* **1985**, *4*, 187–190.

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
International Journal of Pharmaceutics 321 (2006) 27–34
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Crystal modification of dipyridamole using different solvents and crystallization conditions

R. Adhiyaman [a,1], Sanat Kumar Basu [b,*]

[a] *Department of Pharmaceutics, Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India*
[b] *Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India*

Received 21 March 2005; received in revised form 10 April 2006; accepted 17 April 2006
Available online 13 May 2006

Abstract

Dipyridamole crystals having different types of habits, improved dissolution rate were prepared by recrystallization from selected solvents, such as acetonitrile, benzene and methanol (Method I); crystals have also been made by solvent change using methanolic solution of dipyridamole in the presence of 2% solutions of Tween-80, Povidone K_{30} and polyethylene glycol (PEG) 4000 (Method II). Scanning electron microscopy, X-ray powder diffractometry, IR spectrometry and differential scanning calorimetry were used to investigate the physicochemical characteristics of the crystals. The comparative dissolution behavior of the newly developed crystals and that of the untreated dipyridamole were also studied. It was found that the newly developed crystals were different from each other with respect to physical properties but are chemically identical. The crystals, obtained (Method I) from benzene and acetonitrile, produced needle shaped crystals and that obtained from methanol produced rectangular shaped crystals. But the crystals obtained (Method II) with the methanolic solution of the drug in the presence of Tween-80, Povidone K_{30} and PEG-4000 produced smooth needle shaped crystals. X-ray diffraction spectra and differential scanning calorimetry study of the newly developed crystals, clearly indicate that dipyridamole exist in different crystal modification. The dissolution rate of newly developed crystals was found to be greater than the pure drug dipyridamole. Stability studies at 40 °C (75% RH) for 1 month for the modified crystals as well as the pure drug did show some changes in the XRD and DSC but not in IR studies.

Keywords: Dipyridamole; Recrystallization; Physicochemical characterization

1. Introduction

Different physiological and formulation factors are responsible for the bioavailability of drug from the dosage form. One of the most important physical factors, which affect the bioavailability and therapeutic efficacy of drug, is the existence of active ingredients in various crystal forms having different internal structure and physical properties (Kapoor et al., 1998). The different crystal form of a drug have different physicochemical characteristics, namely crystal shape, crystal size, melting point, density, flow properties solubility pattern, dissolution characteristics and XRD pattern, though they are chemically identical. A physical form having improved dissolution rate and solubility is useful for improving the bioavailability of a drug (Burt and Mitchell, 1980; Watanable et al., 1982). The crystal habit is an important variable in pharmaceutical manufacturing, where some factors, such as the polarity of crystallization solvent and the presence of impurities in the solvent, affect crystallization (Chow et al., 1985; Femi-Oyewo and Spring, 1994; Garekani et al., 2000). Among them, solvent strongly affects the habit of crystalline materials; however, the role-played by solvent interactions in enhancing or inhibiting crystal growth is still not completely understood (Lahra and Leiserowitz, 2001). The drug dipyridamole used herein is practically insoluble in water. Its main use in therapy as antiplatelet aggregating and peripheral vasodilating effect is well known. But the water insolubility and the poor bioavailability are the limitations of its effective use clinically. Keeping this in view, crystal modification of dipyridamole has been undertaken to improve dissolution and bioavailability. Dipyridamole is a derivative of 1,3,5,7-tetra azanaphthalene and used mainly for cardiovascular diseases for the above-mentioned purposes. It has been recrystallized

* Corresponding author.
E-mail addresses: eskebee@yahoo.com (R. Adhiyaman), basusanat_kumar@hotmail.com (S.K. Basu).
[1] Present address: J.S.S. College of Pharmacy, The Rocklands, Ootacamund-643001, Tamil Nadu, India.

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.04.021

from selected solvents and solvent system. The newly developed dipyridamole crystals were characterized by some physicochemical approaches.

2. Materials and methods

2.1. Materials

Dipyridamole was obtained as generous gift from German Remedies (Mumbai, India). The solvents used for the present work were acetone, benzene, methanol, obtained from Ranbaxy Chemical Laboratories (S.A.S. Nagar, India) and Tween-80, Povidone K_{30} and polyethylene glycol (PEG) 4000 were obtained from SDS Chemical Limited (Boisar, India).

2.2. Preparation of dipyridamole crystals

Two different methods used in this study to observe the effect of solvents on the development of crystal habits in the changed environment are given below.

2.2.1. Method I

One gram of dipyridamole was dissolved separately in 50 ml of selected solvents in a conical flask. The solution was heated at the boiling point of the respective solvents and filtered, concentrated and the solution was left at room temperature (28–30 °C) until the solvent was completely evaporated. The crystals were further dried under vacuum at room temperature and stored in appropriate airtight container for further use.

2.2.2. Method II

One gram of dipyridamole was dissolved in 40 ml of methanol in a conical flask and the solution was heated and filtered. The resultant solution was concentrated at 60 °C and then cooled down at room temperature (28–30 °C). The clear solution, thus obtained, was rapidly added to equal volume of cold water (5 °C) containing 2% solution of Tween-80, PVP K_{30} and PEG-4000, separately under agitation by means of a glass rod and then left for 1 h at 10–15 °C. The crystals were then recovered by filtration under vacuum using a sintered glass funnel. They were then kept in airtight container for further use.

2.3. Stability studies

One month's accelerated stability test was carried out for each sample after preparation, when the crystals were kept in humidity chambers (75% RH) and at a temperature 40 °C and the physicochemical changes of the crystals as observed are compared with that of the drug dipyridamole under identical conditions. The results are summarized in Figs. 9 (XRD) and 10 (DSC), respectively.

2.4. Scanning electron microscopy

Electron micrograph of crystals was obtained using a scanning electron microscope (JEOL JSM—5200) operating between 5 and 24 kV. The specimens were mounted on a metal stub (with double side adhesive tape) and coated under vacuum with gold in an argon atmosphere prior to observation.

2.5. X-ray powder diffraction

The cavity of the metal sample holder of X-ray diffractometer was filled with ground sample powder and then smoothed out with a spatula. X-ray diffraction pattern of dipyridamole crystals were obtained using the X-ray diffractometer (Rich Seifert Model 3000P) at 30 kV, 30 mA over a range of 10–100 2θ, using Cu Kα radiation wavelength 1.5405 Å.

2.6. Infrared spectroscopy

The spectra were recorded on an IR spectrophotometer (PERKIN-ELMER USA MODEL—248), after respective samples were mixed with dried KBr powder and compressed to a 12 mm disc by a hydraulic press at 10 tonnes compression for 30 s.

2.7. Thermal analysis

Differential scanning calorimetry (DSC) of the samples, 10 mg, was carried out using a thermal analysis system (METTLER TA 4000 System). Calibration with standard was undertaken prior to subjecting the samples, which were heated at 10 °C/min in an aluminum pan under a nitrogen atmosphere and a similar empty pan was used as the reference. The instrument automatically calculated onsets of melting points and enthalpy of fusion.

2.8. Dissolution studies

Dipyridamole and its crystals, 25 mg in each case were accurately weighed and dissolution profile of the drug was determined in a USP Type II Dissolution test apparatus at 37 °C, with basket (100 mesh) with a stirring speed of 50 rpm. The dissolution medium was 600 ml of phosphate buffer pH 4.0, I.P. (Indian Pharmacopoeia). Samples were withdrawn from the dissolution vessels at selected time intervals and analyzed for dipyridamole content at 285 nm on a UV spectrophotometer (BECKMAN-UM-64). The results are shown as the graphical plots in Figs. 7 and 8, respectively.

3. Results and discussion

3.1. Morphology of crystals

Fig. 1 shows the scanning electron micrographs (SEM) of untreated and recrystallized dipyridamole from different solvents under solvent evaporation method (Method I). It is clear from the figure that the untreated dipyridamole is having small irregular needle shaped crystals (Fig. 1d), whereas the crystals obtained from acetonitrile is needle shaped (Fig. 1c) and that from benzene is rod shaped (Fig. 1b). Recrystallization of dipyridamole from methanol solution with the same method produced

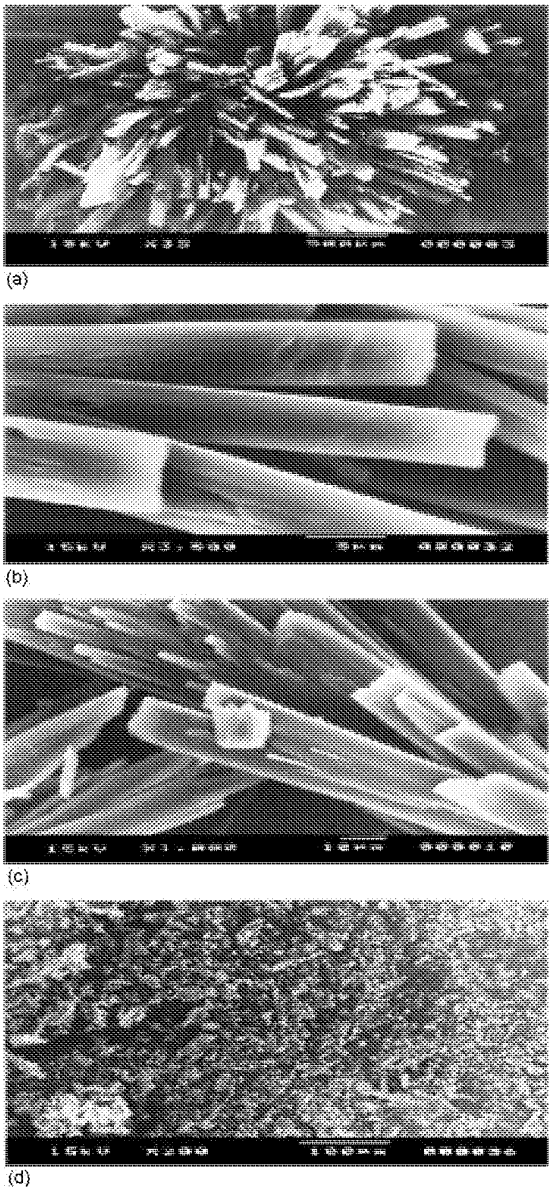


Fig. 1. Scanning electron micrographs of dipyridamole recrystallized from (a) methanol, (b) benzene, (c) acetonitrile and (d) untreated dipyridamole.

rectangular needle shaped crystals (Fig. 1a), while using solvent change method (Method II), the shape of crystals changes to fine needles (Fig. 2a–c). The results also showed that the size of crystals produced from Methods I and II are somewhat different from the size of untreated dipyridamole and follows the order, i.e. Method I > Method II (compare the magnification of the SEM in Figs. 1 and 2). Therefore, it can be concluded that cooling rate decreases the crystal size due to incomplete growth of large number of small crystals (Garekani et al., 1999).

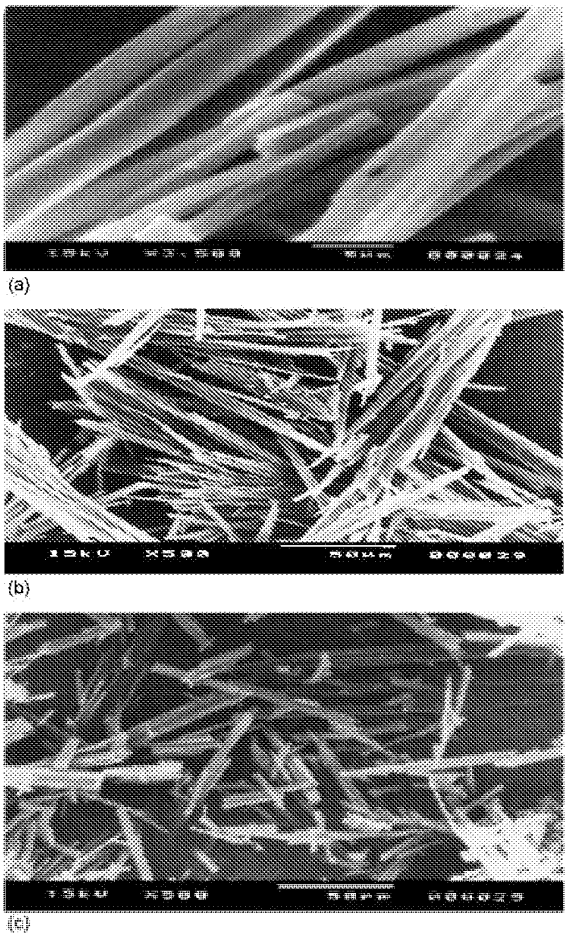


Fig. 2. Scanning electron micrographs of dipyridamole recrystallized from methanol with 2% solutions of (a) Tween-80 (SCT); (b) PEG-4000 (SCPEG); (c) $PVPK_{30}$ (SCPVP).

3.2. X-ray diffraction

To obtain information on the physicochemical characteristics of the prepared crystals, X-ray powder diffraction measurements were conducted.

XRD spectra for all crystals are presented in Figs. 3 and 4. In the powder diffractogram sharp peak at diffraction angle (2θ) 30.04, 20.74, 20.81, 12.33, 17.45, 10.25, and 20.93 were obtained in case of drug dipyridamole and the modified crystals obtained from methanol, benzene, acetonitrile, Tween-80, PEG-400, PVP K_{30}, respectively. The presences of these sharp peaks are clearly evident in the comparative diffractogram presented in Figs. 3 and 4 and the data recorded therein. From the data recorded, it is clearly evident that there is significant difference in the entire diffraction pattern or d-spacing values between treated and untreated dipyridamole samples. The intensity of the peak in methanol is the highest than that of all other modified crystals reported herein. This is probably due to higher crystal

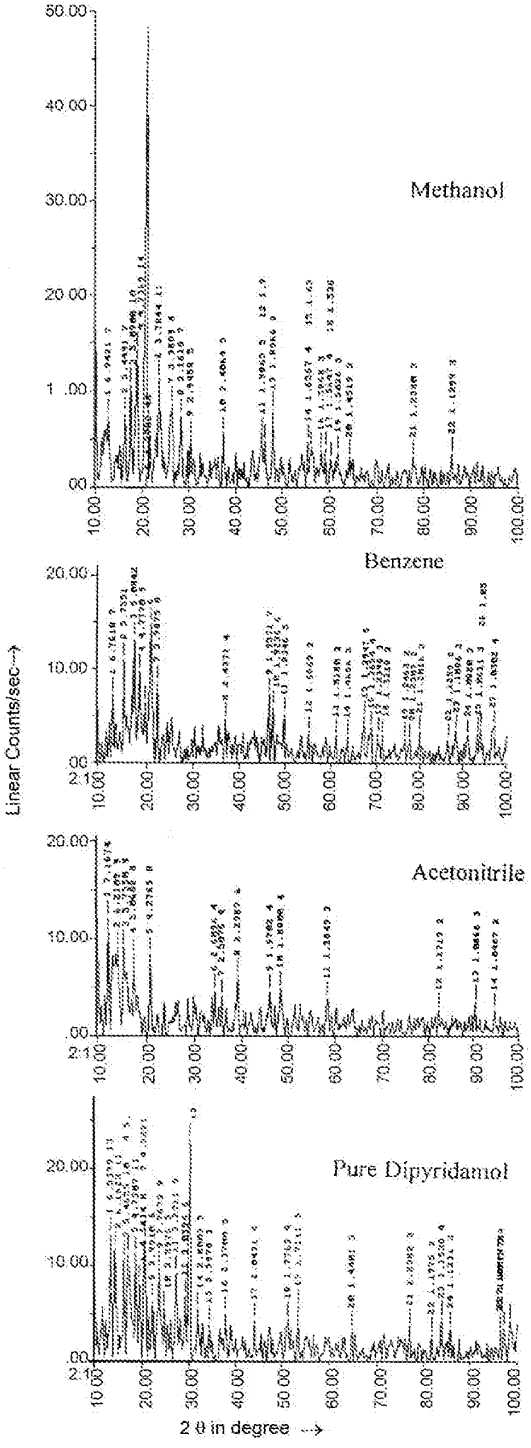


Fig. 3. X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from methanol; benzene; acetonitrile.

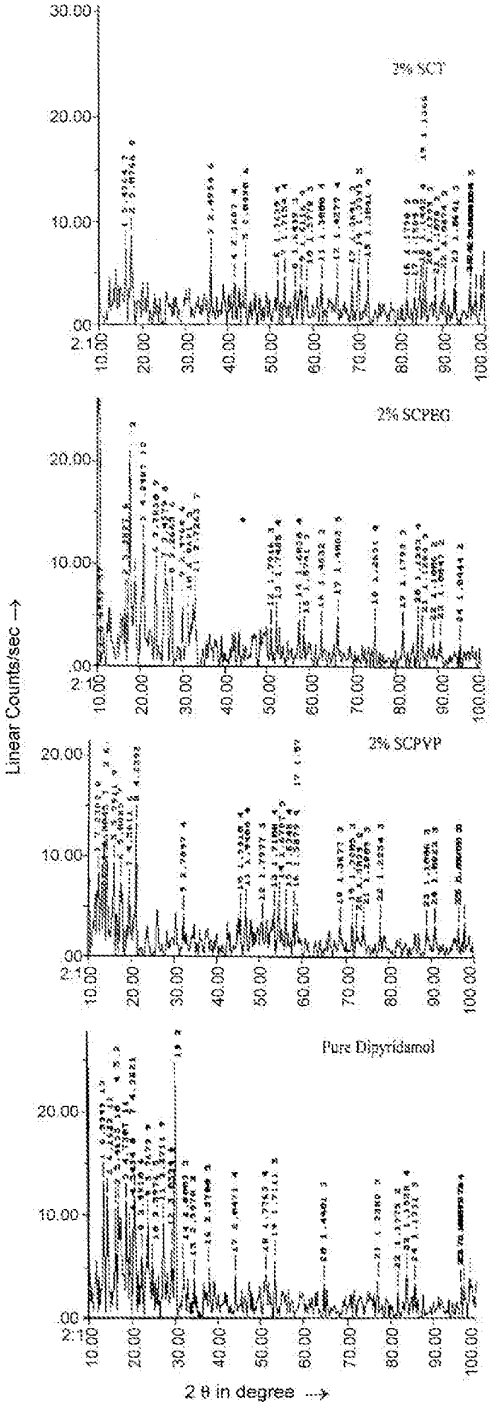


Fig. 4. X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from methanol with 2% solutions of Tween-80 (SCT); PEG-4000 (SCPEG); PVP K_{30} (SCPVP).

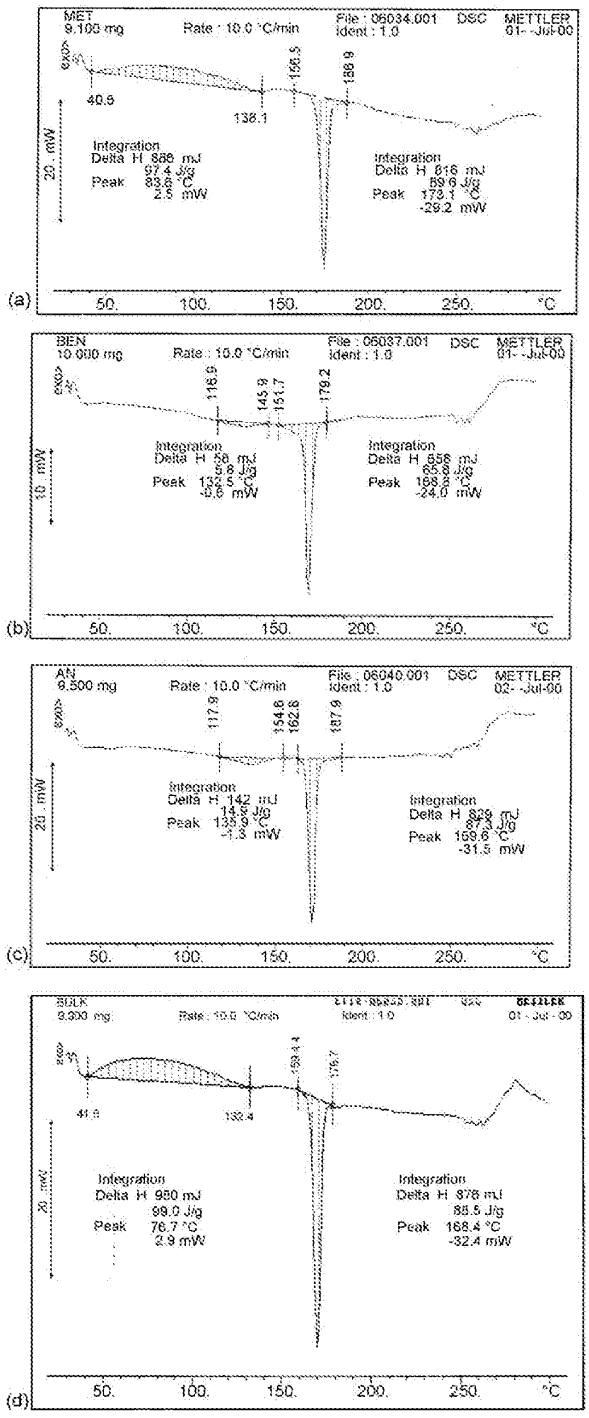


Fig. 5. Differential scanning calorimetric thermographs of dipyridamole recrystalized from (a) methanol; (b) benzene; (c) acetonitrile; (d) untreated dipyridamole.

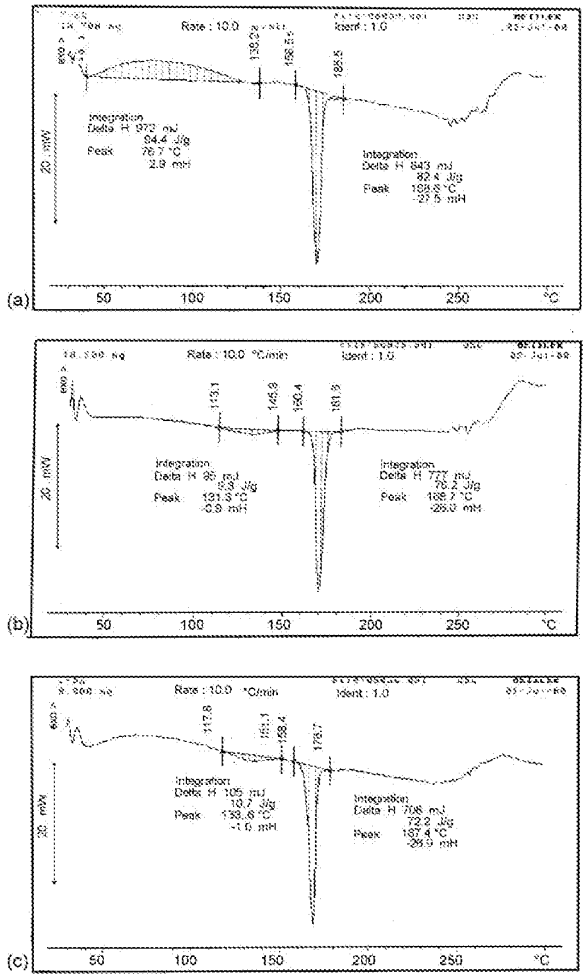


Fig. 6. Differential Scanning Calorimetric thermographs of dipyridamole recrystalized from methanol with 2% solution of (a) Tween-80(SCT); (b) PEG-4000 (SCPEG); (c) PVP K_{30} (SCPVP).

perfection in this condition of crystallization (Nokhodchi et al., 2003).

3.3. Infrared spectroscopy

The spectra of all modified crystals were identical and the main absorption bands of dipyridamole appeared in all of the spectra. This indicates that there were no difference between the internal structure and conformations of these samples, because these were not associated with changes at molecular level.

3.4. Thermal analysis

The DSC data for drug dipyridamole (untreated) and the modified crystals are shown in Figs. 5 and 6. It should be noted that the DSC thermo grams (Figs. 5 and 6) of all modified crystals showed only slight variation. However, the modified crystal

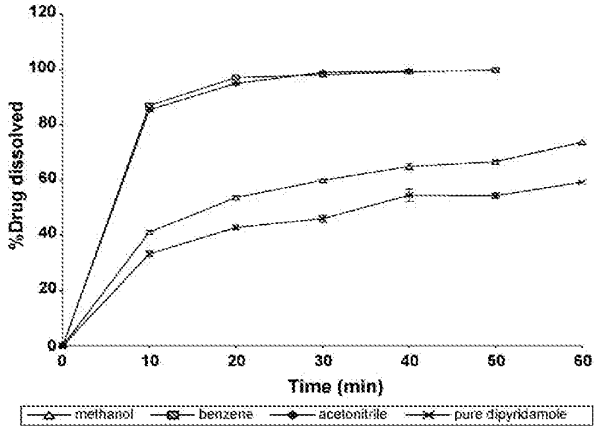


Fig. 7. Dissolution profile of pure Dipyridamole and modified crystals obtained using various solvents in phosphate buffer pH 4.0. (I.P). (a) Methanol; (b) benzene; (c) acetonitrile; (d) untreated dipyridamole.

obtained from methanol shows significant changes due to high crystal perfection.

The DSC curve of crystals from SCT (2%, v/v) and methanol shows broad exothermic peaks and very slight but insignificant variation in transition temperature and a little difference (not significant) in enthalpy of fusion. This may be due to oxidation or phase transformation. Crystals obtained by using acetonitrile, benzene, SCPVP (2%, w/v) and SCPEG (2%, w/v) show a weak endothermic peak and there is no significant variation in transition temperature, but significant difference in enthalpy of fusion is observed in case of acetonitrile, SCPEG (2%, w/v) and SCPVP (2%, w/v) while compared with the thermo gram obtained in case of benzene. The appearance of weak endothermic peaks in this case may be due to solvation of the crystals (Gordon and Chow, 1992).

Results from IR spectroscopy, X-ray diffraction analysis and DSC taken together led to the conclusion that only habit modifications were observed during recrystallization of dipyridamole under various conditions of the crystallization.

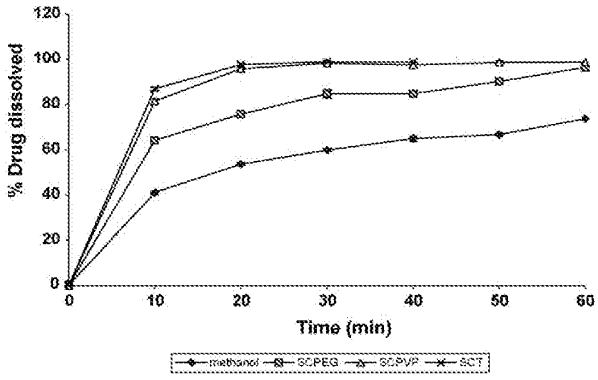


Fig. 8. Dissolution profile of modified crystals of dipyridamole from methanol and also from methanol with 2% solutions of PEG-4000, PVP K_{30}, and Tween-80 in phosphate buffer pH 4.0. (I.P). (a) Methanol; (b) Tween-80 (SCT); (c) PEG-4000 (SCPEG); (d) PVP K_{30} (SCPVP).

3.5. Dissolution studies

The dissolution profile of dipyridamole and its modified crystals from different solvents are shown in Figs. 7 and 8, respectively.

Recrystallization of the parent drug from various solvents, given earlier (Method I), resulted in the increase of the dissolution rate of different modified crystals than dipyridamole. Especially, crystals obtained from benzene and acetonitrile, show higher dissolution rate than untreated dipyridamole because of the better crystallinity of the modified crystals in these cases. Crystals obtained using only methanol show lower dissolution rate than other crystals obtained (Method II). However, it is evident that after the addition of Tween-80 and other polymer solution, the dissolution rates were increased. This may be due to

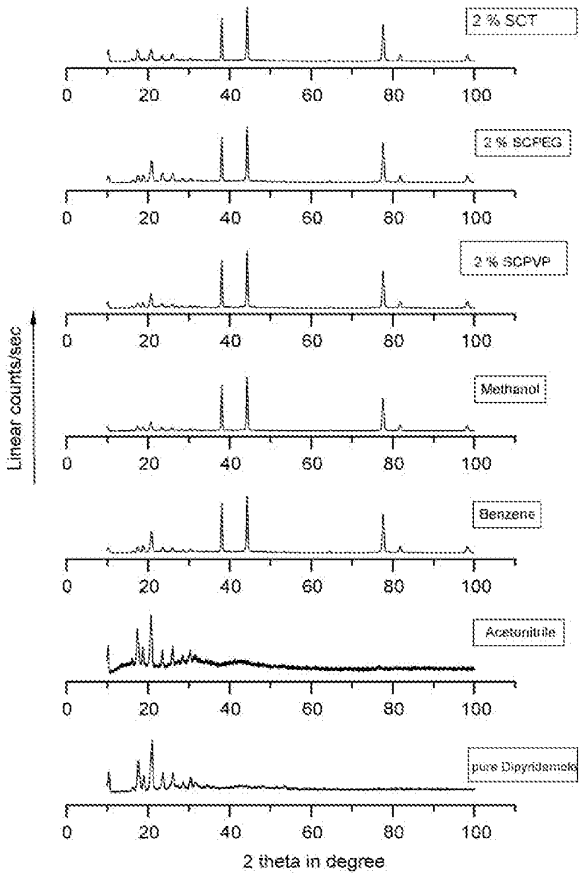


Fig. 9. Comparative X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from acetonitrile; benzene; methanol and dipyridamole recrystallized from methanol with 2% solutions of PEG-4000 (SCPEG), $PVPK_{30}$ (SCPVP), Tween-80 (SCT) and kept at elevated temperature (40 °C) and 75% RH for one month.

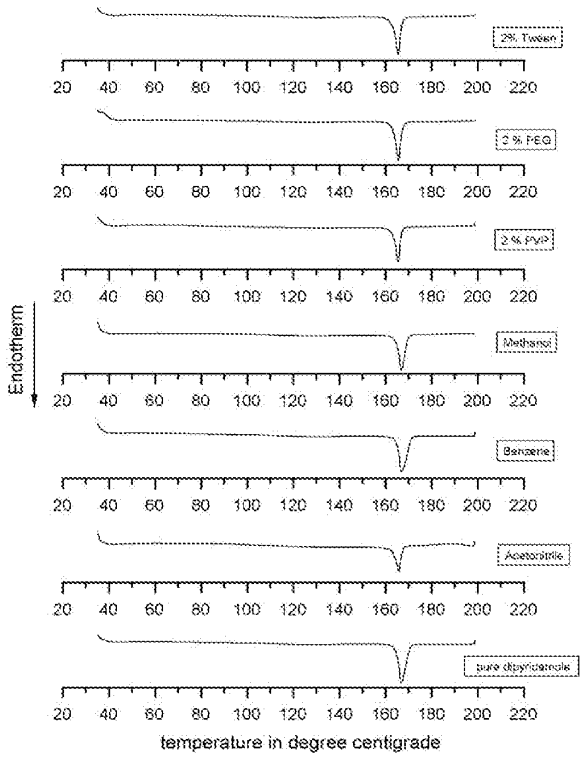


Fig. 10. Comparative Differential scanning calorimetric thermographs of pure dipyridamole and dipyridamole recrystallized from acetonitrile; benzene; methanol and dipyridamole recrystallized from methanol with 2% solutions of PEG-4000 (SCPEG), $PVPK_{30}$ (SCPVP), Tween-80 (SCT) and kept under elevated temperature (40 °C) and 75% RH for one month.

the adsorption of surfactant and polymers on the crystal surface (Majumdar et al., 1992).

3.6. Stability studies

The results obtained in the stability test showed slight changes in XRD, DSC data for all samples under investigation. XRD spectra for all the crystals kept at the elevated temperature (40 °C) are presented in Fig. 9. In the powder diffractogram of dipyridamole and the modified crystals, sharp peak at diffraction angle (2θ), 20.98, 20.79 and 44.25, respectively were obtained incase of drug dipyridamole and crystals from acetonitrile and benzene. But in the case of crystals obtained from methanol (Method I) and other crystals obtained (Method II), all of them showed the sharp peak at diffraction angle (2θ), 44.5. These clearly indicate that under the circumstance all retain the same state. However, there is significant difference in the d-spacing values between the freshly prepared crystals and the crystals obtained after storing at elevated temperature including pure drug. This is probably due to the existence of different crystal habits in the crystalline materials at elevated temperature. The DSC data for drug dipyridamole (untreated) and the modified crystals kept at elevated temperature are shown in Fig. 10. It is clearly evident from the DSC thermo grams for all the samples including pure dipyridamole under investigation that the modified crystals (Methods I and II) showed slight change in the value of enthalpy and the heat of fusion. However, the DSC curve of crystals from SCT (2%, v/v) and PEG (2%, w/v), very weak exothermic peaks were seen in a position significantly different from the samples, studied under ambient conditions, leading to significant variation in transition temperature and in enthalpy of fusion. This may probably be due to oxidation or phase transformation under such stress condition. But it is very much interesting to note that none of the samples studied under such stress condition did show any change in the IR spectrum confirming the presence of its chemical identity.

4. Conclusion

In conclusion, it can be said that the crystallization conditions and the medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves. This suggests that the newly developed crystals of dipyridamole under ambient conditions exist in different crystalline modification facilitating significantly improved dissolution rate as compared to dipyridamole. There are enough references (Dalton et al., 2001; el-Yazigi and Sawchuk, 1985) available in the literature wherein it has been proved that in vitro dissolution data are good predictor of in vivo performance in reality. Therefore, it can be safely concluded that the improvement obtained in the present study in the modified crystals will give better bioavailability and better therapeutic activity clinically. But the stability study undertaken at 40 °C and a relative humidity of 75% shows some physical changes probably due to some phase transitions but retaining the chemical identity. The effect of such changes in reality needs to be explored in actual situations, if any.

Acknowledgements

The authors thank Indian Association for the Cultivation of Science, Kolkata, India; Bengal Engineering and Science University, Shibpur, Howrah, India; University Science and Instrumentation Centre, Jadavpur University, Kolkata, India for their help during instrumental analysis of samples.

References

Burt, H.M., Mitchell, A.G., 1980. Effect of habit modification on dissolution rate. Int. J. Pharm. 5, 239–251.

Chow, A.H.L., Chow, P.K.K., Wang, Z., Grant, D.G.W., 1985. Modification of acetaminophen crystals; influence of growth in aqueous solutions containing.p-aceto oxyacetanilibe on crystal properties. Int. J. Pharm. 23, 239–258.

Dalton, J.T., Straughn, A.B., Dickason, D.A., Grandolfi, G.P., 2001. Predictive ability of level A in vitro–in vivo correlation for ringcap controlled-release acetaminophen tablets. Pharm. Res. 18, 1729–1734.

el-Yazigi, A., Sawchuk, R.J., 1985. In vitro–in vivo correlation and dissolution studies with oral theophylline dosage forms. J. Pharm. Sci. 74, 161–164.

Femi-Oyewo, M.N., Spring, M.S., 1994. Studies on paracetamol crystals produced by growth in aqueous solutions. Int. J. Pharm. 112, 17–28.

Garekani, H.A., Ford, J.L., Rubinstein, M.H., Rajabi-Siah-boomi, A.P., 1999. Formation and compression characteristics of prismatic polyhedral crystal and thin plate like crystals of paracetamol. Int. J. Pharm. 187, 77–89.

Garekani, H.A., Ford, J.L., Rubinstein, M.H., Rajabi-Siah-boomi, A.P., 2000. Highly compressible paracetamol; compression properties. Int. J. Pharm. 208, 101–110.

Gordon, J.D., Chow, A.H.L., 1992. Modification of phenytoin crystals: influence of 3-propanolozymethyl-5,5-diphenly-hydantoin on solution-phase crystallization and related crystal properties. Int. J. Pharm. 79, 171–181.

Kapoor, A., Majumdar, D.K., Yadav, M.R., 1998. Crystal forms of nimesulide – a sulfonanilide (non-steriodal anti inflammatory drug). Indian J. Chem. 37B, 572–575.

Lahra, M., Leiserowitz, L., 2001. The effect of solvent on crystal growth and morphology. Chem. Eng. Sci. 56, 2245–2253.

Nokhodchi, A., Bolourtchian, W., Dinarvand, R., 2003. Crystal modification of phenytoin using different solvents and crystallization conditions 250, 85–97.

Watanable, A., Yamaoka, Y., Takada, K., 1982. Crystal habits and dissolution behavior of aspirin. Chem. Pharm. Bull. 30, 2958–2963.

Thermochimica Acta, 5 (1972) 173–220

PURITY DETERMINATION BY DIFFERENTIAL SCANNING CALORIMETRY

ERWIN E. MARTI

Central Research Services Department, Ciba–Geigy Ltd., Basel (Switzerland)

(Received April 3rd, 1972; revised June 23rd, 1972)

ABSTRACT

A review of the literature on the DSC method for purity determination is presented, with a discussion of the most important aspects, *i.e.* theory, sample handling, calibration of the instrument, evaluation of melting curves, and the conditions and accuracy of the measurement of eutectic impurities.

A number of mathematical descriptions of the solid–liquid equilibrium for eutectic binary systems is applied to the calculation of theoretical phase diagrams and specific heat functions, which are then compared with experimental phase diagrams and melting curves. The applicability of the DSC method to systems of solid solutions is discussed.

Both the experimental procedure and the evaluation by computer methods required to obtain accurate impurity determinations by DSC are presented. A number of practical examples is included.

INTRODUCTION

The measurement of the melting point of a substance as a method of identification dates back to the early days of chemistry. Many different observations on organic and inorganic substances were made during the thermal treatment necessary for a melting point determination.

The observations were summarized and interpreted in terms of phenomena like polymorphism, sublimation, thermal decomposition, solid solutions, eutectic systems, congruently-melting compounds, glass transitions and others. Kofler[1] turned the melting point determination by microscopic observation into an extremely useful method in the field of analytical chemistry. Kofler's treatise on purity determinations is excellent, but of course, today, it is not easy to agree with the statement in *Thermomikromethoden*: "The method of purity determination with the microscopical observation of the melting point, however, will finally replace all the others". Somehow, the development of the analytical methods for purity determination since 1950 has appeared to prove the opposite, namely that all the other analytical methods would replace the melting point determinations. Kofler's melting point method is nowadays performed with many different types of apparatus. The method is used

because it is the simplest analytical method for getting information about the purity and about the crystal form of the sample under investigation. The melting point method is based on the determination of the absolute temperature of the substance assuming an infinitely small amount of solid substance in the solid–liquid equilibrium. A reference standard of a high purity is required to make the temperature measurement only a relative one. This high purity standard is also used for the relation between the purity and the melting point difference given in Eqn. (1)

$$\Delta T = T_1 - T_s = x_0 \cdot k_r \tag{1}$$

where ΔT is the melting point difference in °K, T_1 is the melting point of the high purity standard in °K, T_s is the melting point of the sample in °K, x_0 is the mole fraction of the impurity, and k_r is the cryoscopic constant in °K.

The cryoscopic constant is defined as

$$k_r = \frac{RT_1^2}{\Delta H_{f,1}} \tag{2}$$

where R is the gas constant and $\Delta H_{f,1}$ is the molar heat of fusion of the high purity standard, and is experimentally determined by means of Eqn. (1) or with a measurement of the heat of fusion $\Delta H_{f,1}$ and the melting point of the reference standard.

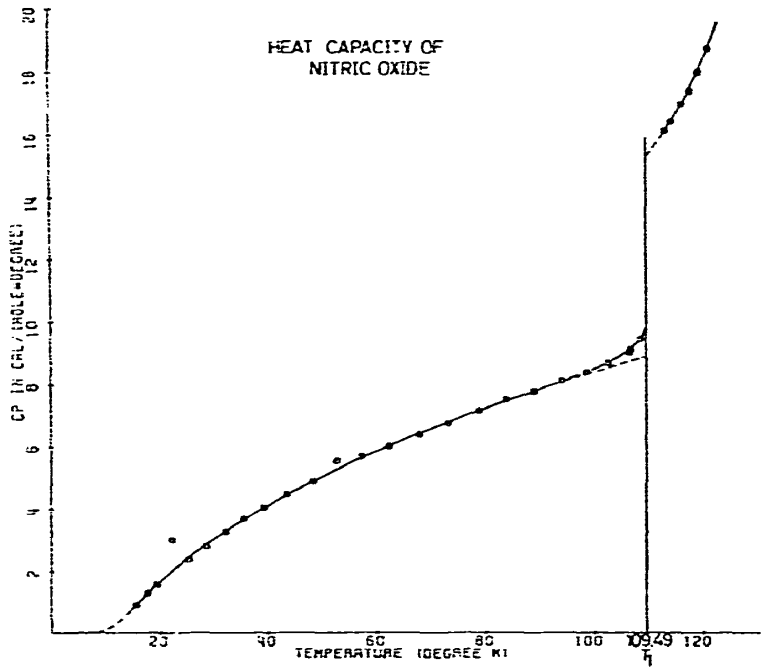


Fig. 1. Heat capacity of nitric oxide measured by Johnston and Giauque[3]. (Melting point T_1 = 109.49°K.)

Today, a second method seems to replace at least partially the microscopic observation of the melting point. This second method is known as differential scanning calorimetry (DSC). The DSC method measures the endothermic amount of energy which is afforded by the premelting process of substances. The method of premelting as a purity determination dates back to the 1920's in a form used by Eucken and Karwat[2] and Johnston and Giauque[3] for the measurement of the heat capacity of nitric oxide in the melting point region. In 1929, Johnston and Giauque[3] reported from the Chemical Laboratory of the University of California in Berkeley on the heat capacity of nitric oxide from 14°K to the boiling point.

The paper of Johnston and Giauque is interesting enough for a brief discussion. In Fig. 1, the heat capacity of nitric oxide is shown as a function of temperature, according to the measurements of Johnston and Giauque. The extremely sharp melting region of the nitric oxide sample at about 110°K should be noted. The nitric oxide used by Johnston and Giauque was produced by the reaction of potassium nitrite and potassium iodide in distilled water. The generated nitric oxide was purified over several distillation steps.

As an example, the same purified sample, containing $n_0 = 3.769$ moles of nitric oxide, was used for the premelting measurements and also the measurements of the heat capacity, the heat of fusion, and the melting point. Johnston and Giauque measured the following values for this sample of nitric oxide: molar heat of fusion, $\Delta H_{f,1} = 549.5 \pm 1.0$ cal.mole^{-1}; melting point, $T_1 \cong T_s = 109.49 \pm 0.05$°K.

The purity of the nitric oxide was calculated by applying Eqn. (3), which holds for low concentration of impurities

$$x_{0,2} = \frac{\Delta H_{f,1}}{RT_1^2}(T_1 - T)r \tag{3}$$

where $x_{0,2}$ is the eutectic impurity of the sample as mole fraction, $\Delta H_{f,1}$ is the molar heat of fusion of the pure nitric oxide, T_1 is the melting point of the pure nitric oxide, T is the temperature of the solid–liquid equilibrium, r is the molten fraction of the system at temperature T, and R is the gas constant.

The heat of premelting Δq_p, necessary for a temperature rise of the solid–liquid equilibrium from T' to T'', is related to the corresponding molten fractions of the sample r' and r''. We can write the equation

$$\Delta q_p = \Delta H_{f,1} n_0 (r'' - r') \tag{4}$$

The method of Johnston and Giauque enables the measurement of the total amount of heat Δq for a temperature rise of the substance from T' to T''. This total amount of heat is the sum of the heat of premelting Δq_p and an amount Δq_c given by the specific heat of the substance and the known temperature interval $\Delta T = T'' - T'$

$$\Delta q = \Delta q_p + \Delta q_c \tag{5}$$

The calculation of the heat of premelting (Δq_p) is possible from Eqn. (5), with the

measurement of the heat capacity of nitric oxide (Δq) and with an extrapolation of the specific heat from a region with practically no premelting into the selected region of premelting. The eutectic impurity of the nitric oxide is calculated for a corresponding set of temperatures and molten fractions (T', r'; T'', r'') and with the aid of Eqns. (3) and (4).

$$x_{0,2} = \frac{\Delta q_p}{n_0 R T_i^2} \cdot \frac{(T_1 - T'')(T_1 - T')}{T'' - T'} \tag{6}$$

With Eqn. (6) and the values of the measurements on nitric oxide, it is possible to calculate exactly the same values of eutectic impurities as found by Johnston and Giauque. The values and results are presented in Table I.

TABLE I
PREMELTING MEASUREMENTS ON NITRIC OXIDE

Temperatures (°K)		*Heat of premelting between T' and T'', q_p (cal)*	*Eutectic impurities $x_{0,2}$ (mole fraction)*
T'	T''		
104.71	108.59	0.171	7.9×10^{-6}
107.63	109.15	0.365	6.4×10^{-6}

Johnston and Giauque came to the conclusion that the nitric oxide used in their measurements contained less than 10^{-3} mole percent of eutectic impurities, or, the so-called purity is of the order of 99.999%. The authors excluded the possibility of noneutectic impurities because of the method of preparation of the nitric oxide used for these investigations. Johnston and Giauque explained that no analyses of the purified gas were made since accurate melting point and heat capacity data provide a more sensitive test of impurity than that given by chemical analysis. Johnston and Giauque made an equivalent statement to Kofler's about the measurement of impurities by the melting point method. It seems to be clear that such excellent investigators as Giauque and Kofler did not emphasize the melting point and premelting method in such a way without being deeply impressed by the possibilities of these two methods.

If we want to compare the excellent work from the low temperature laboratory at the University of California in Berkeley (the laboratory was named Giauque Hall in 1967) with the premelting measurements, mainly DSC and DTA, performed in the 1970's, we have to consider several points. The difference between the calorimetric method of Johnston and Giauque and the DSC or DTA method is not in thermodynamics but rather in the instrumentation and in the properties of the methods of measurement.

In Table II we compare some of the aspects of the two methods, selecting the DSC-IB of the Perkin–Elmer Corporation for the second group.

TABLE II

COMPARISON OF THE PREMELTING METHOD OF JOHNSTON AND GIAUQUE AND THE PURITY DETERMINATION WITH THE DSC-1B

Condition or property measured	*Calorimetric method of Johnston and Giauque*[3]	*DSC-1B (Perkin–Elmer Corp.)*
Weight of the sample	100 g	3 mg
Accuracy of the absolute temperatures	$\pm 10^{-2}$ °K	$\pm 3 \times 10^{-1}$ °K
Accuracy of the relative temperatures	$\pm 2 \times 10^{-3}$ °K	$\pm 10^{-2}$ °K
Accuracy of the measured heat of fusion	$\pm 2 \times 10^{-1}$ %	±5%
Accuracy in the purity value for high-purity substances	$\pm 10^{-4}$ %	$\pm 5 \times 10^{-2}$ %
Time for a premelting measurement	2–4 days	20 min

The great disadvantage of the calorimetric method developed by Johnston and Giauque, especially in industrial use, is the extremely long running time required for one measurement which is of course due to the enormous sample weights and the necessity for an equilibrium between the liquid and solid phases of the sample at all temperature points[4]. It is also clear, however, that somehow one has to pay for such a high accuracy in purity measurements. Between the measurements on purity with thermoanalytical methods of the 1920's and the 1970's, a great number of papers were published on purity measurements by the freezing point method. We mention only one paper, which we regard as representative of all the papers on thermoanalytical purity measurements produced during this period: Determination of Purity by Measurement of Freezing Points, by Glasgow, Krouskop, Beadle, Axilrod and Rossini[5].

Following these preliminary and historical remarks, we will concentrate on purity work performed with the DSC-IB, an instrument of the Perkin–Elmer Corporation. The development of new DSC- and DTA-systems will certainly change the issue of the purity determination, *e.g.* enhance the accuracy of the measurement of eutectic impurities and solid solutions without increasing the running time for one measurement.

DISCUSSION ON THE DSC LITERATURE ON PURITY

In this discussion we shall not attempt a complete report of the DSC literature. We will arrange our discussion according to theoretical and experimental points of the DSC-purity method.

(*a*) *Theory of the purity measurements*

As far as we know, all DSC results on purity in the literature are calculated

according to the following equation

$$T = T_1 - \frac{x_{0,2} RT_1^2}{\Delta H_{f,1}} \cdot \frac{1}{r} \tag{7}$$

[for symbols see Eqn. (3)].

Eqn. (7) is derived under the following approximations and conditions: (*i*) The components form a eutectic phase diagram; (*ii*) the system is at constant pressure; (*iii*) the impurity or impurities form ideal solutions with the molten part of the main component; (*iv*) the impurity is restricted to low concentrations; and (*v*) the heat of fusion is independent of temperature.

A second equation discussed by Driscoll and coworkers[6] describes systems containing eutectic impurities and impurities forming solid solutions with the main component. The systems of solid solutions are characterized according to Driscoll by a partition coefficient, this being the ratio of the concentrations of the impurity between the solid and liquid phases.

$$K = \frac{k'}{k} \tag{8}$$

leading with Eqn. (7) to the relationship

$$T = T_1 - \frac{x_{0,2} \cdot RT_1^2}{\Delta H_{f,1}} \frac{1}{\frac{K}{1-K} + r} \tag{9}$$

The discussion of systems with eutectic impurities and impurities forming solid solutions is rather inconsistent.

With regard to this relationship, Driscoll *et al.* state: "Systems which form true solid solutions, however, cannot be handled by this method of analysis". Joy and coworkers[7] declare in their abstract: "Because the DSC technique is "blind" to equilibrium solid solution formation, DSC values should not be used as a sole criterion of purity". Mastrangelo and Dornte[8] reported on a mixture of 2,2-dimethylbutane and 2,3-dimethylbutane. These two substances are known to form solid solutions. Mastrangelo and Dornte find a reasonable agreement between the theoretical temperature relation of Eqn. (9) and the experimental findings.

We have found no complete experimental proof of Eqn. (9) in the literature. Such a proof would require the independent determination of the parameters and thermodynamic constants such as temperature T, mole fractions of the main component and the impurities, molten fraction r, the partition coefficient K, the melting point T_1, and the heat of fusion of the main component $\Delta H_{f,1}$. Investigations of this kind should result in an assessment of the equilibrium with respect to temperature and concentrations.

Reconsidering Eqn. (7), we find the limitation of this equation discussed by several authors with respect to the allowable concentration of impurities. The limit is not properly defined because the definition would require the introduction of an absolute deviation between the theoretical amount of eutectic impurities and the sum of eutectic impurities as determined by DSC. With the lack of such a definition it is not surprising that the limitation of Eqn. (7) is estimated with considerable differences: Davis and Porter[10] assumed a limitation of Eqn. (7), with respect to the concentration of eutectic impurities, of 5%; De Angelis and Papariello[11] assumed a limitation of 1%; and Joy *et al.*[7], one of 2%.

These limitations on the amount of eutectic impurities for the premelting method can be overcome by a method suggested by De Angelis and Papariello[11]. Samples of high impurity concentration (>1%) are diluted with the pure main component to extend the limit of the applicability of the DSC method. Such a dilution method was applied by De Angelis and Papariello to 4 different organic systems with actual purities of 95.5–97.0 mole-%. The DSC purity values of these samples without dilution gave results in a narrow range from 97.4 to 97.8 mole-%. The absolute differences between the true and the experimental purity values were, therefore, of the order of 1–2 mole-%. DSC results with such high inaccuracies are not sufficient for analytical purposes. The experiments of De Angelis and Papariello performed with the same compounds, but with a dilution of the main impurities with the corresponding main component to a purity level above 99 mole-%, resulted in excellent agreement between DSC values and the actual purity. Schumacher and Felder[12] present similar results in DSC purity values determined directly and after dilution with a substituted benztriazole as the main component.

The differences between the actual purity and the experimental values determined without dilution are explained by the authors of both papers[11,12] in terms of an inconsistency between Eqn. (7) and the actual melting behaviour of organic substances in a purity region below 99 mole-%. We found that such an explanation of the differences of theoretical and experimental purities appears to be, however, only one of several possibilities. Another possible explanation for the differences is that the DSC method without dilution, used by Papariello and Schumacher, is only applicable to substances with a purity of at least 99 mole-%. In contrary to the findings of Papariello and Schumacher, we observed for many substances that the method without dilution gave correct values for impurities in the case of samples with substantially higher concentrations of impurities. We found that highly accurate purity values can only be achieved by selecting a scan speed appropriate to both the impurity concentration and the evaluation procedure. Thus, using the simplest Perkin–Elmer type of evaluation[13], a scan speed of 0.625 °C min^{-1} will yield valid results only in the purity range above 98 mole-%. The accuracy of a melting curve evaluation is improved by a data collection and evaluation at more than the 5–7 points within the important melting region, as suggested by Perkin–Elmer[13]. The more sophisticated the data collection and evaluation, the less important are the experimental conditions—scan speed, weight of sample and sensitivity—for getting a purity value of a high accuracy.

If one observes differences between the experimental and the actual purity values one has to check the experimental conditions, including the type of sample pan used, the data collection, the evaluation procedure of the melting curve, and the melting behaviour of the substance. If after all these investigations the differences in the experimental and the actual purity persist, an inconsistency between Eqn. (7) and the melting behaviour of this specific system is highly probable.

The dilution method introduced by Papariello[11] is excellent for the solution of special problems. Its pratical use in an analytical laboratory is, however, limited by the amount of work involved. Therefore, the question of the limitation of the DSC method to a region of high purity substances (*e.g.* to a purity better than 98 mole-%) has to be reexamined because such a strong limitation would diminish the value of the whole method. Such an investigation of the purity region, in which the DSC method is a useful analytical tool, should be performed with binary systems. It would be very helpful if the phase diagrams of the selected binary systems were known from literature. With such a binary system, all kinds of possible parameters and conditions have to be varied; the ratio of the two compounds, the sample weight, the sample pan, the scan speed, the sensitivity, the first, second and following melting curves of the same sample if possible, and the data collection and evaluation. The results thus obtained may be discussed with respect to discrepancies between theoretical and experimental values of the purity, the heat of fusion, and the melting points. They can, moreover, reveal properties of the two components such as thermal stability, high vapor pressure in the melting region for one or both of the compounds, polymorphism, and anomalous behaviours demonstrated by the phase diagram and by the melting curve. Having completed these investigations on some binary systems one could perform a similar program on multi-component systems. All these results should give us information on the limitation of Eqn. (7).

(*b*) *Handling of the samples*

Gray[13] suggested the use of the volatile sample pan with an inside cover. This inside cover is made from aluminum to fit into the bottom part of the volatile pan. Driscoll *et al.*[6], Barral and Diller[14], Reubke and Mollica[15], and others regard the volatile sample pan with an inside cover as the best solution to avoid volatilization. The sample handling and the variation of the temperature treatment are most important for substances with polymorphism, in the presence of impurities with a high vapor pressure in the melting region of the main component, and with substances which are unstable in the melting region.

Difficulties also arise with the sample holders of the DSC-IB. The sample pans, the aluminum dome lids and the outside cover of the sample holders have to be carefully placed in the correct positions[14,16].

Barrall and Diller[14] make a good point on the preparation of samples whereby great care has to be taken in selecting test samples or in mixing of low concentration standards, because the sample size in DSC measurements has to be in the region of a few milligrams. For quantitative work with the DSC-IB, the sample size should be

between 1 and 5 mg. Results with a high reproducibility are only possible with special care in the handling procedure.

(*c*) *Calibration of the DSC apparatus*

The calibration of the temperature axis of the DSC with high purity standards should be performed in the way indicated by Barrall and Diller[14]. The calibration of the sensitivity of the DSC in calories per unit area presents no problems. Important for high purity measurements is the careful calibration of the thermal resistance between the sample pan holder and the sample pan with standards like indium, tin and lead; this is also shown in the interesting investigations performed by Barrall and Diller[14].

The question arises whether or not one is allowed to use inorganic materials as standards for the measurement of the thermal resistance, which can then be used in the purity determination of organic substances. However, the DSC-IB is nearly independent of the thermal resistance of the sample, as long as the sample consists of crystals of a rather small size[17].

(*d*) *Instrumental conditions for a purity determination*

The instrumental conditions for a purity determination are sensitivity or the calorimetric range, the scan speed, and the sample pan. There are mutual relationships between these experimental conditions and some of the properties of the instrumentation and the sample. As an exemple, the appropriate calorimetric range used in a purity determination depends on several conditions, *i.e.* heat of fusion of the main component, sample size, scan speed, concentration of impurities, and recording system or data collection.

The scan speed, as indicated in the literature[6,7,14], is in general kept at the lower end of the range, *i.e.* 0.625 or 1.25°C/min. Such low values of the scan speed are

TABLE III

EFFECT OF SAMPLE SIZE AND HEATING RATE ON CALCULATED PURITY (BARRALL AND DILLER[14])

Mixture	*Sample size (mg)*	*Heating rate (°C/min)*	*Purity (mole-%)*	
			Found[a]	*Known*[b]
Lead in tin	3.084	1.25	0.425	0.419
Lead in tin	3.084	5.0	0.185	0.419
Lead in tin	3.084	20.0	0.0828	0.419
Lead in tin	4.300	1.25	0.321	0.419
Lead in tin	6.284	5.0	0.857	1.16
Lead in tin	6.284	1.25	0.871	1.16
Lead in tin	6.284	0.625	0.899	1.16

[a]Calculated with partial areas considered to the vertex of the endotherm. [b]Determined by atomic absorption of lead with a Perkin–Elmer Model 303 spectrophotometer, using nitrous oxide as oxidizing agent to dissociate the tin compounds.

required for high purity measurements. Low values of the scan speed are necessary as the sample is probably not at thermal equilibrium during rapid rates of heating, according to Barrall and Diller[14]. The effects of sample size and heating rate on the measured purity in mixtures of lead in tin[14] are presented in Table III.

Three parameters are varied in Table III; sample size, scan speed, and purity level. The mixture with the lower concentration of lead seems to be strongly sensitive to changes of the heating rate with respect to the concentrations of lead calculated from melting curves. Conclusions from Table III are only typical for the applied conditions, such as the data collection and evaluation procedure. Generalizations are only possible after performing the investigations mentioned in part (*a*).

(*e*) *Evaluation of the melting curves*

The calibration of the instrument, the handling of the samples, and the determination of the correct instrumental conditions for obtaining a melting curve which may be easily handled by an evaluation procedure, are all possible with some care in the experimental work. However, understanding and performing the purity calculations from melting curves is rather complicated. Therefore, the literature about this subject is quite extensive. No review of evaluation methods is available in the literature.

A given procedure for the evaluation of a melting curve can be checked in several different ways; there are a great many internal and external checks possible. We will discuss here the external checks which are performed with the values resulting from a normal evaluation of a melting curve; *i.e.* (*i*) the melting point of the sample, (*ii*) the melting point of the pure main component, (*iii*) the heat of fusion of the pure main component, and (*iv*) the purity value of the sample. The melting point and the heat of fusion of the sample calculated by the evaluation procedure may be compared with the values measured directly on the melting curve by applying the calibration factors. The melting point and the heat of fusion of the pure main component can probably be found in the literature. Such literature values permit a comparison with the results from the evaluation of melting curves.

For test substances, the measured DSC purity value may be compared with the actual purity value known from mixing. A second method is to compare the DSC purity value with the purity information obtained from a separate analytical procedure. In the case of disagreement between the DSC purity value and the actual purity value, several points must be considered with regard to the DSC method; *i.e.* the instrumental conditions used in getting the melting curve; the physical and chemical behaviour of the main component and the impurities; and the evaluation procedure, and the use of the thermodynamic relationship for the description of the solid–liquid equilibrium.

All the considerations given in this section, which are necessary in case of discrepancies between the values evaluated from melting curves (*i.e.* purity, melting points, heat of fusion) and values found in the literature, receive practically no mention in the published work on DSC-purity determination.

The evaluation of melting curves by hand, suggested by Perkin–Elmer[13], is practicable but too cumbersome for routine work. Computer programs used in the evaluation give higher accuracies in purity and thermodynamic values, and are much faster. Programs were developed by Driscoll *et al.*[6], Scott and Gray[18], Barrall and Diller[14], Davis and Porter[10], Heuvel and Lind[19], Gent[20], and others.

The basic problems of the evaluation of melting curves by computer or by hand are the same. Referring to Eqn. (7), one has to fit the experimental DSC-curve to a straight line in the $(1/r, T)$-diagram, as it was first shown by Pitzer and Scott[21].

The evaluation procedures cited above consist of: (*1*) the fit of the experimental points from a melting curve to a given thermodynamic function, together with the determination of the true heat of fusion of the main component[6,10]; (*2*) the linearization with an appropriate mathematical method[6,18]; and (*3*) the calculation of the purity value and the thermodynamic constants of the sample and of the corresponding main component. It is not always possible to separate a given evaluation procedure into these three parts. However, the literature of the evaluation procedures is more easily discussed by such a partition.

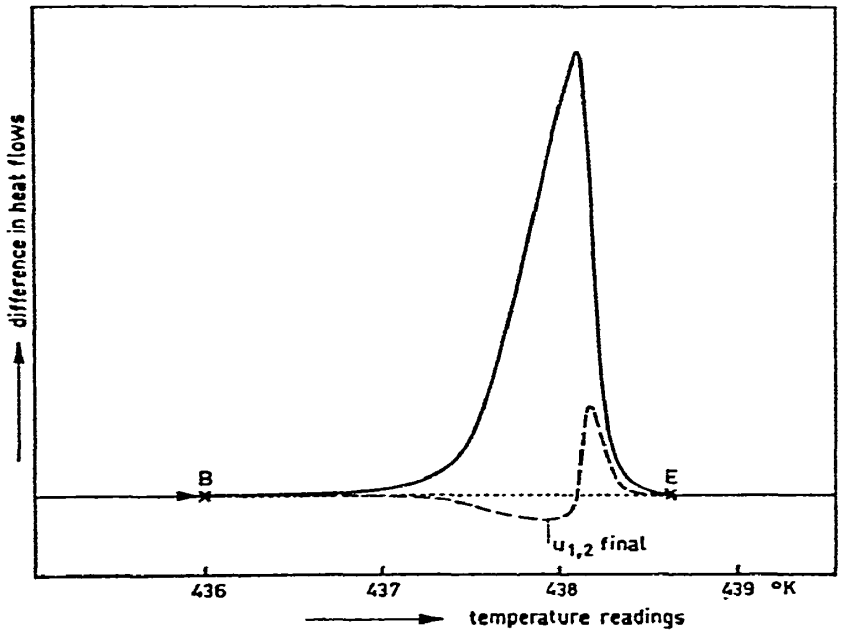


Fig. 2. Melting trace of benzanilide with a baseline $u_{1,2}$ *final* calculated by Heuvel and Lind[19].

The problem of evaluating the true heat of fusion exists because the DSC is measuring the difference in the heat necessary to maintain a given and constant temperature rise in the reference and the sample cell. The baseline of the instrument during an exothermic or endothermic change of the sample can only be determined

approximately by a connection of the recorder lines before and after such an energy change. Heuvel and Lind[19] stated, "Under certain conditions of instrument operation, *e.g.* fast scanning rates, the course of the base line deviates to a large extent from simple interpolation between pre-transition and post-transition baselines". Fig. 2 shows the melting trace of benzanilide from the paper of Heuvel and Lind. The indicated baselines are given by (*i*) a straight line from point B to E, and (*ii*) $U_{1,2}$ final; a function of the heating rate, the heat capacity of the sample, and the thermal resistance from the sample holder to the sample[17] according to the calculations of Heuvel and Lind[19].

For a sharp transition, as shown in Fig. 2, both baselines give the same calculated value for the heat of fusion, which is a conclusion of the paper of Heuvel and Lind[19].

The discussion of the heat of fusion is presented in two parts: (*1*) with high purity substances, and (*2*) with substances having lower purity values.

Table IV shows the heats of fusion for several high-purity substances. The values are directly calculated from melting curves in applying a straight baseline, as shown in Fig. 2.

TABLE IV

HEAT OF FUSION FROM DSC MELTING CURVES FOR SUBSTANCES OF A HIGH PURITY VALUE

Substances	*DSC purity (mole %)*	*Heat of fusion*		$\frac{\Delta H_{f,DSC}-\Delta H_{f,Lit.}}{\Delta H_{f,Lit.}} \times 100$ (%)
		DSC uncorrected baseline $\Delta H_{f,DSC}$ ($cal.mole^{-1}$)	*Lit. values* $H_{f,Lit.}$ ($cal.mole^{-1}$)	
Benzene	99.8	2352[a]	2349 (Ref. 22)	+0.1
Benzene	99.05	2237[a]	2349 (Ref. 22)	−4.8
Benzamide	99.25	4590[b]	4899 (Ref. 23)	−6.3
Benzoic acid		3945[b]	4300 (Ref. 24)	−8.3
Anthrachinon	99.94	7725[b]	7830 (Ref. 25)	−1.3
Potassium nitrate		2370[b]	2295 (Ref. 26)	+3.3
Distilled water	99.97	1400[b]	1434 (Ref. 27)	−2.4
Butazolidine	99.56 ±0.28[c]	5710[b] ±680[b,c]		

[a]DSC values by Driscoll *et al.*[6]. [b]DSC values by Marti and Heiber (unpublished). [c]Error in a single measurement on 95% confidence limits.

The agreement of the DSC with the literature values for the heat of fusion is reasonable in the case of high purity substances. The reproducibility of the heat of fusion, according to measurements on butazolidine, is indicative of a normal-precision, and certainly not of a high-precision instrument. A better precision in the determination of energies are expected from new instruments, *e.g.* Mettler DTA 2000[28] and Perkin–Elmer DSC-2[29].

The determination of the heat of fusion from melting curves of samples with low purity values reveals a completely different picture compared to that presented in Table IV. The results are presented in Table V. The measurements were performed by Driscoll *et al.*[6] for an impurity content of $\leqslant 2.80$ mole-%, and for highest value of impurities by a measurement in our laboratory. The determination of the heat of fusion was performed with an uncorrected baseline, as described in Fig. 2.

TABLE V

HEAT OF FUSION FROM DSC MELTING CURVES FOR BENZENE WITH VARIOUS AMOUNTS OF EUTECTIC IMPURITIES

Substance	*DSC purity (mole-%)*	*Heat of fusion DSC, uncorrected baseline* $\Delta H_{f,DSC}$ *(cal.mole*$^{-1}$*)*	$\frac{\Delta H_{f,DSC} - \Delta H_{f,Lit.}}{\Delta H_{f,Lit.}} \times 100$ (%)
Benzene	99.8	2352	+0.1
	99.05	2237	−4.8
	99.10	2131	−9.3
	97.14	1788	−23.9
	91.5	1293	−49.2

A correction of the heat of fusion for substances with purities below 99% is absolutely necessary. For example (see Table V, benzene, purity 91.5%), the eutectic impurity calculated in applying Eqn. (7) would be too low by as much as 50% for the theoretical impurity value of 8.5 mole-%. A similar picture of the difference between the heat of fusion according to the literature values and the DSC measurements was shown by Davis and Porter[10]. The difference in the values of the heats of fusion can be explained by (*i*) the fact that the instrument has a limited sensitivity, and (*ii*) a eutectic and premelting region which is unrecorded because the eutectic point may be far below the melting point of the main component.

The incorrect baseline measured by the DSC has not only an influence on the heat of fusion, but also on the evaluation procedure. The melting curve allows a calculation of the fraction of the substance melted as a function of temperature. The temperature indicated on the DSC-IB must be corrected to the temperature of the sample. The correction is performed with a temperature calibration curve of the instrument and with calibration measurements on the thermal lag between the sample holder and the sample[30]. The plot of the temperature of the sample as a function of the reciprocal molten fraction—the $1/r$, (T)-diagram—should give a straight line according to Eqn. (7). A straight line in the $(1/r, T)$-diagram can be observed occasionally for substances with an extremely high purity. All other substances give only a straight line after a trial and error correction of the baseline, the so callep linearization[13]. A new position of the baseline yields a new value of the heat of fusion. The linearization procedure corrects, at least partially, for the energy unrecorded

through the instrument's limitation and for the premelting region which is not observed. The linearization procedure leads to a more accurate determination of the heat of fusion of the main component.

The linearization in the $(1/r, T)$-diagram is only possible within a certain region of the melting curve. The limits of the linearization region are discussed in the paper by Driscoll *et al*[6]. They used for their linearization a constant value for the lower limit of the fraction melted with 2% and for the upper limit, a value based on the fraction melted at the point where the rate of heat input reaches half of its maximum value. This defines the upper limit from about 12.5% for a pure sample to about 40% for a sample with approximately 2 mole-% of eutectic impurities. The influence of the limits of the linearization interval on the calculated impurity values is shown in Table VI, taken from the work of Driscoll *et al.*[6].

TABLE VI

CALCULATED IMPURITY VALUES FOR NBS OCTANE WITH A CERTIFIED IMPURITY OF 0.06±0.04%

Linearization limits, fraction melted (%)		*Calculated impurity (mole-%)*
Lower limit	*Upper limit*	
2	10	0.016
2	18	0.23
2.5	36	0.52
2.5	40	0.59
2.5	50	0.78
10	50	2.69

Driscoll and coworkers emphasized the importance of the linearization limits, which are applied for the calculation of impurity values. Only with a comprehensive investigation on melting curves of substances which are close to an ideal melting behaviour, is one able to find proper values for the linearization limits. The necessity for an investigation of the linearization limits is clearly demonstrated in comparing the values of the linearization limits and the calculated impurities in Table VI. These calculated impurity values differ by two orders of magnitude from the NBS value.

In general, the performance of the linearization permits the calculation of the heat of fusion of the main component $\Delta H_{f,1}$; the melting point of the main component T_1, obtained from the intercept of the T-axis with the straight line of the corrected data for the melting curve in the $(1/r, T)$-diagram; and the melting point of the sample T_s as the temperature value where $r = 1$. The calculation of the eutectic impurity using Eqn. (7) can then be made without any difficulty.

Apart from the computing procedures for the determination of the concentration of eutectic impurity, there is another method suggested by Plato and Glasgow[31]. These two authors reported their experiences with 95 different organic compounds analyzed with the DSC: "An experienced analyst can estimate the purity of an

unweighed sample to within about 0.2 mole-% by visual inspection of the DSC curve produced in a 3-min scan". This remark can be regarded as the introduction of a new evaluation method. However, this new evaluation method of melting curves would demand, in our opinion, the following procedure, especially to reach the goal of an accuracy in eutectic impurities of ± 0.2 mole-%: (*1*) Preparation of a set of reference melting curves; the range of eutectic impurities and the instrumental conditions required to set up reference curves should be appropriate to the samples which have to be measured for analytical purposes. (*2*) Measurement of the melting curves of samples with the same instrumental conditions as used for the reference curves. (*3*) Comparison of the melting curves and the reference curves. Using the improved method of Plato and Glasgow the computer program for the calculation of purity values can be partially replaced.

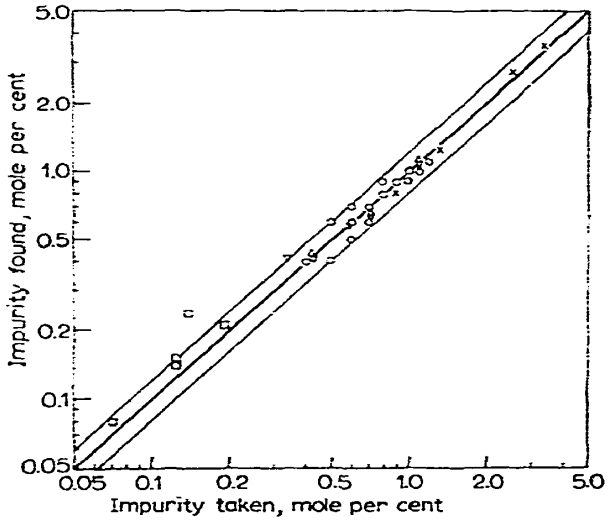


Fig. 3. Relation between actual and experimental impurity value (Joy *et al.*[7]).

(f) Accuracy of measurements of eutectic impurities with DSC

All authors agree that the accuracy of the impurity values measured with DSC decreases with increasing content of eutectic impurities. Barrall and Diller[14] claim a high relative accuracy of $\pm 3\%$ with respect to the eutectic impurity, but only in case of high-purity substances. Reubke and Mollica[15] reported on substances in the purity region of 99 to 100%. These authors claim an absolute error in the eutectic impurities of 0.1%, which means more than 10% relative to the amount of impurities. Joy and coworkers[7] make the interesting remark that the upper limit in purity measurements with DSC are 99.95 mole-%. Higher numerical values of the purity seem to bear no significance. The statement of Joy and coworkers of the existence of an absolute error in eutectic impurities of only 0.05 mole-% is quite close to the value given by Reubke and Mollica. This minimum value of the absolute error of the

impurity measurements is of course typical for the DSC-IB. An extensive comparison of theoretical and measured DSC values is presented in Fig. 3. The substances are selected by Joy *et al.* and the purity of most of these substances is in the region of $99.0 \pm 0.5\%$.

The upper and lower lines on the graph indicate the $+20\%$ and -20% relative error limit. It should be added, however, that only substances with no problems in melting (*e.g.* suspected solid solution formation, incomplete solubility of the impurities in the melt, or other disturbances) were chosen by Joy for presentation in Fig. 3. De Angelis and Papariello[11] give examples of organic substances with absolute errors between the actual and DSC purity values of 0.5 mole-% at the 98-% purity level and 2.5 mole-% at the 95-% level. The actual values of the purity are given by dry mixing the main component and the impurity. Dry mixing of substances in the purity range of less than 99% should be without any problems. Therefore actual values of the purity known from mixing of the main component and impurities are expected to be very close to the true purity values. The relative accuracies of eutectic impurities, according to the measurements and calculations of De Angelis and Papariello, are within 25% for the 98-% purity level and 50% for the 95-% level. De Angelis and Papariello explain, "We have not yet encountered any system in which accurate results were obtained beyond 1.5 mole-% impurity and it is indicated that DSC purity values of less than 99% are likely to be in error". From statements in the literature on accuracy in the determination of eutectic impurities the following conclusion can be made. There are two regions of purity with an arbitrary separation limit of 99 mole-%. The probability of a good agreement of actual and measured impurity values is high in the high-purity region, and low in the low-purity region. A first step to a clearer situation in the low-purity region could be reached by an extensive study on any system which shows great differences in actual and experimental purity values.

(*g*) *Application of the purity determination to substances which are unstable in the melting region*

Reubke and Mollica[15] reported, "Samples were selected which would melt without decomposition". Plato and Glasgow[31] stated, "Purity of chemicals that decompose near their melting points cannot be determined by the DSC method". Throughout the literature one can find the statement that the DSC method is unable to handle substances which decompose during melting.

THEORY OF THE PURITY DETERMINATION USING THE METHOD OF PREMELTING ON BINARY SYSTEMS WITH A EUTECTIC PHASE DIAGRAM

The theory of the purity determination with the method of premelting was discussed by Marti *et al.*[32] for a binary system with a eutectic phase diagram. A binary system is the simplest system for a theoretical discussion of the purity determination by DSC, also for experimental work it is easy to collect all the necessary information from the literature or by measurements. The understanding of the

melting behaviour of a binary system from a theoretical and experimental point of view is certainly the most important part of the attempt to understand the melting behaviour of a multicomponent system.

The melting behaviour of a eutectic system consisting of only two components is commonly described approximately by a thermodynamic relationship in the region of solid–liquid equilibria. Such a description is given in Eqn. (10)[33] for an ideal mixture of non-electrolytes under isobaric conditions with a heat of fusion independent of temperature.

$$\ln (x_i) = \frac{\Delta H_{f,i}}{R}\left(\frac{1}{T_i} - \frac{1}{T}\right) \qquad (i = 1, 2) \tag{10}$$

where x_i is the mole fraction of the component i in the liquid phase, $\Delta H_{f,i}$ is the heat of fusion of the pure component i at the melting point, R is the gas constant, T_i is the melting point of the pure component i in °K, and T is the temperature in °K.

The mole fractions for a binary mixture are connected by the equation

$$x_1 + x_2 = 1 \tag{11}$$

At low values of one of the components, *e.g.* component 2, we can write Eqn. (10) in the form

$$x_2 = \frac{\Delta H_{f,1}}{R}\left(\frac{1}{T} - \frac{1}{T_1}\right) \tag{12}$$

More exactly, the solubility equilibrium (Eqn. 10) is found by introducing a heat of fusion, ΔH_i, which is a function of temperature[34]. In this case we can write

$$\Delta H_i = \Delta H_{f,i} + \Delta C_{0,i}(T - T_i) \tag{13}$$

where $\Delta C_{0,i}$ is the difference of the molar heat capacities of the pure component i at constant pressure for the liquid and the solid phase.

Eqn. (13), applied to the solubility equilibrium of an ideal mixture, leads to the following relationship

$$\ln (x_i) = \frac{\Delta H_{f,i}}{R}\left(\frac{1}{T_i} - \frac{1}{T}\right) - \frac{\Delta C_{0,i}}{R}\left(1 - \frac{T_i}{T} + \ln \frac{T_i}{T}\right) \tag{14}$$

The following abbreviation will be used for Eqn. (14)

$$\ln (x_i) = \mathrm{A}(T) \tag{15}$$

Eqns. (10), (12) and (14) enable us to construct the isobaric melting point diagram for binary systems, which are ideal mixtures on the basis of the properties of the main components alone (namely melting points, heats of fusion, and heat capacities).

A mixture of phenacetin and benzamide was chosen as an example of a binary system. The thermodynamic values used for the calculation of the phase diagram are given in Table VII.

TABLE VII
THERMODYNAMIC PROPERTIES OF PHENACETIN AND BENZAMIDE

Thermodynamic values	*Phenacetin*		*Benzamide*	
	Author's values by DSC	*Lit. values*	*Author's values by DSC*	*Lit. values*
Melting point T_t (°K)	407 ±0.3	407–408[35]	400 ±0.3	400–400.7[36]
Heat of fusion $\Delta H_{f,t}$ (cal/mole)	7750 ±600	7880[a]	4900 ±600	4900[37]
Difference between the molar heat capacities in the liquid and the solid phase $\Delta C_{0,t}$ (cal/mole °K)	12.5 ±0.5		13.3 ±0.5	

[a]Calculated from the heat of sublimation ($\Delta H_s = 27.60$ kcal mole^{-1})[38] and the heat of evaporation ($\Delta H_v = 19.72$ kcal mole^{-1})[39].

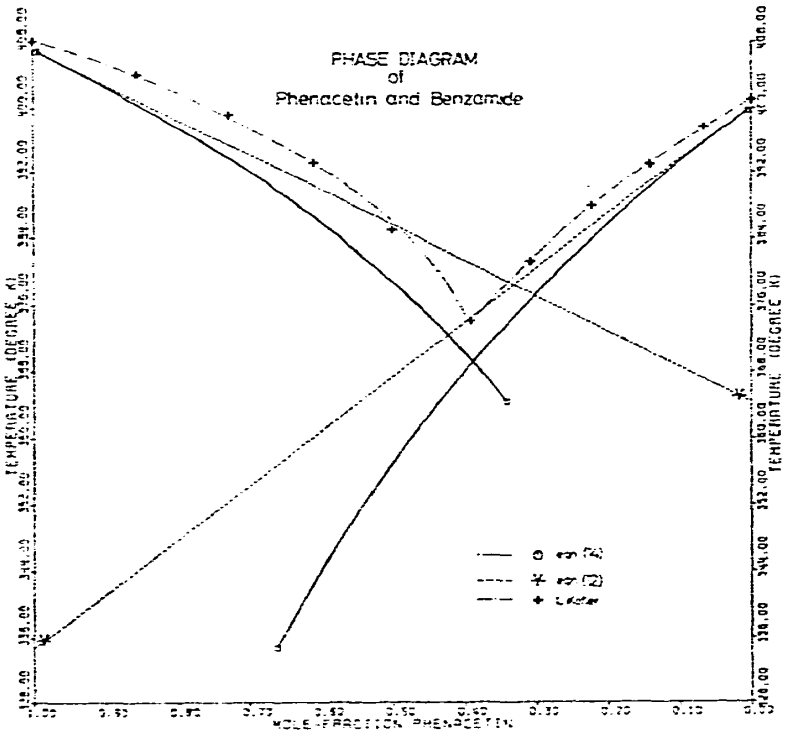


Fig. 4. Comparison of theoretical and experimental phase diagrams for phenacetin and benzamide (Kofler, Marti *et al.*[32]).

In Fig. 4, the melting point diagrams calculated from Eqns. (12) and (14) are compared with a diagram from measurements by Kofler[40]. The differences between the two theoretical phase diagrams are easily understood by the two levels of approximation applied to Eqns. (12) and (14). However, the differences between the theoretical

phase diagram calculated with Eqn. (14) and the experimental phase diagram are certainly caused by the difference between the activities of the components, and the concentration itself. A smaller part of the differences may be due to experimental conditions. A discussion of the activity can be attempted by introducing the relation[41]

$$a_i = x_i f_i \tag{16}$$

where a_i is the activity of the component i in the liquid phase, and f_i is the activity coefficient of the component i.

The activity coefficient, f_i, of a binary liquid mixture of non-electrolytes is defined by Eqn. (17)

$$\mu_i = \mu_i^\circ + RT \ln (x_i f_i) \qquad (i = 1, 2) \tag{17}$$

where μ_i is the chemical potential of component i in the liquid phase and μ_i° is the chemical potential of pure liquid component i at the same pressure and temperature.

A relationship exists between the activity coefficients f_1 and f_2 because of the mutual interaction of the substances in a binary system and because of the equilibrium between the phases of a heterogeneous system. The equilibrium condition demands that all the phases must have the same temperature T, the same pressure P, and the chemical potential of each component must have the same value of μ_i in all the phases.

The relationship between the activity coefficients follows from a combination of Eqns. (11) and (17) and in the application of the Gibbs–Duhem relationship

$$x_1 \left(\frac{\delta \ln f_1}{\delta x_1}\right)_{T,P} + (1 - x_1) \left(\frac{\delta \ln f_2}{\delta x_1}\right)_{T,P} = 0 \tag{18}$$

The activity coefficient f_i is a function of temperature, pressure and the mole fraction of the components; it is determined by a solubility measurement of each component at the temperature T and at isobaric conditions according to Eqn. (19), which follows from Eqns. (14) and (16)

$$\ln (x_i f_i) = \frac{\Delta H_{f,i}}{R} \left(\frac{1}{T_i} - \frac{1}{T}\right) - \frac{\Delta C_{0,i}}{R} \left(1 - \frac{T_i}{T} + \ln \frac{T_i}{T}\right) \tag{19}$$

The point-to-point determination of the activity coefficient is cumbersome. The problem may be solved relatively easily if we can specify the form of the function of the activity coefficient $f_1 (T, x_1)$. This function is then found, to a certain approximation, by combining only a few solubility data from Eqn. (19) along with the temperature dependence of the activity coefficient. The temperature dependence at a given concentration x_1 is related to the temperature dependence of the chemical potential by

$$\left(\frac{\delta \ln f_1}{\delta T}\right)_{P,x_1} = -\frac{\Delta H_1^*}{RT^2} \tag{20}$$

where ΔH_1^* is the differential heat of mixing given by the following equation

$$\Delta H_1^* = H_1 - H_1^\circ \tag{21}$$

where H_1^0 is the molar enthalpy of the pure liquid component 1 and H_1 is the partial molar enthalpy of component 1 in the mixture.

With the determination of the function $f_1(T, x_1)$ from measurements on a binary system, the function $f_2(T, x_2)$ is also known according to Eqn. (18). Each branch of the melting point diagram of a binary system under isobaric conditions is described to a good approximation by Eqn. (19) and the thermodynamic constants of the corresponding main component known from the literature or from the measurements of heat of fusion, melting point, difference of the molar heat capacities for the liquid and the solid phase, and the activity coefficient.

Next, theoretical melting curves for different values of the ratio of the components for a given binary system are calculated. The calculation is based on the Eqns. (12) and (14) under the restriction to ideal mixtures. A melting curve can be defined by the rate of heat flow to the sample which, in a solid–liquid equilibrium, is a function of the temperature. The melting curve is further dependent on the ratio of the components and on the phase diagram of the binary system. Such a representation of the melting curves by the rate of heat flow as a function of temperature is experimentally obtained by the DSC method. In contrast to the paper by O'Neill[17], which presents a fusion analysis with the rate of heat flow, our discussion of the melting curves is based on the specific heat function. The relation between rate of heat flow, specific heat function and scan speed is given in Eqn. (22)

$$\frac{dH}{dT} = \frac{dH}{dt} \cdot \frac{dt}{dT} \tag{22}$$

where dH/dT is the specific heat function at constant pressure (cal $°K^{-1}$ $mole^{-1}$), dH/dt is the rate of heat flow (cal sec^{-1} $mole^{-1}$), and dT/dt is the scan speed in $°K\ sec^{-1}$. There is no difference, in principle, in discussing the melting behaviour with the rate of heat flow or the specific heat function.

One branch of the phase diagram is selected with the condition for component 2 of

$$x_{0,2} < x_{e,2} \tag{23}$$

where $x_{0,2}$ is the mole fraction of component 2 in the binary mixture and $x_{e,2}$ is the mole fraction of the component 2 at the eutectic point.

The condition in Eqn. (23) defines component 1 as the main component of the mixture. The eutectic melting at the eutectic temperature T_e is neglected by setting the temperature limits for Eqns. (12) and (14)

$$T_1 > T > T_e \tag{24}$$

The relation between the mole fraction x_2 of component 2 in the liquid phase and the mole fraction of this component in the given system ($x_{0,2}$) is

$$x_2 = \frac{x_{0,2}}{r} \tag{25}$$

where r is the molten fraction of the mixture.

By inserting Eqn. (25) into the equation for the solubility equilibrium in Eqn. (12), we obtain

$$\frac{x_{0,2}}{r} = \frac{\Delta H_{f,1}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_1}\right) \tag{26}$$

The introduction of the molten fraction r enables us to write the specific heat function as

$$\frac{dH}{dT} = \frac{dH}{dr} \cdot \frac{dr}{dT}. \tag{27}$$

The derivative dr/dT is calculated from Eqn. (26). The other derivative dH/dr can be formed using the following relation

$$H_r = \Delta H_{f,1} \cdot r \tag{28}$$

The relation in Eqn. (28) holds because of the limitation to eutectic systems and because of the restriction to ideal mixtures. Insertion of dH/dr and dr/dT into Eqn. (27) leads to the specific heat function

$$\left(\frac{dH}{dT}\right)_1 = x_{0,2} \frac{RT_1^2}{(T_1 - T)^2} \tag{29}$$

The integration of Eqn. (29) can be performed between the limits T_a and $T_{s,1}$

$$\int_{T_a}^{T_{s,1}} \left(\frac{dH}{dT}\right)_1 dT = x_{0,2} \cdot RT_1^2 \int_{T_a}^{T_{s,1}} \frac{dT}{(T_1 - T)^2} \tag{30}$$

The upper limit $T_{s,1}$ is the melting point of the given mixture and is approximated [see Eqn. (26), $r = 1$] by

$$T_{s,1} = T_1 - x_{0,2} \cdot \frac{RT_1^2}{\Delta H_{f,1}} \tag{31}$$

The lower limit is more or less arbitrarily chosen as

$$T_a = T_1 - \frac{RT_1^2}{\Delta H_{f,1}} \tag{32}$$

defining r as equal to $x_{0,2}$ for this lower limit.

The definition of the lower limit of the specific heat functions excludes the energetic change of binary systems at the eutectic points. There is no continuity between the eutectic point and the melting region which would demand a mutual discussion of both phenomena.

By repeating the procedure, which gave Eqn. (29) from Eqn. (26), with the solubility equilibrium from Eqn. (14) we obtain the specific heat function

$$\left(\frac{dH}{dT}\right)_1 = \frac{x_{0,2}}{RT^2} \cdot [\Delta H_{f,1} + \Delta C_{0,1}(T - T_1)]^2 \cdot \frac{1}{e^{A(T)} + e^{-A(T)} - 2} \tag{33}$$

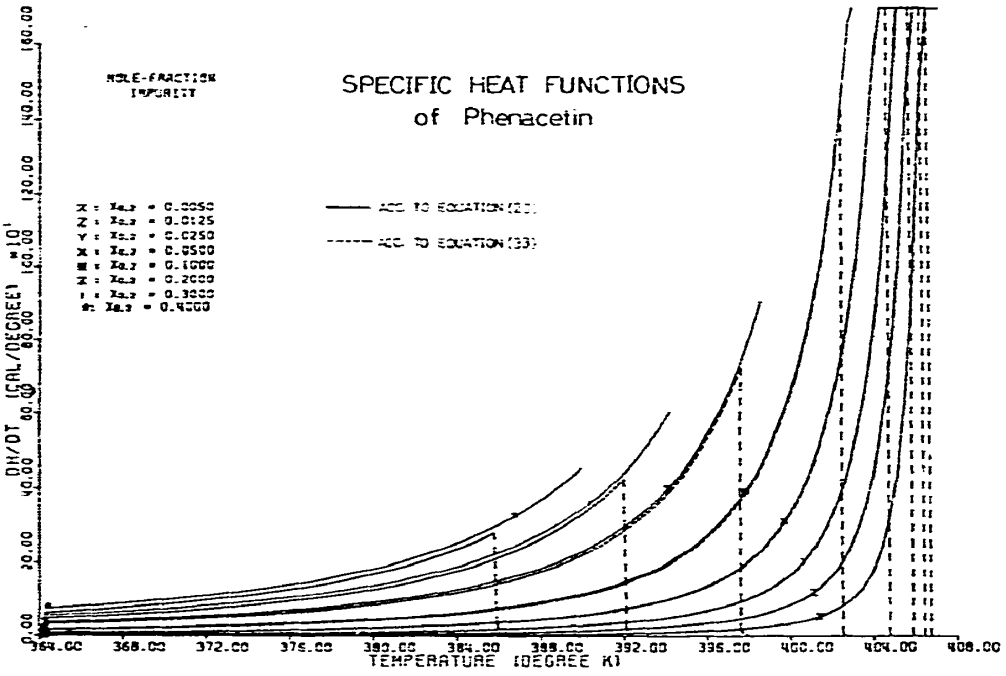


Fig. 5. Specific heat functions of phenacetin from Eqns. (29) and (33).

For a given mole fraction, the theoretical specific heat functions according to Eqns. (29) and (33) depend only on the properties of the main components. Specific heat functions of phenacetin and benzamide are presented in Figs. 5 and 6. The sets of specific heat functions for phenacetin are calculated according to Eqns. (29) and (33), whereas for benzamide, only one set of curves from Eqn. (29) is shown. The thermodynamic values of the main components used for our calculations are shown in Table VII. The following mole fractions of component 2 (impurity) were used for the presentation of the specific heat functions in the case of phenacetin and benzamide; $x_{0,2} = 0.005$, 0.0125, 0.025, 0.05, 0.10, 0.20, 0.30. In addition, for phenacetin as the main component, a curve with $x_{0,2} = 0.40$ was also plotted. The upper and lower limits of the specific heat functions calculated according to Eqn. (29) are determined by Eqns. (31) and (32). In Figs. 5 and 6, the curves are plotted between these limits if the selected range of the specific heat of 1600 cal $°K^{-1}$ allows such a presentation. As

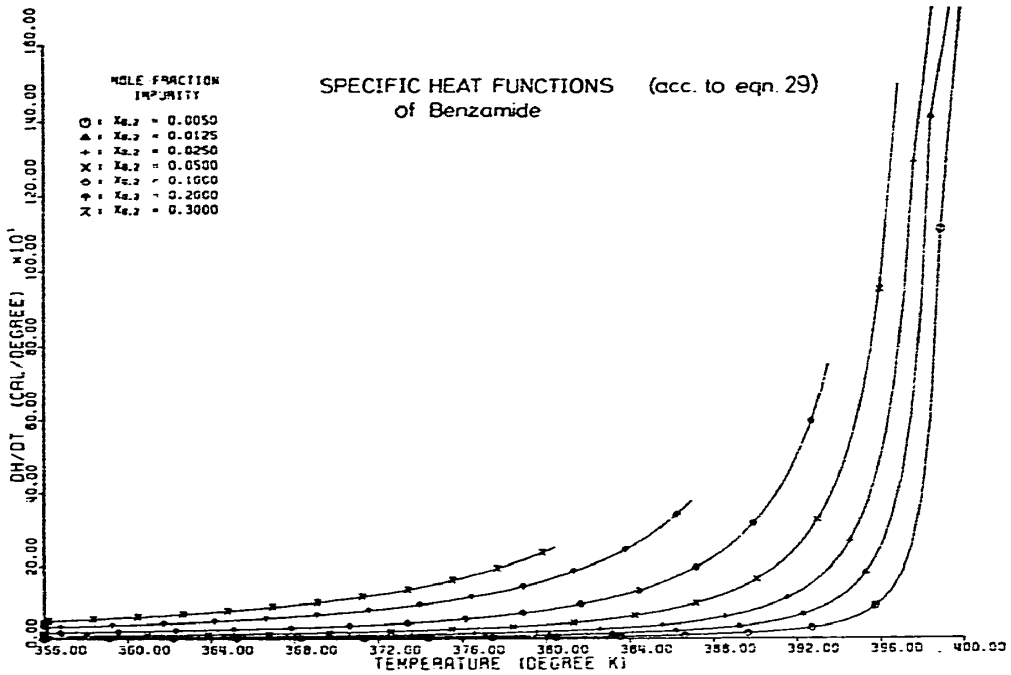


Fig. 6. Specific heat functions of benzamide from Eqn. (29) (Marti *et al.*[32]).

an example, a comparison of the specific heat functions for phenacetin and benzamide with a mole fraction of component 2 ($x_{0,2} = 0.3$) shows differences caused mainly by the difference in the melting points and the heat of fusions. Among other subjects, there is an investigation in a subsequent part of this paper into the difference of specific heat functions calculated using Eqn. (29) for main components which differ in their thermodynamic constants.

The differences of the specific heat functions from Eqns. (29) and (33) are shown in Fig. 5. Significant differences between the specific heat functions (shape of curves and upper limits) are only seen for extremely high impurity values (component 2). For the specific heat functions of Eqn. (29) the upper limits (melting points) were evaluated from Eqn. (31). Eqn. (31) is only a poor approximation in a region of the mole fraction of component 2 between 0.4 and 0.1. The absolute values and relative differences of the specific heat functions of phenacetin as main component, calculated from Eqns. (29) and (33), are presented for selected temperature values and mole fractions in Table VIII.

The relative differences of the specific heat functions calculated with Eqns. (29) and (33) are strongly dependent on temperature but are practically independent of the mole fraction of the main component. Table VIII clearly shows that the differences in the assumptions used for the calculation of specific heat functions are not really

TABLE VIII

COMPARISON OF SPECIFIC HEAT FUNCTIONS CALCULATED FROM EQN. (29) OR EQN. (33)

Temperature	*Mole fraction of component 2*	(dH/dT) *from Eqn. (29)*	(dH/dT) *from Eqn. (33)*	$\frac{(dH/dT)_{29}-(dH/dT)_{33}}{(dH/dT)_{29}} \times 100$
364.3	0.30	54.4	46.0	15.5
391.8	0.30	428.2	412.8	3.8
364.3	0.05	9.1	7.7	15.4
391.8	0.05	71.5	68.9	3.6

essential for the shape of the curves and, therefore, calculated impurity values in mole fractions change only a little when using either Eqn. (29) or (33).

It is even possible to go one step further in saying that to a certain approximation the specific heat function is only dependent on the mole fraction of the second component (impurity) as long as the restriction of eutectic systems holds. To explain this statement and to show the closeness of this approximation, Eqn. (29) may be written in the transformed form

$$\left(\frac{dH}{dT}\right)_1 = x_{0,2}\,\frac{R}{1-2\frac{T}{T_1}+\left(\frac{T}{T_1}\right)^2} \tag{34}$$

Now, two mixtures with different main components and melting points of the pure substances T_1 and T_2, respectively can be compared. The following temperature difference, ΔT, is introduced

$$\Delta T = T_2 - T_1 \tag{35}$$

The specific heat function of mixture number 2 is represented on a shifted scale, namely

$$T' = T - \Delta T \tag{36}$$

The specific heat functions are indicated with the indices 1 and 2 and the mole fraction of the impurity in both cases is made the same. The ratio of the two specific heat functions formed with Eqn. (34) can be written as

$$\frac{\left(\frac{dH}{dT}\right)_1}{\left(\frac{dH}{dT}\right)_2} = \frac{1}{\left(1+\frac{\Delta T}{T_1}\right)^2} \tag{37}$$

Obviously, the ratio of the specific heat functions taken at corresponding temperatures is constant.

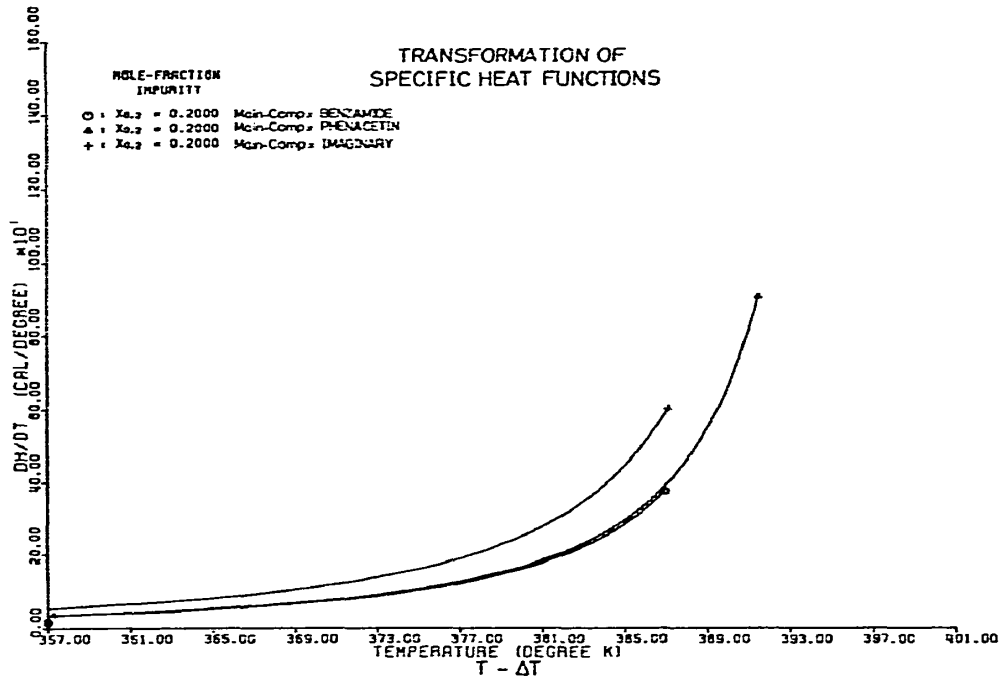


Fig. 7. Transformation of specific heat functions (Marti *et al.*[32]).

As an example, three specific heat functions on a transformed temperature scale are represented in Fig. 7 with a constant mole fraction $x_{0,2} = 0.2$. The substances and thermodynamic values used in these examples are shown in Table IX.

TABLE IX
THERMODYNAMIC PROPERTIES OF THE COMPOUNDS USED FOR FIG. 7

Substance	*Heat of fusion* $\Delta H_{f,1}$ *(cal/mole)*	*Melting point* T_1 *(°K)*	*Temp. shift* T *(°K)*
1. Benzamide	4900	400	0
2. Phenacetin	7750	407	7
3. Imaginary substance	7750	500	100

Eqn. (37) and the values in Table IX give the ratios

$$\frac{\left(\frac{dH}{dT}\right)_1}{\left(\frac{dH}{dT}\right)_2} = 0.966 \quad \text{and} \quad \frac{\left(\frac{dH}{dT}\right)_1}{\left(\frac{dH}{dT}\right)_3} = 0.64$$

which agree fully with the ratios taken directly from the specific heat functions in Fig. 7.

A second point observed in Fig. 7 is the position of the upper limit of the specific heat functions. This limit is given by Eqn. (31) and, for a constant mole fraction of the impurity, depends only on the cryoscopic constant of the main component.

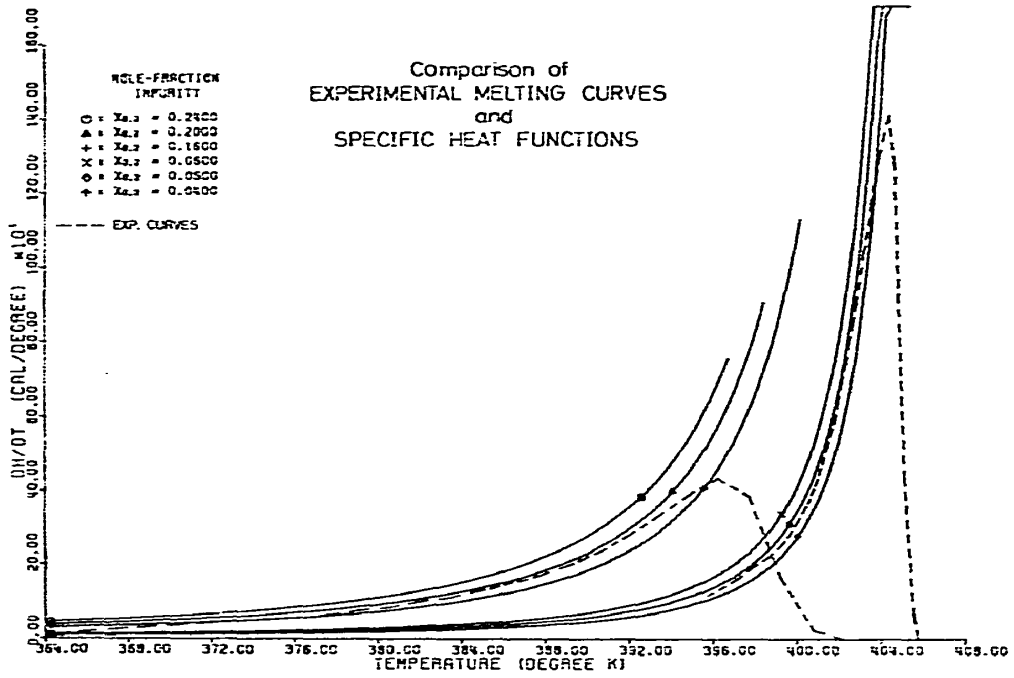


Fig. 8. Comparison of experimental melting curves and specific heat functions for phenacetin as main component and benzamide as impurity (Marti *et al.*[32]). The actual impurity values in mole fraction of the two mixtures are $x_{0,2}^{exp} = 0.2$ and 0.05.

In Fig. 8, theoretical specific heat functions for phenacetin calculated with Eqn. (29) and experimental curves recorded with the DSC-IB instrument of the Perkin–Elmer Corp. are compared. The samples were carefully mixed from phenacetin OAS (main component) and crystalline benzamide (impurity). The purities of both components used for the preparation of the mixtures were determined with the DSC-IB and evaluated with a computer program. The mean values of eutectic impurities of the two components are: phenacetin OAS, 0.25 ± 0.05 mole %; benzamide cryst. 0.66 ± 0.10 mole %.

The mixtures under investigation can be regarded as pseudo-binary systems with components of such a purity level, especially for the chosen mole fractions of the components. In Fig. 8, the experimental curves for $x_{0,2} = 0.2$ and 0.05 (concentration

of the impurity) are presented after transforming the ordinates $Y_{exp.}$ with a factor f_{DSC} to reach the same scale as applied for the theoretical curves.

$$Y = Y_{exp.} \cdot f_{DSC} \tag{38}$$

The factor is determined by the equation

$$f_{DSC} = \frac{B}{\dot{T}} \cdot R_f \cdot \frac{M}{m} \tag{39}$$

where B = recorder speed (cm min^{-1}), $\dot{T}$ = scan speed (°K min^{-1}), R_f = range factor (cal cm^{-2}), M = molecular weight of the main component (g), and m = sample weight of main component (g).

The temperature scale was only shifted according to a temperature calibration curve used for the measurements with the DSC-IB. No correction of the experimental curves for the thermal resistance of the DSC instrument were applied. Correction is certainly necessary for tan α values (thermal resistance) of less than 20.

The experimental conditions for the melting curves presented in Fig. 8 are as follows:

Mole fraction of benzamide	*Sample weight (mg)*	*Range (mcal sec^{-1})*	*Scan speed (°K min^{-1})*
0.2	3.09	4	16
0.05	3.20	4	4

Fig. 8 shows the agreement between the theoretical and experimental curves at least for the important melting region used for the determination of purity values.

DISCUSSION OF THE MEASUREMENTS OF SYSTEMS WITH IMPURITIES FORMING SOLID SOLUTIONS WITH THE MAIN COMPONENT

The two basic forms of phase diagrams are eutectic systems and systems with a complete range of solid solutions. One observes, normally, in the region of low concentration of one component either the form of a eutectic system or a solid solution. All other effects are restricted mainly to a mole-fraction region from about 0.1 to 0.9. As an example one is unlikely to find a congruently- or incongruently-melting compound in the concentration range of 0–0.1.

A theoretical representation of the melting curves for systems of solid solutions is not as easy as in the case of substances with a eutectic phase diagram. This difficulty arises because the concentrations of the components are normally a function of temperature, (*i*) in eutectic systems only in the liquid phase, and (*ii*) in systems of solid solutions in the liquid phase as well as in the solid phase. We shall now discuss a

temperature change for a eutectic system and a system of a solid solution within the temperature region of the solid–liquid equilibrium.

A system at equilibrium conditions at a given temperature is brought to a non-equilibrium condition by an infinitely-fast temperature change. The system will recover from these non-equilibrium conditions with two relaxation processes, a heat flow and a mass transport. The mass transport is caused by the temperature change and, therefore, the mass transport is consecutive to the heat flow. Equilibrium concentrations of the components are attained anew by diffusion of the components inside the phase. In eutectic systems, the diffusion is restricted to the liquid phase. In systems of solid solutions the diffusion of components occurs in the liquid and in the solid phase. The difference in the relaxation processes for eutectic systems and systems of solid solutions is mainly due to the difference in the diffusion rates in the liquid or in the solid phase. Therefore these diffusion rates which determine the relaxation times differ in order of magnitude.

Another difficulty in systems of solid solutions is caused by the crystallization of the substances. The crystallization conditions have an influence on the crystals formed. The solid phase may consist of so-called "zone crystals", which differ according to the conditions of crystallization in their concentration profile over cross-sections of any single crystal. Melting curves of zone crystals, which are measured at different non-equilibrium conditions are influenced by the actual concentration profile of the crystals.

Investigations into equilibrium or non-equilibrium conditions during the melting of systems of solid solutions are important for a similar treatment of purity determination in eutectic systems and systems of solid solutions. A similar purity determination for systems of solid solutions does not exist on the same level as in the case of eutectic systems.

The systems of solid solutions are only discussed phenomenologically and the possibilities of the DSC method are explained for a specific system, namely benzene–thiophene. The measurements published by Driscoll *et al.*[6] on benzene–thiophene are presented in Table X. The measurements were made on a pseudobinary system, of

TABLE X

DSC MEASUREMENTS OF SOLID SOLUTIONS IN THE BENZENE–THIOPHENE SYSTEM BY DRISCOLL *et al.*[6]

Sample	*Impurity*	*Added impurity (mole-%)*	*True impurity (mole-%)*	*Measured impurity (mole-%)*	ΔH_f *(cal/mole)*
Benzene	Thiophene	0		0.11	2352
		0.07	0.18	0.21	2209
		0.20	0.31	0.13	2364
		0.44	0.55	0.13	2370
		1.27	1.38	0.30	2438
		3.04	3.15	0.43	2291
		5.27	5.38	0.97	2468

benzene and thiophene. These two compounds are known to form solid solutions. The added impurity in Table X refers to thiophene. The true impurity is the added impurity (thiophene), corrected with the eutectic impurity (benzene), and could be measured with the DSC if benzene and thiophene could form a eutectic system.

The measured impurity, the impurity of benzene, and the heat of fusion of the samples were determined with the DSC apparatus. The measured impurity is only about 13% of the value of the true impurity, at least for the addition of more than 1 mole-% of thiophene. From the investigations by Driscoll *et al.*, one can calculate a distribution ratio of the impurity between the solid and the liquid phase of about $K \approx 4$. The calculation is, of course, only a rough approximation. We do not know if the distribution ratio is a function of the conditions prevalent during the measurements.

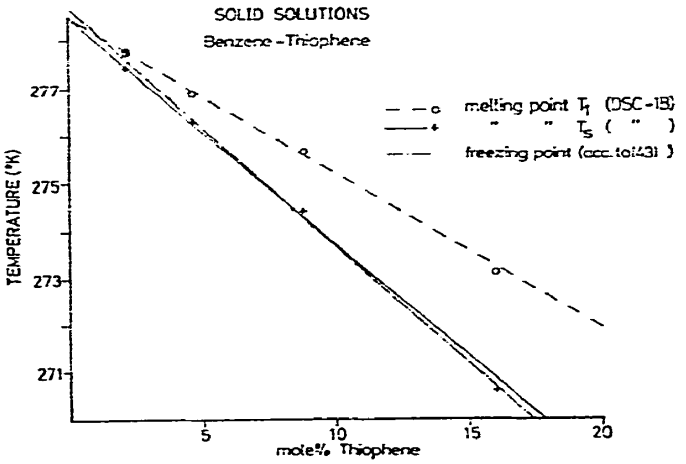


Fig. 9. Solid solutions of benzene–thiophene. Presentation of melting point *vs.* concentration of thiophene in the measured systems.

The heat of fusion is, in contrast to eutectic systems, practically constant with increasing amounts of impurity. As reported at the Perkin–Elmer meeting in Zurich[42], in 1969, we obtained similar values for the impurity and heat of fusion for the same system as reported by Driscoll *et al.*[6]. With careful calibration of the temperature scale of the DSC-IB, we were able to get absolute values of the melting points within ± 0.3 °C of the benzene–thiophene samples. In Fig. 9 the melting points of the samples calculated with a computer program based on Eqn. (7) are presented as a function of the thiophene concentration. The melting points of the samples, T_s, agree with literature values found from freezing point measurements by Fawcett and Rasmussen[43]. The decrease of the melting point of the pure component, T_1, is incompatible with the assumption of a eutectic system.

The decrease of T_1 with an increasing concentration of thiophene is explained by the phase diagram of a complete series of solid solutions of benzene–thiophene.

From our measurements, we conclude that a temperature accuracy of the DSC-IB in the order of $\pm 0.3\,^{\circ}C$ enables the detection of at least 1 mole-% of thiophene. In order to measure the amount of impurity forming solid solutions in a binary system, one has to determine, with test measurements on the same binary system, the shift of the melting point as a function of the concentration of the impurity. For binary and multicomponent systems with unknown impurities, a significant change of the melting point T_i indicates one or several possible effects, *e.g.* solid solutions, polymorphism, salt or solvate formation of the main component, decomposition, etc.

In conclusion, we can state that in a great number of pseudobinary systems, it is possible to measure impurities forming solid solutions with the main component but with the restriction that the impurities are known. At present, one is not able to measure absolute amounts of impurities in systems of solid solutions with unknown components.

THE PRACTICAL ASPECTS OF DSC PURITY MEASUREMENTS

(*a*) *Experimental technique*

The experimental procedure for a purity determination with the DSC apparatus, in the case of a substance investigated for the first time, is as follows.

DSC curves of the substance under investigation are recorded from room temperature, or from at least 30 °C below the melting region up to 100 °C above the melting point in the case of low-melting substances. The curves are measured with a high scan speed (*e.g.* $dT/dt = 16\,^{\circ}C\ min^{-1}$) in the volatile as well as in the open sample pan. This procedure enables energy changes to be observed in addition to the heat of fusion, caused by effects such as; modification changes, eutectic points, evaporation of impurities, loss of crystal water, and decompositions. The measured DSC curves enable us to form the substances into three groups: (*1*) Substances with no effect observed other than the melting in the given temperature region; (*2*) substances with effects clearly separated from the melting region; and (*3*) substances with effects interfering with the melting region.

This discussion is restricted to substances without effects interfering with the melting region; such an effect is one that occurs within the linearization region used in the computing procedure of the purity value. The investigation of effects other than the melting observed with the DSC apparatus is an analytical problem involving thermogravimetrical analysis, X-ray and spectroscopical methods and other appropriate methods.

The same samples used for the first DSC curves are cooled down for recrystallization and heated up again to get a second melting curve, if possible. The second melting curve yields information about a modification change which could occur during the recrystallization, about the stability of the substance in the temperature region scanned, and about effects which can be observed by a comparison of the curves from the first and second melts.

If a second melting curve cannot be obtained because the substance has

decomposed during melting or did not recrystallize, the stability is determined by keeping a sample at constant temperature for 3–30 minutes at about 10 °C below the melting point. Such a procedure, with respect to the thermal treatment, is not completely equivalent to a second melting.

If such a thermal treatment indicates a decomposition of the substance, the samples are enclosed in volatile sample pans inside a glove box filled with nitrogen gas. The measurements in a nitrogen atmosphere reveal the answer to the question of the oxygen sensitivity of the substance under investigation.

Finally, some melting curves are selected for an evaluation with the computer. The results of the computer program are listed and then compared with the corresponding melting curve and with the information available from other analytical methods. After following this procedure there is a strong basis for setting up an instruction for routine work on the same substance.

Routine analyses are normally performed with one or two melting curves under appropriate conditions. In the case of routine substances without any anomaly, the evaluation of the melting curve is performed according to the method suggested by Plato and Glasgow[31] or with a computer program.

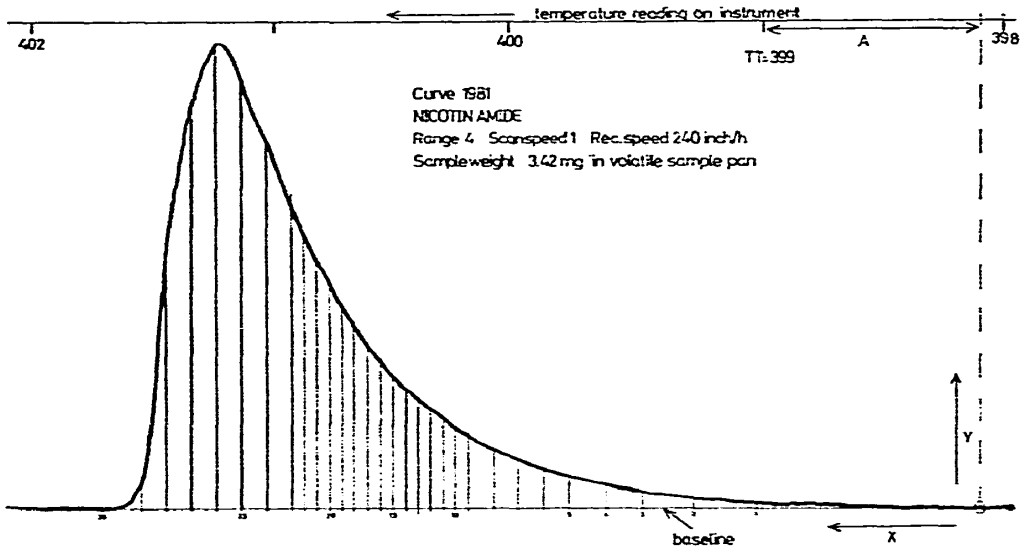


Fig. 10. DSC melting curve of nicotinamide (number of curve 2, 1981).

(b) The evaluation of melting curves in this laboratory

The evaluation of melting curves and the basic computer program for the purity determination will be discussed in some detail because of the importance of the evaluation with respect to the whole issue of the purity determination by DSC. The evaluation of melting curves will be discussed with nicotinamide as an example. A

typical melting curve of a sample of nicotinamide measured with the DSC-IB is shown in Fig. 10. The conditions for this melting curve are: sample weight, 3.42 mg; range, 4 mcal sec^{-1}; scan speed, 1 °C min^{-1}; and recorder speed, 240 in. h^{-1}.

The evaluation of the melting curve starts with the drawing of a baseline, as shown in Fig. 10. The baseline connects the pretransition to the posttransition region with a straight line. The points 0–30 are marked on the baseline, observing the rule that the density of the points should be greater in the expected linearization region than in the other parts of the melting curve. The temperature TT (399 °K in the case of nicotinamide) and the distance A are necessary for the connection of the points on the x-axis (see Fig. 10) to the temperature marking of the instrument. The point number zero is the zero point for both the x- and y-ordinates. The values (x_i, y_i) for the points $i = 0$–30 are used as a representation of the melting curve in the computer program. The main program calls up the subroutines. Each of the subroutines can be briefly described as follows:

Subroutine INPUT: Reads and writes experimental data.

Subroutine DATEN: Contains the calibration factors of the instrument and calculates the constants for the temperature correction.

Subroutine INTGR: Performs the integration of the melting curves and corrects the temperature for all experimental points.

Subroutine SUBXQ: Calculates the molten fraction r using the values obtained from subroutine INTGR.

The molten fraction r_i is given by

$$r_i = \frac{\sum_{n=1}^{i} a_n}{\sum_{n=1}^{30} a_n} = \frac{a_{0,i}}{a_{0,30}} \tag{40}$$

where a_n is the area bounded by the baseline, the melting curve and the lines perpendicular to the baseline through the points i and $i-1$ ($i = 1$–30). The baseline is shifted with the parameter ax set to zero at the beginning of the linearization and set to 10 cm^2 for the first linearization step. The molten fraction r_i' is calculated according to the equation

$$r_i' = \frac{a_{0,i} + ax}{a_{0,30} + ax} \tag{41}$$

The variation of the parameter ax within the linearization procedure is performed in subroutine VORFIT.

Subroutine VORFIT: This subroutine tests the curvature of the transformed melting curve in the $(1/r, T)$-diagram inside the given linearization region (for a molten fraction of about 10–40%). The curvature is determined with segments between transformed points i and $i+j$, with i and $i+j$ restricted inside the linearization region, and for $j = 2, 3, 4, 5, 6$, etc.

When the curvature of the transformed melting curve is convex downwards, the distance between the curve and segments (calculated only for the transformed experimental points) is positive according to our definition, and is counted as an element of class *N1*. When the curvature is convex upwards, all the points are counted as class *N2*. The predominant curvature inside the linearization region is proportional to the absolute value of $\Delta N = N1 - N2$ and the sign of ΔN indicates whether the curvature is convex upwards or downwards. The sign of the parameter ax for the first linearization step is the same as the sign of ΔN calculated at the beginning of the linearization. Again, the values of *N1*, *N2* and ΔN are determined with the shifted values of the molten fraction according to Eqn. (41). If the sign of ΔN is not changing, the parameter $2ax$ is used for the second linearization step. If the sign of ΔN changes in the second linearization step, the parameter $0.5ax$ is applied to the next linearization. The linearization with the diminution of the parameter ax is performed until one of the following conditions is reached: (*i*) $|N1 - N2| \leq 1$; (*ii*) $|ax_{old} - ax_{new}| \leq 0.5\ cm^2$; or (*iii*) the number of steps is greater than 50.

The value of the parameter ax for the last linearization step is called POPTA, given in the printout as the value relative to the total area of the melting curve $a_{0,30}$

$$\text{POPT} = \frac{\text{POPTA}}{a_{0,30}} \times 100 \tag{42}$$

With a parameter value of $ax = 10\ cm^2$ at the beginning of the linearization, condition (ii) can be fulfilled within 5 linearization steps.

Subroutine FIT: The linearization in this subroutine starts with the last parameter value POPTA taken from the subroutine VORFIT. The subroutine performs a least-square fit with the parameter values POPTA, POPTA$\pm AX$. The parameter value AX is set to $0.5\ cm^2$ at the beginning. The sum of the squares of the deviation of the transformed points with respect to the regression line is minimised by changing the parameter AX. The least-square fit terminates under any of the following conditions: (*i*) $AX \leq 10^{-2}\ cm^2$; (*ii*) when the number of steps is greater than 30; or (*iii*) when the sum of the squares of the deviation of the transformed points from the regression lines is $\leq 10^{-5}$.

Subroutine KONZ: The subroutine KONZ calculates with the aid of Eqn. (7) the information which one can get from the regression line and the conditions of the last linearization step.

Subroutine CHECK: The subroutine CHECK calculates the distance between the transformed points and the regression line. The greatest distance within the linearization region is called DTMAX. The distance of transformed points outside the linearization region is compared with DTMAX. Any point with a distance less than DTMAX is added to the linearization region. The first point, beginning with the linearization limits, with a distance greater than DTMAX interrupts the enlargement of the region in this specific direction. If the distances of the points adjacent to the limits of linearization are greater than DTMAX, the enlargement of the linearization region is attempted by multiplying DTMAX by a factor f. The values of the factor f

```
2  1981         NICOTINSAEUREAMID
-----------------------------------------------------------------

EINWAAGE              =      3,4200  (IN MGR)                    TG-ALPHA   =     3.1200
MOLEKULARGEWICHT      =    122,1300  (IN GR)                     ALPHA      =    -0.1030
RANGE                 =      4,0000  (IN MCAL/SEC)               BETA       =    41.2900  (IN GRAD)
RANGE-FAKTOR          =      0,9640  (IN MCAL/(CM**2))           A          =     8.9000  (IN CM)
SCAN SPEED            =      1,0000  (IN GRAD/MIN)               B          =     9.8000  (IN CM)
                                                                 TT         =   399.0000  (IN GRAD KELVIN)
                                                                 ZVOR       =   240.0000  (IN INCH/H)
DTMAX                 = 0,488E-02  GRAD                          NPOT       =     1       (1=FLUESSIGKEITS-,2=NORMALBEHAELTER)

RESULTATE DES FIT-PROGRAMMS (MIT   0*DTMAX):

GRENZEN   J1 = 10 ;   J2 = 22                       SCHMELZBEREICH B(J1) =  12.18 %   B(J2) =  38.04 %
POPT                        =     2,06 %            VON FN(30)                          =   160.74              CM**2
SCHMELZTEMPERATUR   (TO)    =  127,27   GRAD CELSIUS  DELTA-H-F                         =  5647.40              CAL/MOL
SCHMELZTEMPERATUR   (TS)    =  127,22   GRAD CELSIUS  KONZENTRATION DER VERUNREINIGUNG  =   0.0953 +- 0.0029MOL-%
STEIGUNG DER GERADEN (S)    =   -0,0541 +-  0,0016  KRYOSKOPISCHE KONSTANTE             =   -0.57               GRAD/MOL-%
Q.-SUMME DER Y-ABWEICHUNGEN: M-1/B GROSS =  0,1214E-03   M-1/B KLEIN =  0,4099E-04
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

RESULTATE DES FIT-PROGRAMMS (MIT   1*DTMAX):

GRENZEN   J1 = 10 ;   J2 = 26                       SCHMELZBEREICH B(J1) =  10.34 %   B(J2) =  73.43 %
POPT                        =    -0,03 %            VON FN(30)                          =   160.74              CM**2
SCHMELZTEMPERATUR   (TO)    =  127,24   GRAD CELSIUS  DELTA-H-F                         =  5531.76              CAL/MOL
SCHMELZTEMPERATUR   (TS)    =  127,20   GRAD CELSIUS  KONZENTRATION DER VERUNREINIGUNG  =   0.0737 +- 0.0081MOL-%
STEIGUNG DER GERADEN (S)    =   -0,0427 +-  0,0047  KRYOSKOPISCHE KONSTANTE             =   -0.58               GRAD/MOL-%
Q.-SUMME DER Y-ABWEICHUNGEN: M-1/B GROSS =  0,2955E-03   M-1/B KLEIN =  0,5659E-03
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

RESULTATE DES FIT-PROGRAMMS (MIT   5*DTMAX):

GRENZEN   J1 =  9 ;   J2 = 26                       SCHMELZBEREICH B(J1) =  10.04 %   B(J2) =  73.65 %
POPT                        =     0,82 %            VON FN(30)                          =   160.74              CM**2
SCHMELZTEMPERATUR   (TO)    =  127,24   GRAD CELSIUS  DELTA-H-F                         =  5578.55              CAL/MOL
SCHMELZTEMPERATUR   (TS)    =  127,20   GRAD CELSIUS  KONZENTRATION DER VERUNREINIGUNG  =   0.0788 +- 0.0087MOL-%
STEIGUNG DER GERADEN (S)    =   -0,0453 +-  0,0050  KRYOSKOPISCHE KONSTANTE             =   -0.57               GRAD/MOL-%
Q.-SUMME DER Y-ABWEICHUNGEN: M-1/B GROSS =  0,4241E-03   M-1/B KLEIN =  0,5955E-03
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

RESULTATE DES FIT-PROGRAMMS (MIT  10*DTMAX):

GRENZEN   J1 =  8 ;   J2 = 26                       SCHMELZBEREICH B(J1) =   9.16 %   B(J2) =  73.91 %
POPT                        =     1,83 %            VON FN(30)                          =   160.74              CM**2
SCHMELZTEMPERATUR   (TO)    =  127,25   GRAD CELSIUS  DELTA-H-F                         =  5634.50              CAL/MOL
SCHMELZTEMPERATUR   (TS)    =  127,20   GRAD CELSIUS  KONZENTRATION DER VERUNREINIGUNG  =   0.0857 +- 0.0094MOL-%
STEIGUNG DER GERADEN (S)    =   -0,0488 +-  0,0053  KRYOSKOPISCHE KONSTANTE             =   -0.57               GRAD/MOL-%
Q.-SUMME DER Y-ABWEICHUNGEN: M-1/B GROSS =  0,8071E-03   M-1/B KLEIN =  0,6623E-03
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

Fig. 11. Nicotinamide, printout for curve 2, 1981.

are specified in the program as $f = 5$, 10, 15, 20, etc. The linearization procedure (subroutine VORFIT, FIT and KONZ) is performed again in each linearization region, which is found by enlargement. In routine work the computer program is restricted to three enlargements of the linearization region.

In Fig. 11 the printout for nicotinamide is shown together with the experimental conditions and the calibration factors of the instrument. The distance DTMAX is calculated as 4.8×10^{-3} °C. The first linearization region covers the melting region from 12.18 to 38.04% or from point 10 to point 22. The parameter POPT is 2.06%, which means that the baseline is shifted slighiy downwards. The melting points are: $T_1 = 127.27$ °C and $T_s = 127.22$ °C. The melting points yield a slope of the regression line of -0.05 °C. The heat of fusion is 5650 cal mole^{-1}. The concentration of the impurity is 0.095 ± 0.003 mole-% and the cryoscopic constant according to Eqn. (2) is 0.57 °C mole-%$^{-1}$. The enlargement of the linearization region to a linearization region from 9.16 to 73.91% indicates only small variations in the melting points, heats of fusion and concentrations of impurity.

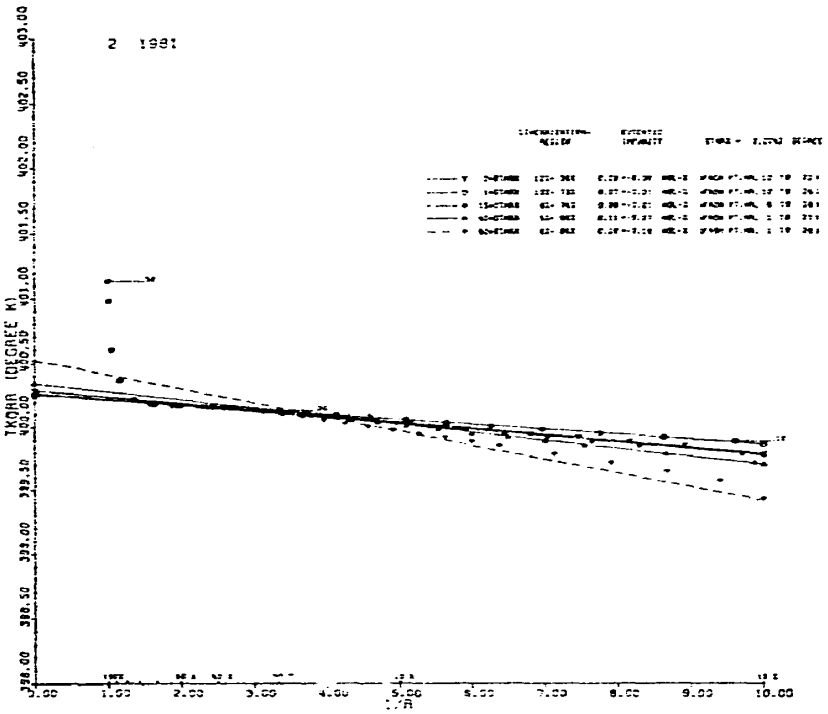


Fig. 12. (1/r, T)-diagram for nicotinamide.

The (1/r, T)-diagram of nicotinamide is presented in Fig. 12. The transformed points of the melting curve of nicotinamide are shown and also the regression lines

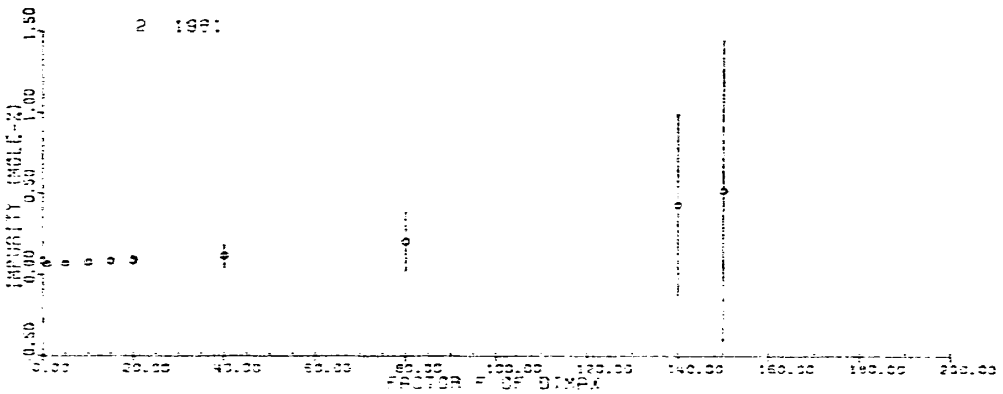


Fig. 13. Presentation of the impurity content of nicotinamide (number of curve 2, 1981) *vs.* the factor *f* of DTMAX.

calculated with points inside the linearization regions. In Fig. 13 the concentration of the impurity as a function of the distance *f* (DTMAX) is presented. The errors of the impurity concentration with 65% confidence limits, calculated with the regression line within the linearization region, are shown as lines through the corresponding points. The linearization region for the *f*-factor of 80 covers the molten fraction from 8 to 96% with a concentration of the impurity of nicotinamide of 0.20 ± 0.18 mole-%; a value which is consistent with the result from the first linearization region. The molten fraction from 8 to 96% covers a part of the melting curve from point 1 to point 28 (see Fig. 10). The point 26 is certainly an upper limit for the linearization region, which in the presented evaluation of the melting curve for nicotinamide implies an *f*-factor of 15 as an upper limit. The agreement between the results for these restricted linearization regions is even better.

The conclusion from such results on impurity values and thermodynamic constants, which are nearly independent of the melting region selected for the calculation, are a necessary but not sufficient condition for an ideal melting behaviour of a substance.

EXPERIMENTS AND DISCUSSION

(*a*) *High purity substances*

A few high purity substances are listed in Table XI. The melting curves of these substances were measured in volatile sample pans and the evaluation was performed according to the method explained in the preceding section, part (*b*). The cryoscopic constants of substances measured with the DSC-IB differ by less than 14% from literature values.

The difference is mainly caused by the heat of fusion measured with the DSC-IB. For high purity substances a 10% error in the evaluated heat of fusion will lead to only a negligible error in the eutectic impurity.

TABLE XI

PROPERTIES OF SOME HIGH PURITY SUBSTANCES

Substance	*Melting points (°K)*		*Difference in melting points* $\Delta T = T_1 - T_2$ (°C)	*Heat of fusion* $\Delta H_{f,1}$ (*cal mole*$^{-1}$)	*Cryoscopic constant* k_f (°C *mole-%*$^{-1}$)	*Eutectic impurity* (*mole-%*)
	T_1	T_2				
Deionized water						
DSC	273.261	273.230	0.031	1650	0.90	0.035
Lit.	273.16			1434	1.04	
Benzene (Merck)						
DSC	278.561	278.490	0.071	2180	0.71	0.10
Lit.	278.66			2350	0.67	
Anthraquinone						
DSC	557.9	557.7	0.043	8400	0.75	0.06
Lit.	558.6			7800	0.86	
Carbazole						
DSC	517.51	517.38	0.013	6500	0.82	0.15
Lit.	518.5			7040	0.86	
4-Acetaminophenol	441.67	441.62	0.051	7280	0.53	0.09
Nicotinamide	400.42	400.37	0.05	5650	0.57	0.09
Irgafen	481.117	481.110	0.007	7140	0.70	0.01
Development compounds						
MA 731, Batch 2	372.55	372,53	0.02	6710	0.33	0.06
MA 849, Batch 1	392.52	392.48	0.04	7770	0.40	0.10

(*b*) *Mixtures of standard substances*

Experiments with mixtures of standard substances are discussed. The mixtures were prepared in a laboratory type ball-mill grinder. In Table XII the actual and measured impurity values for the system phenacetin–*p*-aminobenzoic acid (*p*-ABA) are compared.

TABLE XII

COMPARISON OF THE ACTUAL AND MEASURED IMPURITY VALUES FOR THE PHENACETIN–*p*-AMINOBENZOIC ACID SYSTEM

Main component	*Scan speed* $\dot{T}$ (°C *min*$^{-1}$)	*Added p-ABA (% by weight)*	*Actual impurity* $n_{v,a}$ (*mole-%*)	*Measured impurity* $n_{v,m}$ (*mole-%*)	$\frac{n_{v,a} - n_{v,m}}{n_{v,a}} \times 100$ (%)
Phenacetin	1	0.2	0.43	0.38	+12
	1	0.4	0.70	0.59	+16
	1	0.6	0.96	1.06	−10
	1	0.8	1.23	1.29	−5
	1	1.0	1.50	1.79	−19
	2	1.0	1.50	1.52	−1
	2	1.0	1.50	1.49	+1

The impurities were measured in normal sample pans and the evaluation was performed by the simplest method suggested by Perkin–Elmer[13]. The agreement between actual and measured impurity is reasonable.

Impurity with a high vapor pressure — In the next step, measurements in different sample pans were carried out in the melting region with an impurity of high vapor pressure. The system chosen was phenacetin–acetanilide (ACD), the results are presented in Table XIII. This system was chosen to emphasize the importance of the type of sample pan used for analytical purposes.

TABLE XIII

INFLUENCE OF AN OPEN AND CLOSED SAMPLE PAN ON THE MEASURED IMPURITY IN THE CASE OF AN IMPURITY WITH A HIGH VAPOR PRESSURE

Substance	*Sample pan*	*Number of melt*	*Actual impurity (mole-%)*	*Measured impurity (mole-%)*	*Weight loss of sample pan during melting* $\times 10^{-6}$ *g*
Phenacetin	normal	1		0.20	−7
OAS		2		0.36	−20
		3		0.29	−30
Phenacetin	volatile	1		0.46	−1
OAS		2		0.35	−1
		3		0.28	0
Phenacetin	normal	1	2.4	1.27	−15
OAS		2		1.11	−31
+1.6% (w/w) ACD		3		0.97	−46
		4		0.86	−62
Phenacetin	volatile	1	2.4	1.51	+1
OAS	sample pan	2		1.23	+2
+1.6% (w/w) ACD	without inside	3		1.36	+2
	cover	4		1.44	−2
Phenacetin	volatile	1	2.4	1.73	0
OAS	sample pan	2		1.27	0
+1.6% (w/w) ACD	with inside cover				

Compared with the sample weight of about 3 mg, a remarkable loss of weight from the normal pans is observed. The measurements of the weights of the pans were carried out with a Cahn Electrobalance before and after the melting of the samples. The measured impurity for the pure main component, phenacetin OAS, taken as a mean value of the three consecutive melts, is in the volatile sample pan only 0.1 mole-% higher than in the normal pan. With the absolute value of about 0.4 mole-% for the impurity measured in the volatile sample pan for phenacetin OAS, it was calculated as a rough approximation (setting mole-% equal to % by weight) that the total amount of impurities in a sample was about 12×10^{-6} g. The total amount of impurities is, as may be seen from Table XIII, about equal to the weight loss from a normal pan during each melting run. A loss from the sample pan of 4×10^{-6} g of impurity would have a remarkable influence on the melting curve. No shift of the

eutectic impurities to lower values with increasing number of melts was observed. The conclusion is that the vapor pressure of all the eutectic impurities of phenacetin OAS must be very small compared with the main component. This condition must be fulfilled over the whole temperature range in the region of melting.

The pseudobinary system of phenacetin OAS and acetanilide melted in the normal pan shows a measured value for the eutectic impurity of only 50% of the actual impurity for the first melt, and only 36% for the melt number 4. The total amount of ACD in a sample is 48×10^{-6} g. A loss of 50% of ACD as eutectic impurity during the first melt is equal to 24×10^{-6} g. This amount has to be compared with a total loss out of the sample pan of 15×10^{-6} g. The loss out of the sample pan consists mainly of ACD. Such a conclusion may be drawn from the vapor pressure data of phenacetin and ACD. The vapor pressure of phenacetin for 115°C is 3.2×10^{-2} torr according to the measurements of Wiedemann[38]. For ACD, Cramer[39] has reported a vapor pressure at 115°C of 6.3×10^{-1} torr. If we make the assumption that the loss out of the sample pan is only caused by ACD, we would have to explain the difference between the loss of ACD from the melt and the total loss from the sample pan. There are two possible explanations: (*1*) The heat of evaporation caused by the loss of ACD from the sample pan is superimposed upon the heat of fusion. (*2*) The ACD evaporates from the liquid phase onto positions inside the sample pan which have a lower temperature compared to the melt.

Explanation *1* can be excluded by a rough calculation of the heat necessary for the loss of about 24×10^{-6} g ACD measured from the first melt, when compared with the heat of fusion necessary for the melting of a 3 mg sample; in our example the heat of evaporation is about 2% of the heat of fusion. This energy of evaporation is further spread over the whole temperature region of the melting process. Therefore, the calculated eutectic impurity is influenced only to a rather small extent.

In contrast, point *2* is somewhat more reasonable, because of the great temperature gradient inside the sample pan, caused by the construction of the DSC-IB sample pan holder.

The transport of the ADC inside the sample pans is also indicated by the measurements in the volatile sample pans with and without an inside cover. However, the difference between the actual and the measured impurity is least in the volatile sample pan with an inside cover.

(*c*) *Different evaluation procedures applied to systems of phenacetin and benzamide*

In the next experiments, several evaluation procedures on systems of phenacetin and benzamide are discussed. Phenacetin was chosen as the main component and benzamide as the so-called impurity. The evaluation procedures of the melting curves are compared with the results of the actual and the measured impurity, with the melting points, and with the heats of fusion. The evaluation procedures applied to each of the melting curves are described briefly.

Evaluation procedure "Normal" (*N*) — This evaluation procedure is based on Eqn. (*7*) and is explained in detail in the preceding section, part (*b*).

Evaluation procedure "Square Root" (SR) — The evaluation procedure SR uses the following equation

$$T = \frac{T_1}{2} + \frac{T_1}{2}\left[1 + \frac{4RT_1}{\Delta H_{f,1}}\left(\ln\left(1 - \frac{1}{r}x_{0,2}\right)\right)\right]^{1/2} \tag{43}$$

Eqn. (43) is obtained from Eqn. (14) by the approximation $\Delta c_{0,i} = 0$. The evaluation procedure SR performs a trial and error method in varying the parameters T_1, $\Delta H_{f,1}$, and $x_{0,2}$.

With a set of parameters T_1, $\Delta H_{f,1}$, and $x_{0,2}$, together with chosen experimental values of the molten fraction r, values of T_C may be determined. These calculated temperature values, T_C, are compared with the experimentally obtained values of T, and the sum of the squares of deviation $T_C - T$ is computed for all experimental points within a given region of the molten fraction. With a three-parameter reiteration procedure, the sum of the squares of deviation is brought to a certain small limit, chosen from experience.

Evaluation procedure "heat of fusion" (HF) — The evaluation and linearization of the melting curve is performed with the evaluation procedure "Normal". The only deviation from the evaluation procedure "Normal" is within the subroutine KONZ. In the calculations of the eutectic impurity, using Eqn. (7), the heat of fusion of the main component is taken from the literature or from a measurement of a high-purity sample, whereas in the evaluation procedure "Normal", the heat of fusion used in Eqn. (7) is calculated from the melting curve of the sample under investigation.

Evaluation procedure "correction to the weight of the main component" (CMC) — The evaluation is performed with the procedure "Normal". In the subroutine KONZ we do not use the weight of the sample, but only the weight of the main component.

The results of the measurements on the phenacetin–benzamide system are presented in Table XIV.

In Table XIV are tabulated the concentration of the impurity; the actual impurity, which is known from the benzamide added plus the eutectic impurity of phenacetin OAS; the melting points; the heats of fusion; and the eutectic impurity calculated with the evaluation procedures described, N, SR, HF, and CMC. The measured eutectic impurity $x_{0,2}$ is a function of two parameters; the evaluation procedure and the concentration of the impurity itself.

The melting points will be discussed first. The melting points of the main component, T_1, are nearly constant up to a concentration of 10 mole-% of benzamide. For higher values of the impurity concentration, T_1 is about 3°C too low, but the correction of the thermal lag, caused by a scan speed of 16°C min^{-1}, is not easy. The melting point of the sample, T_s, decreases with increasing impurity content because of the melting point depression. The shift of T_1 for high impurity values, which was explained by experimental reasons, should have pratically the same influence on T_s. Therefore, the temperature difference $\Delta T = T_1 - T_s$, which is important for the

TABLE XIV

RESULTS OF SEVERAL EVALUATION PROCEDURES ON THE PHENACETIN OAS–BENZAMIDE SYSTEM

Main component, phenacetin OAS; impurity, benzamide.

Benzamide added (mole-%)	*Actual impurity (mole-%)*	T_1	T_s	*Evaluation procedure*							
				N		*SR*		*HF*		*CMC*	
				$\Delta H_{f,1}$	$x_{0,2}$	$\Delta H_{f,1}$	$x_{0,2}$	$\Delta H_{f,1}$	$x_{0,2}$	$\Delta H_{f,1}$	$x_{0,2}$
0		133.7	133.6	7860	0.22	7270	0.14	7750	0.21	7860	0.22
1.25	1.47	133.2	133.5	7330	1.39	7300	1.34	7750	1.47	7400	1.40
2.5	2.72	133.2	132.2	7040	2.0 ±0.4	7035	1.9 ±0.3	7750	2.2 ±0.3	7150	2.1 ±0.3
5.0	5.22	133.5	131.4	6960	4.5 ±1.0	7120	4.5 ±1.1	7750	4.9 ±0.8	7190	4.6 ±1.0
10.0	10.2	132.5	129.0	6535	6.9 ±1.0	6710	6.6 ±1.4	7750	8.2 ±0.6	7050	7.5 ±1.0
20.0	20.2	130.7	123.8	6100	13.0 ±1.4	6585	12.7 ±1.0	7750	16.5 ±1.3	7170	15.5 ±1.8
30.0	30.2	130.7	119.0	5896	21.2 ±1.8	6710	20.5 ±1.8	7750	27.8 ±1.9	7570	27.3 ±2.3

calculation of the impurity, should only be affected very slightly by the shift in T_1 and T_s.

The results of the eutectic impurities calculated according to the different evaluation procedures are practically constant for the high-purity substance phenacetin OAS (see Table XIV). The calculated eutectic impurities for systems with concentrations of 1.25, 2.5, and 5 mole-% of benzamide are all well within a normal error limit compared to the actual eutectic impurity (error limit up to ±10% relative to the impurity value).

The differences in eutectic impurities calculated with four different evaluation procedures in the concentration region of 10–30 mole-% of benzamide are remarkable. With the normal evaluation procedure N, also with the evaluation SR, the eutectic impurities are found to be about 30% too low compared with the actual eutectic impurities. In contrast, the evaluation procedures HF and CMC show relative differences of only 10–20% between measured and actual eutectic impurities. The conclusion is that the normal and the square root evaluation methods are only capable of yielding good results in an impurity region up to 5 mole-%. The differences between the two procedures (N, SR) are so small that we do not use the square root method, which requires a much longer computing time than the evaluation procedure "Normal". The heat of fusion method is as easy as the "Normal" evaluation but one needs a high-purity standard or a literature value of the heat of fusion of the main component. The procedure "correction to the weight of the main component" can only be performed with known values of the weight of the main component. The CMC method is of theoretical interest, and the application to systems with unknown impurities would only be a rough approximation.

(d) Variation of scan speed and the use of two different data collection systems for melting curves

Measurements were performed on two systems: (*1*) Diphenyl as a high-purity substance; and (*2*) phenacetin OAS and 2.5 mole-% of benzamide. Also two systems for the collection of data were used for the measurements: (*1*) ERA, Digital Data Acquisition system; (*2*) Mauerhofer system by Ciba–Geigy, Basel. The ERA system collects data in the premelting, melting and postmelting range on magnetic tape. The data collection rate for our measurements was chosen as 20 points sec^{-1}. The Mauerhofer system was built by Ciba–Geigy, Basel. Here the data are collected on paper tape. The data collection rate for this system is 2 points sec^{-1}.

The experimental data collected from melting curves with both of the systems replace the 31 experimental points (x_i, y_i) in the evaluation procedure "Normal". The baseline of the melting curves, which is drawn by hand in the procedure "Normal", is calculated, in the case of the data systems, from points in the premelting and the postmelting ranges. If the number of experimental points in the melting region is too high (ERA system), a reduction in the number of points is obtained by forming a mean value from 10 or 20 adjacent points. Such a data reduction has a smoothing effect on the experimental curve.

The eutectic impurities calculated from melting curves recorded either with the ERA or the Mauerhofer system are presented in Table XV. The experimentally varied parameter in the table is the scan speed.

TABLE XV

EUTECTIC IMPURITY AS A FUNCTION OF SCAN SPEED FROM MELTING CURVES RECORDED WITH THE TWO DIFFERENT DATA COLLECTION SYSTEMS

Scan speed ($°C\ min^{-1}$)	*Eutectic impurity (mole-%)*		
	ERA system	*Mauerhofer system*	*Mettler DTA-2000 and CT system*[c]
Diphenyl			
0.5	0.13	0.08	0.12
1	0.20	0.05	0.13
2	0.16	0.07	0.12
4	0.23	0.05	0.25
8	0.20	[a]	0.88
Phenacetin OAS–2.5 mole-% Benzamide[b]			
4	2.4	2.7	
8	2.2	2.2	
16	2.3	2.2	
32	2.6	[a]	

[a] No evaluation possible. [b] Actual eutectic impurity, 2.7 mole-%. [c] Values added in proof.

The melting of diphenyl is extremely sharp because of the purity level; on the other hand, the melting region of phenacetin–benzamide is rather broad. Equilibrium conditions between temperature and the molten fraction are calculated from Eqn. (7)

and used in the computing procedure. Non-equilibrium conditions, which would be expected at high scan speeds and for high-purity substances, should have an influence on the eutectic impurity calculated with Eqn. (7) with the aid of a linearization in the ($1/r$, T)-diagram. The variations of the calculated impurities with the scan speed for each of the substances within each of the data collection systems are rather small, and there is no significant shift of these eutectic impurities as a function of the applied scan speed.

For diphenyl, the mean value of the eutectic impurity for the scan speed in the range 0.5–8 °C min^{-1} is 0.18 ± 0.04 in using the ERA system for the data collection, and 0.06 ± 0.02 in the scan speed range 0.5–4 °C min^{-1} for the Mauerhofer data system. The difference is rather large, but one has to take into consideration that the melting curves were measured with two different DSC-IB instruments and two different data collection systems. In the case of the phenacetine–benzamide system, the agreement between the actual and the measured eutectic impurities is reasonable for all the scan speeds applied.

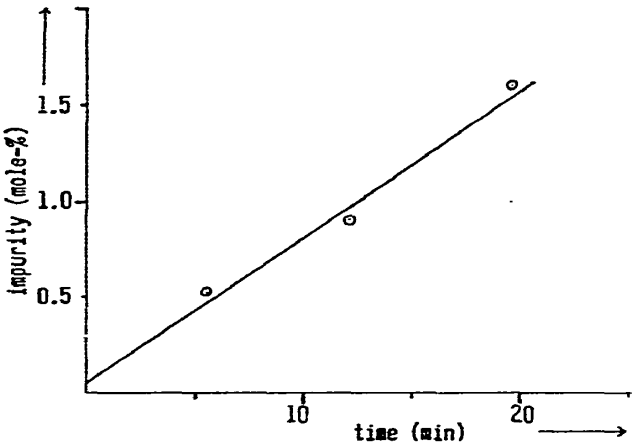


Fig. 14. Presentation of the decomposition of a sample of the development compound MA 1219 in a nitrogen atmosphere.

(*e*) *Purity measurements on substances which decompose in the melting region*

In the preceding section, part (*a*), we explained the necessary investigations on a substance which decomposes in the melting region. Melting curves are measured on the same substance with different thermal treatments. The calculated eutectic impurities of such melting curves are presented as a function of the time for which the substance was kept at a temperature close to the melting point with, of course, a correction for the time necessary for the melting. The measurements are performed in a nitrogen atmosphere in almost every case. An example is given in Fig. 14. The decomposition of this substance in a nitrogen atmosphere is calculated to be

TABLE XVI

COMPARAISON OF PURITY VALUES

Substance	*Batch No.*	*Sum of impurities*			*TLC* (%)	*GLC* (%)	*Titration* (%)	*NMR*
		Thermobalance (*weight-%*)	*DSC*					
			Volatile sample pan (*mole-%*)	*Open pan* (*mole-%*)				
Chlorpromazine-HCl	1	0.40	2.7	1.0	1.0	2.0		
Chlorpromazine-HCl	2	0.40	2.8	1.7	1.8	2.6		
Diazepam (1. Provenance)			0.4		0.10–0.15			
Diazepam (2. Provenance)			4.0		4.5–5.0	4.4		
Ethacrynic acid	1	0.05	1.6		1.1			
Ethacrynic acid	2	0.15	2.4	2.3	0.2			
Tolbutamide	1		1.8		1.6			
Cyclohexenylamine-HCl	1		0.6		pure	0.1		
Cyclohexenylamine-HCl	2		0.7		pure	0.1		
4-Acetaminophenol	1		0.09		0.1			
Nicotinamide	1		0.09		pure	0.02		
MA 731	1		0.9		0.6			
MA 731	2		0.4		0.2			
MA 956	1		0.2		0.05			
MA 956	2		0.1		0.05			
MA 1219	1		1.6		0.2			0.5 H_2O
MA 587	1	1.2	0.8		2–5		1.8–2.3	
MA 1017	2		1.0		pure			
MA 1017	2		1.0		pure	1.05		
MA 1017	4		1.5		0.15	1.2		
MA 1017	5		0.6		0.2	0.07		
MA 1469	3		0.2		0.02	pure		
MA 1769	3		0.4		pure	0.04		

0.075 mole-% min^{-1}. The eutectic impurity of the substance without thermal treatment (extrapolation of the thermal treatment to zero time) is 0.1 ± 0.1 mole-%. With air as the atmosphere for the melting, we observed a similar decomposition of the substance. This example shows the possibility of measuring impurity values of substances which are unstable in the melting region.

COMPARISON OF PURITY INFORMATION ON SEVERAL SUBSTANCES ACCORDING TO DSC, THERMOBALANCE, TLC, GLC, NMR-SPECTROSCOPY AND TITRATION

In Table XVI, a comparison of impurity values on substances obtained from several analytical methods is presented. The values headed "thermobalance" in Table XVI give the loss of weight of the substance up to the melting point. With a high amount of impurities of high vapor pressure in the melting region, one may expect quite a difference in the DSC values measured in the volatile and the open sample pan, as is seen for chlorpromazine–HCl. For many substances shown in Table XVI we see quite a reasonable agreement between the DSC values of impurities compared with the values from other analytical methods. However, there are substances like MA 1219 with a remarkable difference between the given impurity values. Such differences in impurity values obtained by several analytical methods provide a wide spectrum of problems to be solved with the appropriate investigations.

SUMMARY

The identification of substances and the determination of the purity of organic and inorganic substances by measurement of the melting point dates back to the early days of chemistry. In the 1920's Johnston and Giauque[3] introduced another thermal method for the purity determination of substances: the method of premelting. The method of Johnston and Giauque is based on a measurement of the heat of premelting of a substance as a function of temperature. The calorimeters used for the determination of the heat of premelting were built for sample weights up to several hundred grams. Relaxation times for the thermal equilibrium and the equilibrium of mass in the order of hours resulted from the large mass and the geometry of the calorimeters used in these investigations. In the 1960's a calorimeter (DSC) was developed by the Perkin–Elmer Corporation, which allowed the measurement of heats of premelting of a substance for samples of a few milligrams. The relaxation time for the DSC is in the order of parts of a second. The new instrument brought a fast development and a broad application of the method of premelting especially for purity measurements of pharmaceutical and agrochemical substances. Perkin–Elmer improved the DSC with the development of two further instruments: the DSC-IB and the DSC-2. All these three instruments are constructed according to the same basic principle, *i.e.* the measurement of temperature and heat of premelting, but differ in features such as: calorimetric sensitivity, baseline stability, temperature range, temperature calibration linearity and performance of the temperature programmer. Other instruments like

the DTA 2000 from Mettler Corporation, the Du Pont 900 can also be used for purity determinations.

In the literature mainly results on high purity substances are reported. A limitation of the purity region from 95 or even from 99 to 100% is claimed. These limitations of the method are substantiated by several authors because of marked differences between the actual and measured purity values. The discrepancies are explained in literature in terms of an inconsistency between the simplest equation for the solubility equilibrium and the melting behaviour of organic substances. We found that this explanation appears to be only one of several possibilities; other reasons may be the following: anomalous behaviour of main component and impurities, experimental conditions, recording system and data collection, and evaluation procedure—including the chosen equation for the description of the solid–liquid equilibrium—applied to the measured melting curve.

An investigation of these alternative reasons are rather cumbersome in the case of multi-component systems because of the multiplicity of the physical and chemical properties of all the components. Furthermore, the following aspects affecting the purity values should be considered: (i) the measurement of melting curves in open and closed sample pans, (ii) the use of different scan speeds, (iii) the measurement of a first and a second melting curve of the same sample, (iv) the influence of oxygen on the chemical stability of a substance in the melting region, (v) the proof of the ideality of a melting curve, and (vi) the evaluation of melting curves with a high enough number of data points and with the appropriate evaluation procedure.

It may be concluded from statements in the literature on the accuracy of purity values that there are two regions of purity with an arbitrary separation limit of 99 mole-%. The probability of a good agreement of actual and measured purity values is high in the high-purity region, and low in the low-purity region.

The work in our laboratory was concerned with a thorough investigation of effects causing these inconsistencies. Binary eutectic mixtures were selected as test systems, because phase diagrams, physical and chemical properties are easily found in literature. Using different equations for the description of the solubility equilibrium we have calculated theoretical phase diagrams and theoretical melting curves. The comparison of theoretical and experimental phase diagrams gives a measure of the quality of the approximation attained by the chosen equation for the solubility equilibrium. The study should be extended to include the influence of the activity coefficient on theoretical phase diagrams. Theoretical melting curves are useful for a proof of the ideality of experimental curves which is important for the reliability of calculated purity values.

Another application of theoretical melting curves is the determination of purity values by comparison of the experimental curves with a set of theoretical curves. These are calculated by selecting an equation for the solubility equilibrium, and transforming it into a function describing the theoretical melting curve (a so-called specific heat function). Thermal constants of the corresponding main component and a purity value are then inserted into the equation of the specific heat function.

Calculation and presentation of the specific heat functions for a set of purity values can be executed by computer. The comparison of the experimental curve with specific heat functions is performed either visually or by a least-square fit.

The transformation of theoretical melting curves calculated from the simplest equation of the solubility equilibrium on a temperature scale, $T-\Delta T$, is also of theoretical importance. The temperature difference $\Delta T = T_i - T_1$ is given by the difference of the melting point of the corresponding main component T_i and an arbitrarily chosen reference temperature T_1. The ratio of a transformed theoretical melting curve and a reference melting curve with the same concentration of eutectic impurities taken at corresponding temperatures is constant and equal to $[1+(\Delta T/T_1)]^{-2}$. The transformation reveals the fact, that melting curves of different main components with different melting points but equal concentrations of eutectic impurities are rather similar in their shape.

Systems with a complete series of solid solutions are treated at the present time by the measurement of melting or freezing points. A strong restriction in the quantitative determination of solid solutions is the necessity of knowing the melting point of the impurity forming solid solutions with the main component. Of course, it would be even better to know the phase diagram of the main component and the impurity. The accuracy of the purity measurements of solid solutions is related to the accuracy of the temperature measurement of the instrument. The determination of solid solutions is impossible in most of the systems with more than one impurity forming solid solutions.

In the evaluation procedure of melting curves for eutectic impurities one should consider the importance of the following: (i) the evaluation of melting curves measured with scan speeds up to 32°C min^{-1} is only possible in connection with a fast data collection system, (ii) the linearization with a least square fit is not without problems in case of practical melting curves, (iii) the first linearization should be performed in a linearization interval from about 15 to 35% of the substance melted and the interval should be extended for a succeeding linearization, and (iv) the evaluation procedures suggested by several authors (*e.g.* Scott and Gray[18]) are limited to a high purity region. Melting curves of substances with low purity must be treated by an evaluation procedure which corrects among others for the heat of fusion of the main component.

Ten thousand melting curves for more than 500 different compounds were measured and evaluated in our laboratories with three DSC-IB calorimeters since 1968. Several test systems were investigated for an evaluation of practical and theoretical aspects of the purity determination by DSC. Results from other analytical methods, especially for pharmaceutical and agrochemical substances, have been compared with results from the DSC method.

ACKNOWLEDGMENTS

The author wishes to express his thanks to the following persons for collaboration in experimental and theoretical work: O. Heiber, G. Tonn, W. Huber, A.

Geoffroy, B. Humpert and U. Rudolf. The author is also grateful for many helpful discussions with: S. G. Lawrence, J. Meier, H. C. Mez, P. Moser, G. Rihs, K. Wunder and R. Zbinden.

REFERENCES

1 L. Kofler and A. Kofler, *Thermomikromethoden zur Kennzeichnung organischer Stoffe und Stoffgemische*, Verlag Chemie, Weinheim (1954).
2 A. Eucken and E. Karwat, *Z. Phys. Chem.*, 112 (1924) 467.
3 H. L. Johnston and W. F. Giauque, *J. Amer. Chem. Soc.*, 51 (1929) 3194.
4 G. Pilcher, *Anal. Chim. Acta*, 17 (1957) 144.
5 A. R. Glasgow, N. C. Krouskop, J. Beadle, G. D. Axilrod, and F. D. Rossini, *Anal. Chem.*, 20 (1948) 410.
6 G. L. Driscoll, L. N. Duling, and F. Magnotta, *Proc. ACSS on Analytical Calorimetry, San Francisco*, 1968, p. 271.
7 E. F. Joy, J. D. Bonn, and A. J. Barnard Jr., *Thermochim. Acta*, 2 (1971) 57.
8 S. V. R. Mastrangelo and R. W. Dornte, *J. Amer. Chem. Soc.*, 77 (1955) 6200.
9 B. Wunderlich and St. M. Wolpert, *Therm. Anal., Proc. Int. Conf., 3rd, Davos*, 1 (1971) 17.
10 G. J. Davis and R. S. Porter, *J. Therm. Anal.*, 1 (1969) 449.
11 N. J. De Angelis and G. J. Papariello, *J. Pharm. Sci.*, 57 (1968) 1868.
12 R. Schumacher and B. Felder, *Z. Anal. Chem.*, 254 (1971) 265.
13 *Thermal Analysis Newsletter, Nos. 5 and 6*, Analytical Division, Perkin–Elmer Corp., Norwalk, Conn., U. S. A.
14 E. M. Barrall II and R. D. Diller, *Thermochim. Acta*, 1 (1970) 509.
15 R. Reubke and A. Mollica Jr., *J. Pharm. Sci.*, 56 (1967) 822.
16 R. N. Rogers and E. D. Morris Jr., *Anal. Chem.*, 38 (1966) 410.
17 M. J. O'Neill, *Anal. Chem.*, 36 (1964) 1238.
18 L. A. Scott and A. P. Gray, *DSC-Purity and the DSC-4 Computer Program for Purity Analysis*, Norwalk, Conn., U. S. A., 1969.
19 H. M. Heuvel and K. C. J. B. Lind, *Anal. Chem.*, 42 (1970) 1044.
20 W. L. Gent, *J. Sci. Instrum.*, 2 (1969) 69.
21 K. S. Pitzer and D. W. Scott, *J. Amer. Chem. Soc.*, 63 (1941) 2419.
22 D'Ans-Lax, *Taschenbuch für Chemiker und Physiker*, 2 (1964) 1076.
23 *Ibid.*, 2 (1964) 1076.
24 *Ibid.*, 2 (1964) 1075.
25 *Ibid.*, 2 (1964) 1085.
26 *Ibid.*, 1 (1964) 380.
27 *Ibid.*, 1 (1964) 350.
28 W. Perron, *Therm. Anal., Proc. Int. Conf., 3rd, Davos*, 1 (1971) 35.
29 M. J. O'Neill and A. P. Gray, *ibid.*, 1 (1971) 279.
30 A. P. Gray, *Proc. ACSS on Analytical Calorimetry, San Francisco*, 1968, p. 209.
31 C. Plato and A. R. Glasgow Jr., *Anal. Chem.*, 42 (1969) 330.
32 E. Marti, O. Heiber, W. Huber, and G. Tonn, *Therm. Anal., Proc. Int. Conf., 3rd, Davos*, 3 (1971) 83.
33 F. D. Rossini, *Chemical Thermodynamics*, Wiley, New York, (1950) 298.
34 J. H. Hildebrand and R. L. Scott, *The Solubility of Nonelectrolytes*, Dover, New York, (1964) 13.
35 *Beilsteins Handbuch der Organischen Chemie*, 13 (1930) 461.
36 *Ibid.*, 9E1 (1932) 96.
37 H. Staude, *Physikalisch-Chemisches Taschenbuch*, 2 (1949) 1179.
38 H. G. Wiedemann, *Thermochim. Acta*, 3 (1972) 355.
39 J. S. N. Cramer, *Rec. Trav. Chim. Pays-Bas*, 62 (1943) 606.
40 L. Kofler, *Z. Anal. Chem.*, 128 (1948) 533.
41 R. Haase and H. Schönert, *Solid–Liquid Equilibrium*, Pergamon Press, Glasgow, (1969) 60.
42 E. Marti and O. Heiber, *Meet. on the Purity Determination with the DSC-1B*, Perkin–Elmer Corp., Zürich, 1969.
43 F. S. Fawcett and H. E. Rasmussen, *J. Amer. Chem. Soc.*, 67 (1945) 1705.

United States Patent [19]

Aristoff

[11] **4,306,075**

[45] **Dec. 15, 1981**

[54] **COMPOSITION AND PROCESS**

[75] Inventor: **Paul A. Aristoff,** Portage, Mich.

[73] Assignee: **The Upjohn Company,** Kalamazoo, Mich.

[21] Appl. No.: **219,210**

[22] Filed: **Dec. 22, 1980**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 135,055, Mar. 28, 1980, abandoned.

[51] **Int. Cl.**[3] **C07C 177/00**

[52] **U.S. Cl.** ..... **560/56;** 568/734; 568/807; 260/239 BF; 568/808; 260/326.45; 260/465 F; 260/465 D; 260/326.5 C; 544/154; 544/171; 544/176; 544/336; 544/386; 546/203; 546/205; 546/285; 546/314; 546/309; 546/337; 548/250; 560/28; 562/466; 562/451; 562/452; 562/455; 564/80; 564/172; 564/174; 564/88; 564/90; 564/95; 564/158; 568/632; 568/633; 568/634

[58] **Field of Search** ..... 560/56, 28; 562/466, 562/451, 452, 455; 260/239 BF, 326.4 V, 465 F, 465 D, 326.5 C; 544/154, 171, 176, 336, 386; 546/203, 205, 285, 314, 309, 337; 548/280; 564/80, 172, 174, 88, 90, 95, 158; 568/632, 633, 634, 734, 807, 808

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

2017699 10/1979 United Kingdom 810/56

OTHER PUBLICATIONS

Derwent Abstract 48154B/26 J 54063059 05/21/79.

Primary Examiner—PauL J. Killos
Attorney, Agent, or Firm—L. Ruth Hattan; Robert A. Armitage

[57] **ABSTRACT**

The present specification provides novel analogs of carbacyclin (CBA_2), 6a-carba-prostacyclin (6a-carba-PGI_2), which have pronounced prostacyclin-like pharmacological activity, e.g., as platelet antiaggregatory agents. Specifically the novel chemical analogs of CBA_2 are those substituted by fluoro (C-5), alkyl (C-9), interphenylene (C-5), and methano (C-6a,9). Further provided are benzindene analogs of CBA_2 and substituted forms thereof, i.e., 9-deoxy-2',9-methano (or 2',9-metheno)-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-PGF_1compounds. Also provided are a variety of novel chemical intermediates, e.g., substituted bicyclo[3.3.-0]octane intermediates, and chemical process utilizing such intermediates which are useful in the preparation of the novel CBA_2 analogs.

13 Claims, No Drawings

COMPOSITION AND PROCESS

This application is a continuation-in-part of Ser. No. 135,055, filed Mar. 28, 1980, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to novel compositions of matter and novel processes for preparing these compositions of matter. Moreover, there are provided novel methods by which certain of these novel compositions of matter are employed for pharmacologically useful purposes. Further there are provided novel chemical intermediates for preparing these compositions of matter.

The present invention is specifically concerned with novel analogs of prostacyclin or PGI_2. Specifically, the present invention is concerned with analogs of carbacyclin modified at the C-5 or C-9 position, e.g., C-5 interphenylene analogs of carbacyclin, 5-fluoro analogs of carbacyclin, 9β-alkyl analogs of carbacyclin, C-6a,9 tricyclic (cyclopropyl) analogs of carbacyclin, and combinations thereof as well as novel benzidene analogs thereof.

Prostacyclin is an endogenously produced compound in mammalian species, being structurally and biosynthetically related to the prostaglandins (PG's). In particular, prostacyclin exhibits the structure and carbon atom numbering of formula I when the C-5,6 positions are unsaturated. For convenience, prostacyclin is often referred to simply as "PGI_2". Carbacyclin, 6a-carba-PGI_2, exhibits the structure and carbon atom numbering indicated in formula II when the C-5,6 positions are unsaturated. Likewise, for convenience, carbacyclin is referred to simply as "CBA_2".

A stable partially saturated derivative of PGI_2 is PGI_1 or 5,6-dihydro-PGI_2 when the C-5,6 positions are saturated, depicted with carbon atom numbering in formula II when the C-5,6 positions are saturated. The corresponding 5,6-dihydro-CBA_2 is CBA_1, depicted in formula II.

As is apparent from inspection of formulas I and II, prostacyclin and carbacyclin may be trivially named as derivatives of PGF-type compounds, e.g., $PGF_{2\alpha}$ of formula III. Accordingly, prostacyclin is trivially named 9-deoxy-6,9α-epoxy-(5Z)-5,6-didehydro-PGF_1 and carbacyclin is named 9-deoxy-6,9α-methano-(5E)-5,6-didehydro-PGF_1. For description of prostacyclin and its structural identification, see Johnson, et al., Prostaglandins 12:915 (1976).

For convenience, the novel prostacyclin or carbacyclin analogs will be referred to by the trivial, art-recognized system of nomenclature described by N. A. Nelson, J. Med. Chem. 17:911 (1974) for prostaglandins. Accordingly, all of the novel prostacyclin derivatives herein will be named as 9-deoxy-PGF_1-type compounds, PGI_2 derivatives, or preferably as CBA_1 or CBA_2 derivatives.

In the formulas herein, broken line attachments to a ring indicate substituents in the "alpha" (α) configuration, i.e., below the plane of said ring. Heavy solid line attachments to a ring indicate substituents in the "beta" (β) configuration, i.e., above the plane of said ring. The use of wavy lines ($\sim$) herein will represent attachment of substituents in the alpha or beta configuration or attached in a mixture of alpha and beta configurations. Alternatively wavy lines will represent either an E or Z geometric isomeric configuration or the mixture thereof.

A side chain hydroxy at C-15 in the formulas herein is in the S or R configuration as determined by the Cahn-Ingold-Prelog sequence rules, J. Chem. Ed. 41:16 (1964). See also Nature 212:38 (1966) for discussion of the stereochemistry of the prostaglandins which discussion applies to the novel prostacyclin or carbacyclin analogs herein. Molecules of prostacyclin and carbacyclin each have several centers of asymmetry and therefore can exist in optically inactive form or in either of two enantiomeric (optically active) forms, i.e., the dextrorotatory and laveorotatory forms. As drawn, the formula for PGI_2 corresponds to that endogenously produced in the mammalian species. In particular, refer to the stereochemical configuration at C-8 (α), C-9 (α), C-11 (α) and C-12 (β) of endogenously produced prostacyclin. The mirror image of the above formula for prostacyclin represents the other enantiomer. The racemic form of prostacyclin contains equal numbers of both enantiomeric molecules.

For convenience, reference to prostacyclin and carbacyclin will refer to the optically active form thereof. Thus, with reference to prostacyclin, reference is made to the form thereof with the same absolute configuration as that obtained from the mammalian species.

The term "prostacyclin-type" product, as used herein, refers to any cyclopentane derivative herein which is useful for at least one of the same pharmacological purposes for which prostacyclin is employed. A formula as drawn herein which depicts a prostacyclin-type product or an intermediate useful in the preparation thereof, represents that particular stereoisomer of the prostacyclin-type product which is of the same relative stereochemical configuration as prostacyclin obtained from mammalian tissues or the particular stereoisomer of the intermediate which is useful in preparing the above stereoisomer of the prostacyclin type product.

The term "prostacyclin analog" or "carbacyclin analog" represents that stereoisomer of a prostacyclin-type product which is of the same relative stereochemical configuration as prostacyclin obtained from mammalian tissues or a mixture comprising stereoisomer and the enantiomers thereof. In particular, where a formula is used to depict a prostacyclin type product herein, the term "prostacyclin analog" or "carbacyclin analog" refers to the compound of that formula or a mixture comprising that compound and the enantiomer thereof.

PRIOR ART

Carbacyclin and closely related compounds are known in the art. See Japanese Kokia 63,059 and 63,060, also abstracted respectively as Derwent Farmdoc CPI Numbers 48154B/26 and 48155B/26. See also British published specifications 2,012,265 and German Offenlungsschrift 2,900,352, abstracted as Derwent Farmdoc CPI Number 54825B/30. See also British published application Nos. 2,017,699, 2,014,143 and 2,013,661.

The synthesis of carbacyclin and related compounds is also reported in the chemical literature, as follows: Morton, D. R., et al., J. Organic Chemistry, 44:2880 (1979); Shibasaki, M., et al. Tetrahedron Letters, 433–436 (1979); Kojima, K., et al., Tetrahedron Letters, 3743–3746 (1978); Nicolaou, K. C., et al., J. Chem. Soc., Chemical Communications, 1067–1068 (1978); Sugie, A., et al., Tetrahedron Letters 2607–2610 (1979); Shibasaki, M., Chemistry Letters, 1299–1300 (1979),

and Hayashi, M., Chem. Lett. 1437–1440 (1979); and Li, Tsung-tee, "A Facile Synthesis of 9(0)-Methano-prostacyclin", Abstract No. 378, (Organic Chemistry), and P. A. Aristoff, "Synthesis of 6a-Carbaprostacyclin I_2", Abstract No. 236 (Organic Chemistry) both at Abstract of Papers (Part II) Second Congress of the North American Continent, San Francisco, California (Las Vegas, Nevada), USA, 24–29 August 1980.

7-Oxo and 7-hydroxy-CBA_2 compounds are apparently disclosed in U.S. Pat. No. 4,192,891. 19-Hydroxy-CBA_2 compounds are disclosed in U.S. Ser. No. 54,811, filed 5 July 1979. CBA_2 aromatic esters are disclosed in U.S. Pat. No. 4,180,657. 11-Deoxy-Δ^{10}- or Δ^{11}-CBA_2 compounds are described in Japanese Kokai No. 77/24,865, published 24 Feb. 1979.

SUMMARY OF THE INVENTION

The present specification particular by provides:

(a) a carbacyclin intermediate of formula IV, V, VI, VII, VIII, or IX; and

(b) a carbacyclin analog of formula X or XI;

wherein g is 0, 1, 2, or 3;

wherein n is one or 2;

wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro;

wherein M_1 is α-OH:β-R_5 or α-R_5:β-OH, wherein R_5 is hydrogen or methyl;

wherein M_6 is α-OR_{10}:β-R_5 or α-R_5:β-OR_{10}, wherein R_5 is hydrogen or methyl and R_{10} is an acid hydrolyzable protective group;

wherein R_7 is

(1) $—C_mH_{2m}—CH_3$, wherein m is an integer from one to 5, inclusive,

(2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, $(C_1–C_3)$alkyl, or $(C_1–C_3)$alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different,

(3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, $(C_1–C_3)$alkyl, or $(C_1–C_3)$alkoxy, with the proviso that not more than two substituents are other than alkyl,

(4) cis—CH=CH—CH_2—CH_3,

(5) $—(CH_2)_2—CH(OH)—CH_3$, or

(6) $—(CH_2)_3—CH=C(CH_3)_2$;

wherein $—C(L_1)$-R_7 taken together is

(1) $(C_4–C_7)$cycloalkyl optionally substituted by one to 3 $(C_1–C_5)$ alkyl;

(2) 2-(2-furyl)ethyl,

(3) 2-(3-thienyl)ethoxy, or

(4) 3-thienyloxymethyl;

wherein R_8 is hydroxy, hydroxymethyl, or hydrogen;

wherein R_{15} is hydrogen or fluoro;

wherein R_{16} is hydrogen or R_{16} and R_{17} taken together are $—CH_2—$ or R_{16} and R_{47} taken together form a second valence bond between C-6a and C-9 or are $—CH_2—$;

wherein R_{17} is as defined above or is

(1) hydrogen, or

(2) $(C_1–C_4)$alkyl;

wherein R_{18} is hydrogen, hydroxy, hydroxymethyl, $—OR_{10}$ or $—CH_2OR_{10}$, wherein R_{10} is an acid-hydrolyzable protective group; wherein

(1) R_{20}, R_{21}, R_{22}, R_{23}, and R_{24} are all hydrogen with R_{22} being either α-hydrogen or β-hydrogen,

(2) R_{20} is hydrogen, R_{21} and R_{22} taken together form a second valence bond between C-9 and C-6a, and R_{23} and R_{24} taken together form a second valence bond between C-8 and C-9 or are both hydrogen, or

(3) R_{22}, R_{23}, and R_{24} are all hydrogen, with R_{22} being either α-hydrogen or β-hydrogen, and

(a) R_{20} and R_{21} taken together are oxo, or

(b) R_{20} is hydrogen and R_{21} is hydroxy, being α-hydroxy or β-hydroxy;

wherein R_{27} is the same as R_7 except that $—(CH_2)_2—CH(OH)—CH_3$ is $—(CH_2)—CH(OR_{11})—CH_3$;

wherein R_{32} is hydrogen or R_{31}, wherein R_{31} is a hydroxyl hydrogen replacing group;

wherein R_{33} is —CHO or $—CH_2OR_{32}$, wherein R_{32} is as defined above;

wherein R_{47} is as defined above or is

(1) $(C_1–C_4)$alkyl, or

(2) $—CH_2OH$;

wherein X_1 is

(1) $—COOR_1$, wherein R_1 is

(a) hydrogen,

(b) $(C_1–C_{12})$alkyl,

(c) $(C_3–C_{10})$cycloalkyl,

(d) $(C_7–C_{12})$aralkyl,

(e) phenyl, optionally substituted with one, 2 or 3 chloro or $(C_1–C_3)$alkyl,

(f) phenyl substituted in the para position by

(i) $—NH—CO—R_{25}$,

(ii) $—CO—R_{26}$,

(iii) $—O—CO—R_{54}$, or

(iv) $—CH=N—NH—CO—NH_2$ wherein R_{25} is methyl, phenyl, acetamidophenyl, benzamidophenyl, or $—NH_2$; R_{26} is methyl, phenyl, $—NH_2$, or methoxy; and R_{54} is phenyl or acetamidophenyl; inclusive, or

(g) a pharmacologically acceptable cation;

(2) $—CH_2OH$,

(3) $—COL_4$, wherein L_4 is

(a) amino of the formula $—NR_{51}R_{52}$, wherein R_{51} and R_{52} are

(i) hydrogen,

(ii) $(C_1–C_{12})$alkyl,

(iii) $(C_3–C_{10})$cycloalkyl,

(iv) $(C_7–C_{12})$aralkyl,

(v) phenyl, optionally substituted with one, 2 or 3 chloro, $(C_1–C_3)$alkyl, hydroxy, carboxy, $(C_2–C_5)$alkoxycarbonyl, or nitro,

(vi) $(C_2–C_5)$carboxyalkyl,

(vii) $(C_2–C_5)$carbamoylalkyl,

(viii) $(C_2–C_5)$cyanoalkyl,

(ix) $(C_3–C_6)$acetylalkyl,

(x) $(C_7–C_{11})$benzoalkyl, optionally substituted by one, 2 or 3 chloro, $(C_1–C_3)$alkyl, hydroxy, $(C_1–C_3)$alkoxy, carboxy, $(C_2–C_5)$alkoxycarbonyl, or nitro,

(xi) pyridyl, optionally substituted by one, 2 or 3 chloro, $(C_1–C_3)$alkyl, or $(C_1–C_3)$alkoxy,

(xii) $(C_6–C_9)$pyridylalkyl optionally substituted by one, 2 or 3 chloro, $(C_1–C_3)$alkyl, hydroxy, or $(C_1–C_3)$alkyl,

(xiii) $(C_1–C_4)$hydroxyalkyl,

(xiv) $(C_1–C_4)$dihydroxyalkyl,

(xv) (C_1-C_4)trihydroxyalkyl,

with the further proviso that not more than one of R_{51} and R_{52} is other than hydrogen or alkyl,

(b) cycloamino selected from the group consisting of pyrolidino, piperidino, morpholino, piperazino, hexamethyleneimino, pyrrolino, or 3,4-didehydropiperidinyl optionally substituted by one or 2 (C_1-C_{12})alkyl of one to 12 carbon atoms, inclusive,

(c) carbonylamino of the formula $-NR_{53}COR_{51}$, wherein R_{23} is hydrogen or (C_1-C_4)alkyl and R_{51} is other than hydrogen, but otherwise as defined above,

(d) sulfonylamino of the formula $-NR_{53}SO_2R_{51}$, wherein R_{21} and R_{23} are as defined in (c),

(4) $-CH_2NL_2L_3$, wherein L_2 and L_3 are hydrogen or (C_1-C_4)alkyl, being the same or different, or the pharmacologically acceptable acid addition salts thereof when X_1 is $-CH_2NL_2L_3$,

wherein Y_1 is trans—CH=CH—, cis—CH=CH—, $-CH_2CH_2-$, or —C≡C—;

wherein Z_1 is

(1) $-CH_2-(CH_2)_f-C(R_2)_2$, wherein R_2 is hydrogen or fluoro and f is zero, one, 2, or 3,

(2) trans—CH_2—CH=CH—,

(3) —(Ph)—$(CH_2)_g$—, wherein (Ph) is 1,2-, 1,3-, or 1,4-phenylene and g is zero, one, 2, or 3;

wherein Z_4 is $-CH_2-$ or $-(CH_2)_f-CF_2$, wherein f is as defined above;

with the overall proviso that

(1) R_{15}, R_{16}, and R_{17} are all hydrogen only when Z_1 is —(Ph)—$(CH_2)_g$—, and

(2) Z_1 is —(Ph)—$(CH_2)_g$— only when R_{15} is hydrogen.

With regard to the divalent substituents described above (e.g., L_1 and M_1), these divalent radicals are defined as α-R_i:β-R_j, wherein R_i represents the substituent of the divalent moiety in the alpha configuration with respect to the plane of the C-8 to C-12 cyclopentane ring and R_j represents the substituent of the divalent moiety in the beta configuration with respect to the plane of the ring. Accordingly, when M_1 is defined as α-OH:β-R_5, the hydroxy of the M_1 moiety is in the alpha configuration, i.e., as in PGI_2 above, and the R_5 substituent is in the beta configuration.

The carbon atom content of various hydrocarbon-containing moieties is indicated by a prefix designating the minimum and maximum number of carbon atoms in the moiety, i.e., the prefix (C_i-C_j) indicates a moiety of the integer "i" to the integer "j" carbon atoms, inclusive. Thus (C_1-C_3)alkyl refers to alkyl of one to 3 carbon atoms, inclusive, or methyl, ethyl, propyl, and isopropyl.

Certain novel prostacyclin analogs herein, i.e., formula X compounds, are all named as CBA_1 or CBA_2 compounds, respectively, by virtue of the substitution of methylene for oxa in the heterocyclic ring of prostacyclin and the substitution. CBA_2 compounds are those exhibiting the olefinic double bond at C-5,6, while CBA_1 compounds are those saturated at C-5,6. Formula XI compounds are named as PGE_1 or PGF_1 derivatives as hereinafter described.

Novel compounds wherein Z_1 is (Ph)-$(CH_2)_g$ are designated inter-o-, inter-m-, or inter-p-phenylene depending on whether the attachment between C-5 and the $-(CH_2)_g-$ moiety is ortho, meta, or para, respectively.

For those compounds wherein g is zero, one, 2 or 3, the carbacyclin analogs so described are further characterized as 2,3,4-trinor-, 3,4-dinor-, or 4-nor, since in this event the X_1-terminated side chain contains (not including the phenylene) 2, 3, or 4 carbon atoms, respectively, in place of the five carbon atoms contained in PGI_2. The missing carbon atom or atoms are considered to be at the C-4 to C-2 positions such that the phenylene is connected to the C-5 and C-1 to C-3 positions. Accordingly these compounds are named as 1,5- 2,5-, 3,5-, and 4,5-inter-phenylene CBA compounds when g is zero, one, 2, or 3, respectively.

Those CBA analogs wherein Z_1 is $-CH_2-(CH_2)_f-CF_2-$ are characterized as "2,2-difluoro-" compounds. For those compounds wherein f is zero, 2, or 3, the carbacyclin analogs so described are further characterized as 2-nor, 2a-homo, or 2a,2b-dihomo, since in this event the X_1-terminated side chain contains 4, 6, or 7 carbon atoms, respectively, in place of the five carbon atoms contained in CBA_2. The missing carbon atom is considered to be at the C-2 position such that the C-1 carbon atoms is connected to the C-3 position. The additional carbon atom or atoms are considered as though they were inserted between the C-2 and C-3 positions. Accordingly these additional carbon atoms are referred to as C-2a and C-2b, counting from the C-2 to the C-3 position.

Those CBA analogs wherein Z_1 is trans—CH_2—CH=CH— are described as "trans-2,3-didehydro-CBA" compounds.

Those novel compounds where n is 2 are further characterized as 7a-homo-CBA compounds by virtue of the cyclohexyl ring replacing the heterocyclic ring of prostacyclin.

Further, the novel compounds are named as 9β-alkyl-CBA compounds when R_{17} is alkyl.

When R_{16} and R_{17} taken together are $-CH_2-$(methylene), the novel compounds so described are "6$\alpha\beta$,9β-methano-CBA" compounds by virtue of the methylene bridge between C-6a and C-9.

When R_{15} is fluoro, "5-fluoro-CBA" compounds are described.

The formula XI CBA analogs wherein R_{20}, R_{21}, R_{22}, R_{23}, and R_{24} are all hydrogen with R_{22} being β-hydrogen are characterized as "9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" compounds. Corresponding compounds wherein R_{22} is α-hydrogen are characterized as "9-deoxy-2',9β-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" compounds. CBA analogs wherein R_{20}, R_{23}, and R_{24} are all hydrogen and R_{21} and R_{22} taken together form a valence bond between C-9 and C-6a are characterized as "9-deoxo-2',9-metheno-3-oxo-3,4,5-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" compounds. CBA analogs wherein R_{20} is hydrogen and R_{21} and R_{22} taken together form a second valence bond between C-9 and C-6a and R_{23} and R_{24} taken together form a second valence bond between C-7 and C-8 are characterized as "9-deoxo-2',9-metheno-3-oxa-3,4,5-trinor-3,7-(1',3'-inter-phenylene)-7,8-didehydro-PGE_1" compounds. The formula XI CBA analogs wherein R_{22}, R_{23}, and R_{24} are all hydrogen and R_{20} and R_{21} taken together are oxo are characterized as "6a-oxo-9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" or "6a-oxo-9-deoxy-2',9β-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" depending on whether R_{22} is α-hydrogen or β-hydrogen, respectively. Formula XI CBA analogs wherein R_{20}, R_{22}, R_{23}, and R_{24}

are all hydrogen and R_{21} is α-hydroxy are characterized as "6aα-hydroxy-9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" or "6aα-hydroxy-9-deoxy-2',9β-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" compounds depending on whether R_{22} is α-hydrogen or β-hydrogen, respectively. Finally, formula XI TXA analogs wherein R_{20}, R_{22}, R_{23}, and R_{24} are all hydrogen and R_{21} is β-hydroxy are characterized as "6aβ-hydroxy-9-deoxy-2',9β-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" or "6aβ-hydroxy-9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1" compounds depending on whether R_{22} is α-hydrogen or β-hydrogen, respectively. When Z_4 is $—(CH_2)_f—CF_2$ and f is zero, the formula XI CBA analogs are additionally characterized as "2,2-difluoro" compounds. When f is one, 2, or 3, such compounds are additionally characterized as "2a-homo", "2a,2b-dihomo" or "2a,2b,2c-trihomo" compounds.

When R_5 is methyl, the carbacyclin analogs are all named as "15-methyl-CBA" compounds. Further, except for compounds wherein Y_1 is cis—CH=CH—, compounds wherein the M_1 moiety contains an hydroxyl in the beta configuration are additionally named as "15-epi-CBA" compounds.

For the compounds wherein Y_1 is cis—CH=CH—, then compounds wherein the M_1 moiety contains an hydroxyl in the alpha configuration are named as "15-epi-CBA" compounds. For a description of this convention of nomenclature for identifying C-15 epimers, see U.S. Pat. No. 4,016,184, issued 5 Apr. 1977, particularly columns 24–27 thereof.

The novel carbacyclin analogs herein which contain $—(CH_2)_2—$, cis—CH=CH—, or —C≡C— as the Y_1 moiety, are accordingly referred to as "13,14-dihydro", "cis-13", or "13,14-didehydro" compounds, respectively.

When R_7 is straight chained $—C_mH_{2m}—CH_3$, wherein m is as defined above, the compounds so described are named as "19,20-dinor", "20-nor", "20-methyl" or "20-ethyl" compounds when m is one, 2, 4 or 5, respectively. When R_7 is branched chain $—C_mH_{2m}—CH_3$, then the compounds so described are "17-, 18-, 19-, or 20-alkyl" or "17,17-, 17,18-, -17,19-, 17,20-, 18,18-, 18,19-, 18,20-, 19,19-, or 19,20-dialkyl" compounds when m is 4 or 5 and the unbranched portion of the chain is at least n-butyl, e.g., "17,20-dimethyl" compounds are described when m is 5 (1-methylpentyl).

When R_7 is phenyl and neither R_3 and R_4 is methyl, the compounds so described are named as "16-phenyl-17,18,19,20-tetranor" compounds. When R_7 is substituted phenyl, the corresponding compounds are named as "16-(substituted phenyl)-17,18,19,20-tetranor" compounds. When one and only one of R_3 and R_4 is methyl or both R_3 and R_4 are methyl, then the corresponding compounds wherein R_7 is as defined in this paragraph are named as "16-phenyl or 16-(substituted phenyl)-18,19,20-trinor" compounds or "16-methyl-16-phenyl- or 16-(substituted phenyl)-18,19,20-trinor" compounds respectively.

When R_7 is benzyl, the compounds so described are named as "17-phenyl-18,19,20-trinor" compounds. When R_7 is substituted benzyl, the corresponding compounds are named as "17-(substituted phenyl)-18,19,20-trinor" compounds.

When R_7 is phenylethyl, the compounds so described are named as "18-phenyl-19,20-dinor" compounds. When R_7 is substituted phenylethyl, the corresponding compounds are named as "18-(substituted phenyl)-19,20-dinor" compounds.

When R_7 is phenylpropyl, the compounds so described are named as "19-phenyl-20-nor" compounds. When R_7 is substituted phenylpropyl the corresponding compounds are named as "19-(substituted phenyl)-20-nor" compounds.

When R_7 is phenoxy and neither R_3 nor R_4 is methyl, the compounds so described are named as "16-phenoxy-17,18,19,20-tetranor" compounds. When R_7 is substituted phenoxy, the corresponding compounds are named as "16-(substituted phenoxy)-17,18,19,20-tetranor" compounds. When one and only one of R_3 and R_4 is methyl or both R_3 and R_4 are methyl, then the corresponding compounds wherein R_7 is as defined in this paragraph are named as "16-phenoxy or 16-(substituted phenoxy)-18,19,20-trinor" compounds or "16-methyl-16-phenoxy- or 16-(substituted phenoxy)18,19,20-trinor" compounds, respectively.

When R_7 is cis—CH=CH—CH_2CH_3, the compounds so described are named as "cis-17,18-didehydro" compounds.

When R_7 is $—(CH_2)_2—CH(OH)—CH_3$, the compounds so described are named as "19-hydroxy" compounds.

When R_7 is $—(CH_2)_3—CH=C(CH_3)_2$, the compounds so described are named as "20-isopropylidene" compounds.

When $—C(L_1)-R_7$ is optionally substituted cycloalkyl, 2-(2-furyl)ethyl, 2-(3-thienyl)ethyl, or 3-thienyloxymethyl, the compounds so described are respectively 15-cycloalkyl-16,17,18,19,20-pentanor compounds, 17-(2-furyl)-18,19,20-trinor-CBA compounds, 17-(3-thienyl)-18,19,20-trinor compounds, or 16-(3-thienyl-)oxy-17,18,19,20-tetranor compounds.

When at least one of R_3 and R_4 is not hydrogen then (except for the 16-phenoxy or 16-phenyl compounds discussed above) there are described the "16-methyl" (one and only one of R_3 and R_4 is methyl), "16,16-dimethyl" (R_3 and R_4 are both methyl), "16-fluoro" (R_3 or R_4 is fluoro), "16,16-difluoro" (R_3 and R_4 are both fluoro) compounds. For those compounds wherein R_3 and R_4 are different, the prostaglandin analogs so represented contain an asymmetric carbon atom at C-16. Accordingly, two epimeric configurations are possible: "(16S)" and "(16R)". Further, there is described by this invention the C-16 epimeric mixture: "(16RS)".

When X_1 is $—CH_2OH$, the compounds so described are named as "2-decarboxy-2-hydroxymethyl" compounds.

When X_1 is $—CH_2NL_2L_3$, the compounds so described are named as "2-decarboxy-2-aminomethyl" or "2-(substituted amino)methyl" compounds.

When X_1 is $—COL_4$, the novel compounds herein are named as CBA-type amides. Further, when X_1 is $—COOR_1$, the novel compounds herein are named as CBA-type esters and CBA-type salts.

Examples of phenyl esters substituted in the para position (i.e., X_1 is $—COOR_1$, R_1 is p-substituted phenyl) include p-acetamidophenyl ester, p-benzamidophenyl ester, p-(p-acetamidobenzamido)phenyl ester, p-(p-benzamidobenzamido)phenyl ester, p-aminocarbonylaminophenyl ester, p-acetylphenyl ester, p-benzylphenyl ester, p-amidocarbonylphenyl ester, p-methoxycarbonylphenyl ester, p-benzoyloxyphenyl ester, p-(p-acetamidobenzoyloxy)phenyl ester, and p-hydroxybenzaldehyde semicarbazone ester.

Examples of novel amides herein (i.e., X_1 is —COL_4) include the following:

(1) Amides within the scope of alkylamino groups of the formula-$NR_{51}R_{52}$ are methylamide, ethylamide, n-propylamide, n-butylamide, n-pentylamide, n-hexylamide, n-heptylamide, n-octylamide, n-nonylamide, n-decylamide, n-undecylamide, and n-dodecylamide, and isomeric forms thereof. Further examples are dimethylamide, diethylamide, di-n-propylamide, di-n-butylamide, methylethylamide, methylpropylamide, methylbutylamide, ethylpropylamide, ethylbutylamide, and propylbutylamide. Amides within the scope of cycloalkylamino are cyclopropylamide, cyclobutylamide, cyclopentylamide, 2,3-dimethylcyclopentylamide, 2,2-dimethylcyclopentylamide, 2-methylcyclopentylamide, 3-tert-butylcyclopentyl amide, cyclohexylamide, 4-tert-butylcyclohexylamide, 3-isopropylcyclohexylamide, 2,2-dimethylcyclohexylamide, cycloheptylamide, cyclooctylamide, cyclononylamide, cyclodecylamide, N-methyl-N-cyclobutylamide, N-methyl-N-cyclopentylamide, N-methyl-N-cyclohexylamide, N-ethyl-N-cyclopentylamide, and N-ethyl-Ncyclohexylamide. Amides within the scope of aralkylamino are benzylamide, 2-phenylethylamide, and N-methyl-N benzylamide. Amides within the scope nf substituted phenylamide are p-chloroanilide, m-chloroanilide, 2,4-dichloroanilide, 2,4,6-trichloroanilide, m-nitroanilide, p-nitroanilide, p-methoxyanilide, 3,4-dimethoxyanilide, 3,4,5-trimethoxyanilide, p-hydroxymethylanilide, p-methylanilide, m-methyl anilide, p-ethylanilide, t-butylanilide, p-carboxyanilide, p-methoxycarbonyl anilide, p-carboxyanilide and o-hydroxyanilide. Amides within the scope of carboxyalkylamino are carboxyethylamide, carboxypropylamide and carboxymethylamide, carboxybutylamide. Amides within the scope of carbamoylakylamino are carbamoylmethylamide, carbamoylethylamide, carbamoylpropylamide, and carbamoylbutylamide. Amides within the scope of cyanoalkylamino are cyanomethylamide, cyanoethylamide, cyanopropylamide, and cyanobutylamide. Amides withn the scope of acetylalkylamino are acetylmethylamide, acetylethylamide, acetylpropylamide, and acetylbutylamide. Amides within the scope of benzoylalkylamino are benzoylmethylamide, benzoylethylamide, benzoylpropylamide, and benzoylbutylamide. Amides within the scope of substituted benzoylalkylamino are p-chlorobenzoylmethylamide, m-chlorobenzoylmethylamide, 2,4-dichlorobenzoylmethylamide, 2,4,6-trichlorobenzoylmethylamide, m-nitrobenzoylmethylamide, p-nitrobenzoylmethylamide, p-methoxybenzoylmethylamide, 2,4-dimethoxy benzoylmethylamide, 3,4,5-trimethoxybenzoylmethylamide, p-hydroxymethylbenzoylmethylamide, p-methylbenzoylmethylamide, m-methylbenzoylmethylamide, p-ethylbenzoylmethylamide, t-butylbenzoylmethylamide, p-carboxybenzoylmethylamide, m-methoxycarbonylbenzoylmethylamide, o-carboxybenzoylmethylamide, o-hydroxybenzoylmethylamide, p-chlorobenzoylethylamide, m-chlorobenzoylethylamide, 2,4-dichlorobenzoylethylamide, 2,4,6-trichlorobenzoylethylamide, m-nitrobenzoylethylamide, p-nitrobenzoylethylamide, p-methoxybenzoylethylamide, p-methoxybenzoylethylamide, 2,4-dimethoxybenzoylethylamide, 3,4,5trimethoxybenzoylethylamide, p-hydroxymethylbenzoylethylamide, p-methylbenzoylethylamide, m-methylbenzoylethylamide, p-ethylbenzoylethylamide, t-butylbenzoylethylamide, p-carboxybenzoylethylamide, m-methoxycarbonylbenzoylethylamide, o-carboxybenzoylethylamide, o-hydroxybenzoylethylamide, p-chlorobenzoylpropylamide, m-chlorobenzoylpropylamide, 2,4-dichlorobenzoylpropylamide, 2,4,6-trichlorobenzoylpropylamide, m-nitrobenzoylpropylamide, p-nitrobenzoylpropylamide, p-methoxybenzoylpropylamide, 2,4-dimethoxybenzoylpropylamide, 3,4,5-trimethoxybenzoylpropylamide, p-hydroxymethylbenzoylpropylamide, p-methylbenzoylpropylamide, m-methylbenzoylpropylamide, p-ethylbenzoylpropylamide, t-butylbenzoylpropylamide, p-carboxybenzoylpropylamide, m-methoxycarbonylbenzoylpropylamide, o-carboxybenzoylpropylamide, o-hydroxybenzoylpropylamide, p-chlorobenzoylbutylamide, m-chlorobenzoylbutylamide, 2,4-dichlorobenzoylbutylamide, 2,4,6-trichlorobenzoylbutylamide, m-nitrobenzoylmethylamide, p-nitrobenzoylbutylamide, p-methoxybenzoylbutylamine, 2,4-dimethoxybenzoylbutyl-amide, 3,4,5-trimethoxybenzoylbutylamide, p-hydroxymethylbenzoylbutyl-amide, p-methylbenzoylbutyamide, m-methylbenzoylbutylamide, p-ethyl-benzoylbutylamide, m-methylbenzoylbutylamide, p-ethylbenzoylbutyl-amide, t-butylbenzoylbutylamide, p-carboxybenzoylbutylamide, m-methoxycarbonylbenzoylbutylamide, o-carboxybenzoylbutylamide, o-hydroxybenzoylmethylamide. Amides within the scope of pyridylamino are α-pyridylamide, β-pyridylamide, and γ-pyridylamide. Amides within the scope of substituted pyridylamino are 4-methyl-α-pyridylamide, 4-methyl-β-pyridylamide, 4-chloro-α-pyridylamide, and 4-chloro-β-pyridylamide. Amides within the scope of pyridylalkylamino are α-pyridylmethylamide, β-pyridylmethylamide, γ-pyridylmethylamide, α-pyridylethylamide, β-pyridylethylamide, γ-pyridylethylamide, α-pyridylpropylamide, β-pyridylpropylamide, γ-pyridylpropylamide, α-pyridylbutylamide, β-pyridylbutylamide, and γ-pyridylbutylamide. Amides within the scope of substituted pyridylalkylamido are 4-methyl-α-pyridylmethylamide, 4-methyl-β-pyridylmethylamide, 4-chloro-α-pyridylmethylamide, 4-chloro-β-pyridylmethylamide, 4-methyl-α-pyridylpropylamide, 4-methyl-β-pyridylpropylamide, 4-chloro-α-pyridylpropylamide, 4-chloro-β-pyridylpropylamide, 4-methyl-α-pyridylbutylamide, 4-methyl-β-pyridylbutylamide, 4-chloro-α-pyridylbutylamide, 4-chloro-β-pyridylbutylamide, 4-chloro-γ-pyridylbutyl-amide. Amides within the scope of hydroxyalkylamino are hydroxymethylamide, β-hydroxyethylamide, β-hydroxypropylamide, γ-hydroxypropylamide, 1-(hydroxymethyl)ethyl-amide, 1-(hydroxymethyl)-propylamide, (2-hydroxymethyl)propylamide, and α,α-dimethyl-hydroxyethylamide. Amides within the scope of dihydroxyalkylamino are dihydroxymethylamide, β,γ-dihydroxypropylamide, 1-(hydroxymethyl)2-hydroxymethylamide, β,γ-dihydroxybutylamide, β,δ-dihydroxybutyl-amide, γ,δ-dihydroxybutylamide, and 1,1-bis(hydroxymethyl)ethylamide. Amides within the scope of trihydroxyalkylamino are tris(hydroxymethyl)methylamide and 1,3-dihydroxy-2-hydroxymethylpropylamide.

(2) Amides within the scope of cycloamino groups described above are pyrrolidylamide, piperidylamide, morpholinylamide, hexamethyleneiminylamide, piperazinylamide, pyrrolinylamide, and 3,4-didehydropiperidinylamide.

(3) Amides within the scope of carbonylamino of the formula —$NR_{53}COR_{51}$ are methylcarbonylamide, ethylcarbonylamide, phenylcarbonylamide, and benzylcarbonylamide.

(4) Amides within the scope of sulfonylamino of the formula $-NR_{53}SO_2R_{51}$ are methylsulfonylamide, ethylsufonylamide, phenylsulfonylamide, p-tolylsulfonylamide, benzylsulfonylamide.

Examples of alkyl of one to 12 carbon atoms, inclusive, are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, isomeric forms thereof.

Examples of (C_3–C_{10})cycloalkyl which includes alkyl-substituted cycloalkyl, are cyclopropyl, 2-methylcyclopropyl, 2,2-dimethylcyclopropyl, 2,3-diethylcyclopropyl, 2-butylcyclopropyl, cyclobutyl, 2-methylcyclobutyl, 3-propylcyclobutyl, 2,3,4-triethylcyclobutyl, cyclopentyl, 2,2-dimethylcyclopentyl, 2-pentylcyclopentyl, 3-tert-butylcyclopentyl, cyclohexyl, 4-tert-butylcyclohexyl, 3-isopropylcyclohexyl, 2,2-dimethylcyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, and cyclodecyl.

Examples of (C_7–C_{12})aralkyl are benzyl, 2-phenylethyl, 1-phenylethyl, 2-phenylpropyl, 4-phenylbutyl, 3-phenylbutyl, 2-(1-naphthylethyl), and 1-(2-naphthylmethyl).

Examples of phenyl substituted by one to 3 chloro or alkyl of one to 4 carbon atoms, inclusive, are p-chlorophenyl, m-chlorophenyl, 2,4-dichlorophenyl, 2,4,6-trichlorophenyl, p-tolyl, m-tolyl, o-tolyl, p-ethylphenyl, p-tert-butylphenyl, 2,5-dimethylphenyl, 4-chloro-2-methylphenyl, and 2,4-dichloro-3-methylphenyl.

Examples of (C_5–C_7)cycloalkyl optionally substituted by (C_1–C_4)alkyl are cyclobutyl, 1-propylcyclobutyl, 1-butylcyclobutyl, 1-pentylcyclobutyl, 2-methylcyclobutyl, 2-propylcyclobutyl, 3-ethylcyclobutyl, 3-propylcyclobutyl, 2,3,4-triethylcyclobutyl, cyclopentyl, 2,2-dimethylcyclopentyl, 3-ethylcyclopentyl, 3-propylcyclopentyl, 3-butylcyclopentyl, 3-tert-butylcyclopentyl, 1-methyl-3-propylcyclopentyl, 2-methyl-3-propylcyclopentyl, 2-methyl-4-propylcyclopentyl, cyclohexyl, 3-ethylcyclohexyl, 3-isopropylcyclohexyl, 4-methylcyclohexyl, 4-ethylcyclohexyl, 4-propylcyclohexyl, 4-butylcyclohexyl, 4-tert-butylcyclohexyl, 2,6-dimethylcyclohexyl, 2,2-dimethylcyclohexyl, 2,6-dimethyl-4-propylcyclohexyl, and cycloheptyl.

Examples of substituted phenoxy, phenylmethyl, phenylethyl, or phenylpropyl of the R_7 moiety are (o-, m-, or p-)tolyl, (o-, m-, or p-)ethylphenyl, 4-ethyl-o-tolyl, 5-ethyl-m-tolyl, (o-, m-, or p-)-propylphenyl, 2-propyl-(m- or p-)tolyl, 4-isopropyl-2,6-xylyl, 3-propyl-4-ethylphenyl, (2,3,4-, 2,3,5-, 2,3,6-, or 2,4,5-)trimethylphenyl, (o-, m-, or p-)fluorophenyl, 2-fluoro-(m- or p-)tolyl, 4-fluoro-2,5-xylyl, (2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)difluorophenyl, (o-, m-, or p-)chlorophenyl, 2-chloro-p-tolyl, (3-, 4-, 5-, or 6-)-chloro-o-tolyl, 4-chloro-2-propylphenyl, 2-isopropyl-4-chlorophenyl, 4-chloro-3,5-xylyl, (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)dichlorophenyl, 4-chloro-3-fluorophenyl, (3- or 4-)chloro-2-fluorophenyl, (o-, m-, or p-)trifluoromethylphenyl, (o-, m-, or p-)methoxyphenyl, (o-, m-, or p-)ethoxyphenyl, (4- or 5-)chloro-2-methoxyphenyl, 2,4-dichloro-(4- or 6-)methylphenyl, (o-, m-, or p-)tolyloxy, (o-, m-, or p-)ethylphenyloxy, 4-ethyl-o-tolyloxy, 5-ethyl-m-tolyloxy, (o-, m-, or p-)propylphenoxy, 2-propyl-(m- or p-)tolyloxy, 4-isopropyl-2,6-xylyloxy, 3-propyl-4-ethylphenyloxy, (2,3,4-, 2,3,5-, 2,3,6-, or 2,4,5-)-trimethylphenoxy, (o-, m-, or p-)fluorophenoxy, 2-fluoro-(m- or p-)-tolyloxy, 4-fluoro-2,5-xylyloxy, (2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)-difluorophenoxy, (o-, m-, or p-)-chlorophenoxy, 2-chloro-p-tolyloxy, (3, 4, 5, or 6-)chloro-o-tolyloxy, 4-chloro-2-propylphenoxy, 2-isopropyl-4-chlorophenoxy, 4-chloro-3,5-xylyloxy, (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)dichlorophenyloxy, 4-chloro-3-fluorophenoxy, (3-or 4-)chloro-2-fluorophenoxy, (o-, m-, or p-)trifluoromethylphenoxy, (o-, m-, or p-)methoxyphenoxy, (o-, m-, or p-)ethoxyphenoxy, (4- or 5-)chloro-2-methoxyphenoxy, 2,4-dichloro-(5- or 6-)methylphenoxy, (o-, m-, or p-)tolylmethyl, (o-, m-, or p-)ethylphenyl methyl, 4-ethyl-o-tolylmethyl, 5-ethyl-m-tolylmethyl, (o-, m-, or p-)propylphenylmethyl, 2-propyl-(m-, or p-)tolylmethyl, 4-isopropyl-2,6-xylylmethyl, 3-propyl-4-ethylphenylmethyl, (2,3,4-, 2,3,5-, 2,3,6-, or 2,4,5-)trimethylphenylmethyl, (o-, m-, or p-)fluorophenylmethyl, 2-fluoro-(m- or p-)tolylmethyl, 4-fluoro-2,5-xylylmethyl, (2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)difluorophenyl, (o-, m-, or p-)chlorophenylmethyl, 2-chloro-p-tolylmethyl, (3, 4, 5, or 6-)chloro-o-tolylmethyl, 4-chloro-2-propylphenylmethyl, 2-isopropyl-4-chlorophenylmethyl, 4-chloro-3,5-xylylmethyl, (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)dichlorophenylmethyl, 4-chloro-3-fluorophenylmethyl, (3- or 4-)chloro-2-fluorophenylmethyl, (o-, m-, or p-)trifluoromethylphenylmethyl, (o-, m-, or p-)methoxyphenylmethyl, (o-, m-, or p-)ethoxyphenylmethyl, (4- or 5-)chloro-2-methoxyphenylmethyl, and 2,4-dichloro-(4- or 6-)methoxyphenylmethyl.

The novel CBA analogs disclosed herein produce certain prostacyclin-like pharmacological responses.

Accordingly, the novel formula X and XI CBA analogs are used as agents in the study, prevention, control, and treatment of diseases, and other undesirable physiological conditions, in mammals, particularly humans, valuable domestic animals, pets, zoological specimens, and laboratory animals (e.g., mice, rats, rabbits and monkeys). In particular, these compounds have useful application as antithrombotic agents, anti-ulcer agents, and anti-asthma agents, as indicated below.

(a) Platelet Aggregation Inhibition

These novel CBA analogs disclosed herein are useful whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, or to remove or prevent the formation of thrombi in mammals, including man. For example, these compounds are useful in the treatment and prevention of myocardial infarcts, to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, to treat peripheral vascular diseases, and to treat conditions such as atherosclerosis, arteriosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia. Other in vivo applications include geriatric patients to prevent cerebral ischemic attacks and long term prophylaxis following myocardial infarcts and strokes. For these purposes, these compounds are administered systemically, e.g., intravenously, subcutaneously, intramuscularly, and in the form of sterile implants for prolonged action. For rapid response, especially in emergency situations, the intravenous route of administration is preferred. Doses in the range about 0.01 to about 10 mg per kg of body weight per day are used, the exact dose depending on the age, weight, and condition of the patient or animal, and on the frequency and route of administration.

The preferred dosage form for these compounds is oral, although other non-parenteral routes (e.g., buccal, rectal, sublingual) are likewise employed in preference to parenteral routes. Oral dosage forms are conventionally formulated (tablets, capsules, et cetera) and admin-

istered 2–4 times daily. Doses in the range of about 0.05 to 100 mg per kg of body weight per day are effective.

The addition of these compounds to whole blood provides in vitro applications such as storage of whole blood to be used in heart-lung machines. Additionally whole blood containing these compounds can be circulated through organs, e.g., heart and kidneys, which have been removed from a donor prior to transplant. They are also useful in preparing platelet rich concentrates for use in treating thrombocytopenia, chemotherapy, and radiation therapy. In vitro applications utilize a dose of 0.001–1.0 μg per ml of whole blood. For treatment of peripheral vascular diseases, see U.S. Pat. No. 4,103,026.

(b) Gastric Secretion Reduction

These novel CBA analogs disclosed herein are also useful in mammals, including man and certain useful animals, e.g., dogs and pigs, to reduce and control gastric secretion, thereby to reduce or avoid gastrointestinal ulcer formation, and accelerate the healing of such ulcers already present in the gastrointestinal tract. For this purpose, these compounds are injected or infused intravenously, subcutaneously, or intramuscularly in an infusion dose range about 0.1 μg to about 20 μg per kg of body weight per minute, or in a total daily dose by injection or infusion in the range about 0.01 to about 10 mg per kg of body weight per day, the exact dose depending on the age, weight, and condition of the patient or animal, and on the frequency and route of administration.

Preferably, however, these novel compounds are administered orally or by other non-parenteral routes. As employed orally, one to 6 administrations daily in a dosage range of about 1.0 to 100 mg per kg of body weight per day is employed. Once healing of the ulcers has been accomplished the maintenance dosage required to prevent recurrence is adjusted downward so long as the patient or animals remains asymptomatic.

(c) NOSAC-Induced Lesion Inhibition

These novel CBA analogs disclosed herein are also useful in reducing the undesirable gastrointestinal effects resulting from systemic administration of anti-inflammatory prostaglandin synthetase inhibitors, and are useful for that purpose by concomitant administration of the prostaglandin derivative and the anti-inflammatory prostaglandin synthetase inhibitor. See Partridge, et al., U.S. Pat. No. 3,781,429, for a disclosure that the ulcerogenic effect induced by certain non-steroidal anti-inflammatory agents in rats is inhibited by concomitant oral administration of certain prostaglandins. Accordingly these novel CBA analogs herein are useful, for example, in reducing the undesirable gastrointestinal effects resulting from systemic administration of indomethacin, phenylbutazone, and aspirin. These are substances specifically mentioned in Partridge, et al. as non-steroidal, anti-inflammatory agents. These are also known to be prostaglandin synthetase inhibitors.

The anti-inflammatory synthetase inhibitor, for example, indomethacin, aspirin, or phenylbutazone is administered in any of the ways known in the art to alleviate an inflammatory conditions, for example, in any dosage regimen and by any of the known routes of systemic administration.

(d) Bronchodilation (Anti-asthma)

These novel analogs disclosed herein are also useful in the treatment of asthma. For example, these compounds are useful as bronchodilators or as inhibitors of mediator-induced bronchoconstriction, such as SRS-A, and histamine which are released from cells activated by an antigen-antibody complex. Thus, these compounds control spasm and facilitate breathing in conditions such as bronchial bronchitis, bronchiectasis, pneumonia and emphysema. For these purposes, these compounds are administered in a variety of dosage forms, e.g., orally in the form of tablets, capsules, or liquids; rectally in the form of suppositories, parenterally, subcutaneously, or intramuscularly, with intravenous administration being preferred in emergency situations; by inhalation in the form of aerosols or solutions for nebulizers; or by insufflation in the form of powder. Doses in the range of about 0.01 to 5 mg per kg of body weight are used 1 to 4 times a day, the exact dose depending on the age, weight, and condition of the patient and on the frequency and route of administration. For the above use these CBA analogs can be combined advantageously with other anti-asthmatic agents, such as sympathomimetics (isoproterenol, phenylephrine, ephedrine, etc.); xanthine derivatives (theophylline and aminophylline); and corticosteroids (ACTH and prednisolone).

These compounds are effectively administered to human asthma patients by oral inhalation. For administration by the oral inhalation route with conventional nebulizers or by oxygen aerosolization it is convenient to provide the instant active ingredient in dilute solution, preferably at concentrations of about one part of medicament to from about 100 to 200 parts by weight of total solution. Entirely conventional additives may be employed to stabilize these solutions or to provide isotonic media, for example, sodium chloride, sodium citrate, citric acid, sodium bisulfite, and the like can be employed. For administration as a self-propelled dosage unit for administering the active ingredient in aerosol form suitable for inhalation thereapy the composition can comprise the active ingredient suspended in an inert propellant (such as a mixture of dichlorodifluoromethane and dichlorotetrafluoroethane) together with a cosolvent, such as ethanol, flavoring materials and stabilizers. Suitable means to employ the aerosol inhalation therapy technique are described fully in U.S. Pat. No. 3,868,691, for example.

When X_1 is —$COOR_1$, the novel CBA analogs so described are used for the purposes described above in the free acid form, in ester form, or in pharmacologically acceptable salt form. When the ester form is used, the ester is any of those within the above definition of R_1. However, it is preferred that the ester be alkyl of one to 12 carbon atoms, inclusive. Of the alkyl esters, methyl and ethyl are especially preferred for optimum absorption of the compound by the body or experimental animal system; and straight-chain octyl, nonyl, decyl, undecyl, and dodecyl are especially preferred for prolonged activity.

Pharmacologically acceptable salts of the novel prostaglandin analogs of this invention for the purposes described above are those with pharmacologically acceptable metal cations, ammonia, amine cations, or quaternary ammonium cations.

Especially preferred metal cations are those derived from the alkali metals, e.g., lithium, sodium, and potassium, and from the alkaline earth metals, e.g., magnesium and calcium, although cationic forms of other metals, e.g., aluminum, zinc, and iron are within the scope of this invention.

Pharmacologically acceptable amine cations are those derived from primary, secondary, and tertiary

amines. Example of suitable amines are methylamine, dimethylamine, trimethylamine, ethylamine, dibutylamine, triisopropylamine, N-methylhexylamine, decylamine, dodecylamine, allylamine, crotylamine, cyclopentylamine, dicyclohexylamine, benzylamine, dibenzylamine, α-phenylethylamine, β-phenylethylamine, ethylenediamine, diethylenetriamine, adamantylamine, and the like aliphatic, cycloaliphatic, araliphatic amines containing up to and including about 18 carbon atoms, as well as heterocyclic amines, e.g., piperidine, morpholine, pyrrolidine, piperazine, and lower-alkyl derivatives thereto, e.g., 1-methylpiperidine, 4-ethylmorpholine, 1-isopropylpyrrolidine, 2-methylpyrrolidine, 1,4-dimethylpiperazine, 2-methylpiperidine, and the like as well as amines containing water-solubilizing or hydrophilic groups, e.g., mono-, di-, and triethanolamine, ethyldiethanolamine, N-butylethanolamine, 2-amino-1-butanol, 2-amino-2-ethyl,-1,3-propanediol, 2-amino-2-methyl-1-propanol, tris(hydroxymethyl) aminomethane, N-phenylethanolamine, N-(p-tert-amylphenyl)-diethanolamine, galactamine, N-methylglycamine, N-methylglucosamine, ephedrine, phenylephrine, epinephrine, procaine, and the like. Further useful amine salts of the basic amino acid salts, e.g., lysine and arginine.

Examples of suitable pharmacologically acceptable quaternary ammonium cations are tetramethylammonium, tetraethylammonium, benzyltrimethylammonium, phenyltriethylammonium, and the like.

When X_1 is $—CH_2NL_2L_3$, the novel CBA analogs so described are used for the purposes described in either free base or pharmacologically acceptable acid addition salt form.

The acid addition salts of the 2-decarboxy-2-aminomethyl- or 2-(substituted aminomethyl)-CBA analogs provided by this invention are the hydrochlorides, hydrobromides, hydriodides, sulfates, phosphates, cyclohexanesulfamates, methanesulfonates, ethanesulfonates, benzenesulfonates, toluenesulfonates and the like, prepared by reacting the CBA analog with the stoichiometric amount of the acid corresponding to the pharmacologically acceptable acid addition salt.

To obtain the optimum combination of biological response specificity, potency, and duration of activity, certain compounds within the scope of this invention are preferred.

It is preferred that in the X_1-terminated side chain for inter-p-phenylene-CBA compounds, g be zero, for inter-m-phenylene-CBA compounds g be zero or one (especially zero), and for inter-o-phenylene CBA compounds g be zero, one, or 2 (especially one). Inter-o- and inter-m-phenylene-CBA compounds, especially inter-m-phenylene-CBA compounds are preferred. Moreover when Z_1 is $—CH_2—(CH_2)_f—C(R_2)_2$, f is preferably one and R_2 is preferably hydrogen. When R_{17} is $(C_1–C_4)$-alkyl, R_{17} is preferably methyl. Further, when the C-12 side chain contains $—C_mH_{2m}—CH_3$, it is preferred that m be 3, 4, or 5, most preferably 3. When m is 5, more straight chain isomeric forms are preferred, especially methyl-substituted butyl. Further, it is preferred that, when R_7 is aromatic, R_7 be phenoxy, phenyl, or benzyl, including substituted forms thereof. For those compounds wherein R_7 is substituted phenoxy or phenylalkyl, it is preferred there be only one or 2 substituents selected from the group consisting of chloro, fluoro, or trifluoromethyl. Further, for those compounds wherein R_7 is aromatic, it is preferred that R_3 and R_4 both be hydrogen.

Most expecially preferred to biological potency are formula X CBA_2 analogs exhibiting the same C-5 isomeric configuration as CBA_2 itself.

Especially preferred are those compounds which satisfy two or more of the above preferences. Further, the above preferences are expressly intended to describe the preferred compounds within the scope of any generic formula of novel CBA analogs disclosed herein.

Those protective groups within the scope of R_{10} are any group which replaces a hydroxy hydrogen and is neither attacked by nor is reactive to the reagents used in the transformations used herein as a hydroxy is and which is subsequently replaceable by acid hydrolysis with hydrogen in the preparation of the prostaglandin-type compounds. Several such protective groups are known in the art, e.g., tetrahydropyranyl and substituted tetrahydropyranyl. See for reference E. J. Corey, Proceedings of the Robert A. Welch Foundation Conferences on Chemical Research, XII Organic Synthesis, pgs. 51–79 (1969). Those blocking groups which have been found useful include:

(a) tetrahydropyranyl;

(b) tetrahydrofuranyl;

(c) a group of the formula $—C(OR_{11})(R_{12})—CH(R_{13})(R_{14})$, wherein R_{11} is alkyl of one to 18 carbon atoms, inclusive, cycloalkyl of 3 to 10 carbon atoms, inclusive, aralkyl of 7 to 12 carbon atoms, inclusive, phenyl or phenyl substituted with one to 3 alkyl of one to 4 carbon atoms, inclusive, wherein R_{12} and R_{13} are alkyl of one to 4 carbon atoms, inclusive, phenyl, phenyl substituted with one, 2 or 3 alkyl of one to 4 carbon atoms, inclusive, or when R_{12} and R_{13} are taken together $—(CH_2)_a—$ or when R_{12} are R_{13} are taken together $—(CH_2)_b—O—(CH_2)_c$, wherein a is 3, 4, or 5 and b is one, 2, or 3, and c is one, 2, or 3, with the proviso that b plus c is 2, 3, or 4, with the further proviso that R_{12} and R_{13} may be the same or different, and wherein R_{14} is hydrogen or phenyl; and

(d) silyl groups according to R_{28}, as qualified hereinafter.

When the protective group R_{10} is tetrahydropyranyl, the tetrahydropyranyl ether derivative of any hydroxy moieties of the CBA-type intermediates herein is obtained by reaction of the hydroxy-containing compound with 2,3-dihydropyran in an inert solvent, e.g., dichloromethane, in the presence of an acid condensing agent such as p-toluenesulfonic acid or pyridine hydrochloride. The dihydropyran is used in large stoichiometric excess, preferably 4 to 100 times the stoichiometric amount. The reaction is normally complete in less than an hour at 20°–50° C.

When the protective group is tetrahydrofuranyl, 2,3-dihydrofuran is used, as described in the preceding paragraph, in place of the 2,3-dihydropyran.

When the protective group is of the formula $—C(OR_{11})(R_{12})—CH(R_{13})(R_{14})$, wherein R_{11}, R_{12}, R_{13}, and R_{14} are as defined above; a vinyl ether or an unsaturated cyclic or heterocyclic compound, e.g., 1-cyclohexen-1-yl methyl ether, or 5,6-dihydro-4-methoxy-2H-pyran is employed. See C. B. Reese, et al., J. American Chemical Society 89, 3366 (1967). The reaction conditions for such vinyl ethers and unsaturated compounds are similar to those for dihydropyran above.

R_{28} is a silyl protective group of the formula $—Si(G_1)_3$. In some cases, such silylations are general, in that they silylate all hydroxyls of a molecule, while in other cases they are selective, in that while one or more

hydroxyls are silylated at least one other hydroxyl remains unaffected. For any of these silylations, silyl groups within the scope of $-Si(G_1)_3$ include trimethylsilyl, dimethylphenylsilyl, triphenylsilyl, t-butyldimethylsilyl, or methylphenylbenzylsilyl. With regard to G_1, examples of alkyl are methyl, ethyl, propyl, isobutyl, butyl, sec-butyl, tert-butyl, pentyl, and the like. Examples of aralkyl are benzyl, phenethyl, α-phenylethyl, 3-phenyl propyl, α-naphthylmethyl, and 2-(α-naphthyl)ethyl. Examples of phenyl substituted with halo or alkyl are p-chlorophenyl, m-fluorophenyl, o-tolyl, 2,4-dichlorophenyl, p-tert-butylphenyl, 4-chloro-2-methylphenyl, and 2,4-dichloro-3-methylphenyl.

These silyl groups are known in the art. See for example, Pierce "Silylation of Organic Compounds," Pierce Chemical Company, Rockford, Ill. (1968). When silylated products of the charts below are intended to be subjected to chromatographic purification, then the use of silyl groups known to be unstable to chromatography (e.g. trimethylsilyl) is to be avoided. Further, when silyl groups are to be introduced selectively, silylating agents which are readily available and known to be useful in selective silylations are employed. For example, t-butyldimethylsilyl groups are employed when selective introduction is required. Further, when silyl groups are to be selectively hydrolyzed in the presence of protective groups according to R_{10} or acyl protective groups, then the use of silyl groups which are readily available and known to be easily hydrolyzable with tetra-n-butylammonium fluoride are employed. A particularly useful silyl group for this purpose is t-butyldimethylsilyl, while other silyl groups (e.g. trimethylsilyl) are not employed when selective introduction and/or hydrolysis is required.

The protective groups as defined by R_{10} are otherwise removed by mild acidic hydrolysis. For example, by reaction with (1) hydrochloric acid in methanol; (2) a mixture of acetic acid, water, and tetrahydrofuran, or (3) aqueous citric acid or aqueous phosphoric acid in tetrahydrofuran, at temperatures below 55° C., hydrolysis of the blocking group is achieved.

R_{31} is a hydroxy-hydrogen protective group, as indicated above. As such, R_{31} may be an acyl protective group according to R_9, an acid hydrolyzable protective group according to R_{10}, a silyl protective group according to R_{28}, or an arylmethyl hydroxy hydrogen replacing group according to R_{34}.

Acyl protective groups according to R_9 include:

(a) benzoyl;

(b) benzoyl substituted with one to 5 alkyl of one to 4 carbon atoms, inclusive, or phenylalkyl of 7 to 12 carbon atoms, inclusive, or nitro, with the proviso that not more than two substituents are other than alkyl, and that the total number of carbon atoms in the substituents does not exceed 10 carbon atoms, with the further proviso that the substituents are the same or different;

(c) benzoyl substituted with alkoxycarbonyl of 2 to 5 carbon atoms, inclusive;

(d) naphthoyl;

(e) naphthoyl substituted with one to 9, inclusive, alkyl of one to 4 carbon atoms, inclusive, phenylalkyl of 7 to 10 carbon atoms, inclusive, or nitro, with the proviso that not more than two substituents on either of the fused aromatic rings are other than alkyl and that the total number of carbon atoms in the substituents on either of the fused aromatic rings does not exceed 10 carbon atoms, with the further proviso that the various substituents are the same or different; or

(f) alkanoyl of 2 to 12 carbon atoms, inclusive.

In preparing these acyl derivatives of a hydroxy-containing compound herein, methods generally known in the art are employed. Thus, for example, an aromatic acid of the formula R_9OH, wherein R_9 is as defined above (e.g., benzoic acid), is reacted with the hydroxy-containing compound in the presence of a dehydrating agent, e.g. p-toluensulfonyl chloride or dicyclohexylcarbodiimide; or alternatively an anhydride of the aromatic acid of the formula $(R_9)OH$, e.g., benzoic anhydride, is used.

Preferably, however, the process described in the above paragraph proceeds by use of the appropriate acyl halide, e.g., R_9Hal, wherein Hal is chloro, bromo, or iodo. For example, benzoyl chloride is reacted with the hydroxyl-containing compound in the presence of a hydrogen chloride scavenger, e.g. a tertiary amine such as pyridine, triethylamine or the like. The reaction is carried out under a variety of conditions, using procedures generally known in the art. Generally mild conditions are employed: 0°–60° C., contacting the reactants in a liquid medium (e.g., excess pyridine or an inert solvent such as benzene, toluene, or chloroform). The acylating agent is used either in stoichiometric amount or in substantial stoichiometric excess.

As examples of R_9, the following compounds are available as acids (R_9OH), $(R_9)_2O$, or acyl chlorides (R_9Cl): benzoyl; substituted benzoyl, e.g., (2-, 3-, or 4-)methylbenzoyl, (2-, 3-, or 4-) ethylbenzoyl, (2-, 3-, or 4-)isopropylbenzoyl, (2-, 3-, or 4-)tert-butylbenzoyl, 2,4-dimethylbenzoyl, 3,5-dimethylbenzoyl, 2-isopropyltoluyl, 2,4,6-trimethylbenzoyl, pentamethylbenzoyl, phenyl(2-, 3-, or 4-)toluyl, (2-, 3-, or 4-)phenethylbenzoyl, (2-, 3-, or4-)nitrobenzoyl, (2,4, 2,5-, or 2,3-)dinitrobenzoyl, 2,3-dimethyl-2-nitrobenzoyl, 4,5-dimethyl-2-nitrobenzoyl, 2-nitro-6-phenylethylbenzoyl, 3-nitro-2-phenethylbenzoyl, 2-nitro-6-phenethylbenzoyl, 3-nitro-2-phenethylbenzoyl; mono esterified phthaloyl, isophthaloyl, or terephthaloyl; 1- or 2-naphthoyl; substituted naphthoyl, e.g., (2-, 3-, 4-, 5-, 6-, or 7-)methyl-1-naphthoyl, (2- or 4-)ethyl-1-naphthoyl, 2-isopropyl-1-naphthoyl, 4,5-dimethyl-1-naphthoyl, 6-isopropyl-4-methyl-1-naphthoyl, 8-benzyl-1-naphthoyl, (3-, 4-, 5-, or 8-)-nitro-1-naphthoyl, 4,5-dinitro-1-naphthoyl, (3-, 4-, 6-, 7-, or 8-)-methyl-1-naphthoyl, 4-ethyl-2-napthoyl, and (5- or 8-)nitro-2-naphthoyl and acetyl.

There may be employed, therefore, benzoyl chloride, 4-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, or the like, i.e. R_9Cl compounds corresponding to the above R_9 groups. If the acyl chloride is not available, it is prepared from the corresponding acid and phosphorus pentachloride as is known in the art. It is preferred that the R_9OH, $(R_9)_2O$, or R_9Cl reactant does not have bulky hindering substituents, e.g. tert-butyl on both of the ring carbon atoms adjacent to the carbonyl attaching site.

The acyl protective groups, according to R_9, are removed by deacylation. Alkali metal carbonate or hydroxide are employed effectively at ambient temperature for this purpose. For example, potassium carbonate or hydroxide in aqueous methanol at about 25° C. is advantageously employed.

R_{34} is defined as any arylmethyl group which replaces the hydroxy hydrogen of the intermediates in the preparation of the various CBA analogs herein which is subsequently replaceable by hydrogen in the processes

herein for preparation of these respective prostacyclin analogs, being stable with respect to the various reactions to which R_{34}-containing compounds are subjected and being introduced and subsequently removed by hydrogenolysis under conditions which yield substantially quantitative yields of desired products.

Examples of arylmethyl hydroxy-hydrogen replacing groups are

(a) benzyl;

(b) benzyl substituted by one to 5 alkyl of one to 4 carbon atoms, inclusive, chloro, bromo, iodo, fluoro, nitro, phenylalkyl of 7 to 12 carbon atoms, inclusive, with the further proviso that the various substituents are the same or different;

(c) benzhydryl;

(d) benzhydryl substituted by one to 10 alkyl of one to 4 carbon atoms, inclusive, chloro, bromo, iodo, fluoro, nitro, phenylalkyl of 7 to 12 carbon atoms, inclusive, with the further proviso that the various substituents are the same or different on each of the aromatic rings;

(e) trityl;

(f) trityl substituted by one to 15 alkyl of one to 4 carbon atoms, inclusive, chloro, bromo, iodo, fluoro, nitro, phenylalkyl of 7 to 12 carbon atoms, inclusive, with the further proviso that the various substituents are the same or different on each of the aromatic rings.

The introduction of such ether linkages to the hydroxy-containing compounds herein, particularly the benzyl or substituted benzyl ether proceeds by methods known in the art, for example by reaction of the hydroxy-containing compound with the benzyl or substituted benzyl halide (chloride, bromide, or iodide) corresponding to the desired ether. This reaction proceeds in the presence of an appropriate condensing agent (e.g., silver oxide). The mixture is stirred and heated to 50°–80° C. Reaction times of 4 to 20 hours are ordinarily sufficient.

The Charts herein describe the methods whereby the novel intermediates and end products of the present specification are prepared by the novel processes herein. With respect to these charts, g, n, L_1, M_1, M_6, R_7, R_8, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{20}, R_{21}, R_{22}, R_{23}, and R_{24}, R_{28}, R_{31}, X_1, Y_1, Z_1, and Z_4 are as defined above. R_{37} is the same as R_{47}, but other than $—CH_2OH$. R_{38} is $—OR_{31}$, hydrogen, or $—CH_2OR_{31}$, wherein R_{31} is defined as above. R_{27} is same as R_7 except that $—(CH_2)_2—CH(OH)—CH_3$ is $—(CH_2)_2—CH(OR_{10})—CH_3$. R_{37} is the same as R_{17}, buth other than hydrogen. Ac is acetyl. Z_2 is the same as Z_1 but not $—(Ph)—(CH_2)_g—$. Z_3 is the same as Z_1, but not trans$—CH_2—CH=CH—$.

With respect to Chart A, a method is provided whereby the known formula XXI bicyclic lactone is transformed to the carbacyclin intermediate of formula XXV useful in the preparation of formula X CBA compounds wherein R_{17} is alkyl or R_{16} and R_{17} taken together are methano or a second valence bond between C-6a and C-9. With respect to Chart A, the formula XXI compound is transformed to the formula XXII compound by treatment with the anion of dimethyl methylphosphonate. Methods for such a reaction are known in the art. See Dauben, W. G., et al., JACS, 97:4973 (1975), describing a reaction of this type.

The formula XXII lactol is transformed to the formula XXIII diketone by oxidation methods known in the art. For example, Collins reagent or Jones reagent is employed in this oxidative transformation.

The formula XXIII diketone is cyclized to the formula XXIV compound by an intramolecular Horner-Emmons reaction. The chemical methodology for analogous transformations is known in the art. See Piers, E., et al., Tetrahedron Letters, 3279 (1979) and Clark, R. D., et al., Synthetic Communications 5:1 (1975).

The formula XXIV compound is transformed to the novel formula XXV compound wherein R_{16} is hydrogen and R_{37} is alkyl by treatment with lithium dialkyl cuprate. The lithium dialkyl cuprate is prepared by conventional means, e.g., reaction of anhydrous copper iodide in diethyl ether with an alkyllithium in diethyl ether, and thereafter reacted with the formula XXIV compounds, e.g., in diethyl ether.

The formula XXIV compound is transferred to the novel formula XXV compound wherein R_{16} and R_{37} taken together are methylene ($—CH_2—$) by one of two methods. By the first method, the formula XXV compound is prepared by treatment of the formula XXIV compound with the anion of trimethyloxosulfonium iodide. See for reference E. J. Corey, et al., JACS 87:1353 (1965). By this method, the anion is conveniently generated by treatment of trimethyloxosulfonium iodide in sodium hydride.

By a second method, the formula XXIV compound is converted to the formula XXV compound wherein R_{16} and R_{37} taken together are methylene by first converting the formula XXIV compound to the corresponding formula XXVI hydroxymethyl compound by photochemical addition of methanol (e.g., see G. L. Bundy, Tetr. Lett. 1957, 1975), thereafter treating the resulting hydroxymethyl compound with an excess (e.g., two equivalents) of p-toluenesulfonyl chloride in a tertiary amine base to yield the corresponding formula XXVII tosylate, and finally treating the resulting formula XXVII tosylate with base (e.g., potassium t-butoxide) to yield the formula XXV cyclopropyl compound.

With respect to Chart B, a method is provided whereby the formula XXXI compound prepared in accordance with methods of Chart A is transformed to the novel CBA_2 analogs of formula XXXVI.

The formula XXXI compound is transformed to the formula XXXVI compound by methods known in the art for preparing carbacyclin. See for example, British published applications referred to above. Alternatively, the formula XXXI compound is reacted with formula XXXII compound and thereby successively transformed to the formula XXXIII, formula XXXIV and formula XXXV compounds.

The reaction of the formula XXXI compound employing the formula XXXII compound is accomplished by methods known in the art. See Moersch, G. W., J. Organic Chemistry, 36:1149 (1971) and Mulzer, J. et al., Tetrahedron Letters, 2949 (1978). The formula XXXII reactants are known in the art or are prepared by methods known in the art. See Example 4 describing one such method of preparation of a formula XXXII compound.

The formula XXXIII compound is then transformed to the formula XXXIV compound by decarboxylative dehydration. Procedures for this reaction are known in the art. See Eschenmoser, A., et al., Helv. Chim. Acta. 58:1450 (1975), Hara, S., et al., Tetrahedron Letters, 1545 (1975) and Mulzer, J., et al., Tetrahedron Letters, 2953 (1978) and 1909 (1979).

Finally, the formula XXXV compound is prepared from formula XXXIV compound by selective desilylation. Such procedures are known in the art and typi-

cally employ the use of tetra-n-butyl ammonium fluoride and tetrahydrofuran. See Corey, E. J., et al., JACS 94:6190 (1972).

The formula XXXV compound is transformed to various acids, esters, amides, and amines of a formula XXXVI by methods known in the art. Particularly useful in this regard are methods described in the aforementioned British published specifications describing the preparation of carbacyclin analogs.

The preparation of formula XXXVI compounds from the formula XXXV compounds proceeds by, for example, oxidation to the corresponding carboxylic acid, followed by hydrolysis of any protective groups at the C-11 or C-15 position of the molecule. Such carboxylic acids are then esterified by conventional means or amidized by conventional means. Such amides may, for example, then be reduced to corresponding amines (X_1 is $-CH_2NL_2L_3$ by reduction by lithium aluminum hydride. See U.S. Pat. No. 4,073,808. In a preparation of the primary alcohols according to formula XXXVI from the formula XXXV compound, hydrolysis of any protective groups at C-11 or C-15 yields such products directly. Hydrolysis is accomplished by prodcedures described above, e.g., mild acidic conditions at elevated temperatures.

Chart C provides a method whereby the known formula XLI compounds are transformed to the formula XLIV aldehydes employed in Chart D in the preparation of inter-phenylene-CBA_2 compounds therein.

With respect to Chart C, the formula XLII compound is prepared from the formula XLI compounds by reduction. Conventional methods known in the art for the transformation of carboxylic acids to corresponding primary alcohols are employed. For example, one extremely useful conventional means for this reduction is employing lithium aluminum hydride as a reducing agent.

The formula XLIII compound is then prepared from the formula XLII compound by monosilylation. Particularly, formula XLIII compounds are prepared wherein R_{28} represents a relatively stable silyl group, most preferably being t-butyldimethylsilyl or phenyldimethylsilyl. Other silyl groups, particularly trimethyl-silyl (TMS) are not preferred for use in connection with the methods of Chart C.

The formula XLIII monosilyl derivatives are prepared from the formula XLII compound by reacting the formula XLII compounds with about an equal molar amount of the silylating agent. For example, when R_{28} is t-butyldimethylsilyl, a single equivalent of t-butyldimethylsilyl chloride is employed in the transformation. Accordingly, there are prepared both monosilyl derivatives of the formula XLII compound as well as the bis-silyl derivatives corresponding to formula XLII. From this mixture of products, the formula XLIII compound is recovered by conventional means, e.g., column chromatography. Otherwise, the silylation proceeds under conditions conventionally employed for silylating hydroxyl groups. Refer to the discussion hereinabove.

The formula XLIV compound is then prepared from the formula XLIII compound by oxidation of the formula XLIII alcohol to the corresponding aldehyde. Conventional oxidizing agents are employed, e.g., manganese dioxide.

Chart D provides a method whereby the known formula LI ketones are transformed to the formula LX inter-phenylene CBA_2 analogs disclosed herein.

In accordance with Chart D the formula LII compound is prepared from the formula LI compound by reduction of the formula LI ketone to the corresponding secondary alcohol. This reduction proceeds by conventional means, employing readily available reducing agents. Accordingly, sodium, potassium, or lithium borohydride is conveniently employed in this reduction.

Thereafter, the formula LII alcohol is transformed to the corresponding mesylate (methanesulfonate). Conventional methods for the transformation of alcohols to corresponding mesylates are employed. Thus, the formula LII alcohol is reacted with methane-sulfonyl chloride in the presence of a tertiary amine (e.g., tri-ethylamine) in the preparation of the formula LIII compound.

Other sulfonyl derivatives corresponding to the formula LII alcohol may be employed in place of the formula LIII compound in the transformations of Chart D. These other sulfonyl derivatives are preferably those derived from readily available sulfonylating reagents, i.e., the corresponding sulfonyl chlorides. One especially important alternative to the formula LIII compound is the tosylate (toluenesulfonate) corresponding to the formula LII compound.

The formula LIII compound, or an alternate sulfonate corresponding thereto, is transformed to the formula LIV compound by treatment with sodium lithium or potassium thiophenoxide. The thiophenoxide is conveniently prepared just prior to the transformaton by mixing approximately equal molar amounts of thiophenol and base, e.g., potassium t-butoxide.

This formula LIV compound is then oxidized to the corresponding formula LV compound by oxidation with a readily available oxidizing agent such as m-chloroperbenzoic acid.

The formula LV compound is then condensed with the formula XLIV compound prepared according to Chart C by first treatment of the formula LV compound with a strong base, e.g., n-butyllithium, to generate the anion corresponding to the formula LV compound, treatment of the corresponding anion with the aldehyde of formula XLIV and finally treating the resulting adduct with acetic anhydride to yield the formula LVI acetyl compound.

The formula LVI compound is then transformed to the formula LVII compound by reaction with a sodium amalgam. Methods by which the formula LVII olefin is formed form the formula LV compound are analogous to known methods described by Kocienski, P. J., et al., "Scope and Stereochemistry of an Olefin Synthesis from β-Hydroxysulphones", JCS Perkin I, 829–834 (1978).

The formula LVII compound is then transformed to the formula LVIII compound by selective hydrolysis of the silyl group according to R_{28}. Conventional means for this hydrolysis are employed, e.g., tetra-n-butyl ammonium fluoride. Refer to the discussion above for a description of this hydrolysis.

The formula LVIII C-5 diastereomers thusly prepared are conveniently purified into (5-E) and (5-Z) isomeric forms. This transformation proceeds by conventional means, e.g., column chromatography.

Thereafter either the (5E) or (5Z) isomer of formula LVIII is transformed to the formula LIX carboxylic acid or ester by conventional oxidation, followed by optional esterification. One especially convenient means of oxidation is employing the Jones reagent, although

other oxidizing agents are employed. Esterification then proceeds by methods hereinafter described.

Finally, the formula LX products are prepared from the formula LIX compound by first hydrolyzing the protective groups under acidic conditions, e.g., mixtures of water, tetrahydrofuran, and acetic acid. Thereafter, the formula LIX acids and esters are transformed to various other C-1 derivatives by methods hereinafter described.

One especially convenient means of preparing the formula LX compound as a free carboxylic acid (X_1 is —COOH), is by purification of the corresponding methyl ester, followed by saponification under basic conditions (e.g., the treatment with potassium carbonate or sodium or potassium hydroxide).

Charg E provides a method whereby the known formula LXI compound is transformed into formula LXIII intermediate useful in the preparation of the novel CBA_2 analogs.

The procedures for the transformation of the formula LXI compound to the formula LXIII compound are analogous to those describing the transformation in Charts A, B, and D of the formula XXI compound to the formula XXXVI and LX compounds (i.e., corresponding to the transformation of formula LXI compound to the formula LXII compound is the transformation in Chart A of the formula XXI compound to the formula XXV compound and corresponding to the transformation of the formula LXII compound to the formula LXIII compound is the transformation in Chart D of the formula LI compound to the formula LX compound.). For convenience, the protective groups R_{31} and R_{38} may be the same or different, although preferably such protective groups are diffent, whereby the hydrolysis of a protective group according to R_{31} is accomplished in the presence of a protective group according to R_{38}.

Chart F then provides a method whereby the formula LXXI compound prepared according to Chart E is transformed to the formula LXXII carbacyclin analog in accordance with the present invention. With respect to Chart F, the formula LXXI compound is transformed to the formula LXXII compound by selective hydrolysis of the protective group according to R_{31}. Thereafter, the formula LXXII compound is transformed to formula LXXIII compound by methods known in the art, e.g., oxidation of the formula LXXII primary alcohol to the corresponding aldehyde, Wittig oxylacylating the aldehyde, and reduction of the resulting ketone to the secondary or tertiary alcohol corresponding to M_1. For an example of the various transformations employed according to Chart F, see Chart A (part VI) of U.S. Pat. No. 4,107,427, issued Aug. 15 1978.

Chart G provides a method whereby the novel formula LXXXI intermediate, prepared according to Chart A, is transformed to the formula LXXXVIII and LXXXIX isomers of the novel C-6a- and /or C-9-substituted CBA_2 analogs.

With respect to Chart G, the formula LXXXIII compound is prepared from the formula LXXXI ketone by a Wittig ω-carboxyalkylation employing a formula LXXXII triphenylphosphonium compound. The Wittig reaction is undertaken under conventional reaction conditions for preparing prostaglandin-type substances. The formula LXXXIII compound is then optionally hydrolyzed to yield the formula X carboxylic acid products or employed in the further transformations of Chart G in ester form.

The formula LXXXIII compound thusly prepared is thereafter preferably separated directly into C-5 isomers of formulas LXXXVIII and LXXXIX (e.g., by chromatographic means followed by hydrolysis of and protective groups at C-11 or C-15 position of the molecule), or is alternatively transformed to the formula LXXXIV ester by conventional esterification techniques, e.g., ethereal diazomethane treatment or treatment with methyl iodide. The formula LXXXIV ester is then reduced to the corresponding primary alcohol by reduction with a suitable reducing agent, e.g., lithium aluminum hydride, by methods known in the art for preparing prostaglandin-type primary alcohols from corresponding prostaglandin esters.

The formula LXXXV compound represents an especially convenient intermediate for the facile separation of the C-5 diastereomers. Accordingly, the formula LXXXV compound may be separated by conventional means of separation of diastereomeric mixtures, e.g., column chromatography, whereby the formula LXXXVI and formula LXXXVII compounds are prepared in isomerically pure form. These primary alcohols are then conveniently transformed to the formula LXXXVIII and LXXXIX products by methods described above. Refer to the transformations of the formula XXXV compound to the formula XXXVI compound in Chart B.

Chart H provides a method whereby the formula XCVII 5-fluoro-CBA_2 compounds are prepared from the formula XCIII CBA_2 intermediates known in the art. See, for example, British Published Application No. 2,014,143, especially the discussion relative to step (b) of Chart A therein. This formula XCI sulfoximine is transformed to the formula XCII fluorinated sulfoximine by first generating an anion of the formula XCII compound, e.g., by treatment with n-butyllithium in hexane, and treating the resulting anion with a fluorine source. Particulary preferred as a source of fluorine is perchloryl fluoride ($FClO_3$).

The formula XCII compound thusly prepared and the known formula XCIII compound described above are then employed in the preparation of the formula XCIV compound by known methods. Refer again to step (b) of Chart A of British Published Application No. 2,014,143.

The formula XCIV compound thusly prepared is then transformed to the formula XCV primary alcohol by hydrolysis under mild acidic conditions (e.g., mixtures of acetic acid, water, and tetrahydrofuran) as is known in the art. Thereafter, the formula XCV primary alcohol is oxidized to the corresponding formula XCVI carboxylci acid employing conventional means. For example, treatment with oxygen and an aqueous suspension of platinum oxide hydrogenated at ambient temperature and pressure yields the formula LXXVI carboxylic acid. Thereafter, the formula XCVI compound is transformed into the various formula XCVII products by derivatization or transformation of the carboxyl group of the formula XCVI compound.

The C-5 isomers of the formula XCIV to formula XCVII compounds are conveniently separate at any step during the process of Chart H, but are most conveniently and preferably seperated from the formula XCIV diastereomeric mixture. Conventional means, e.g., column chromatography, are employed in the separation.

Chart I provides an optional method whereby the known formula CI compound is transformed to the formula CIII products herein. With respect to Chart I, the formula XCII is prepared from the formula XCI compound by the procedure described in Chart H for the preparation of the formula XCVII compound from the formula XCIII compound. This formula CII CBA_2 intermediate is then transformed to the formula CIII compound by the procedures described in Chart F for the transformation of the formula LXXI to the formula LXXIII compound.

Chart J provides the preferred methods for preparing the formula X CBA analogs wherein Z_1 is trans—CH-2—CH=CH—. With respect to Chart J, R_1 therein is other than hydrogen or a cation, preferably being lower alkyl. The formula CXIV is prepared from the formula CXI compound by first preparing the α-phenylselenyl derivative thereof, dehydrophenylselenizing, whereby the formula CXIII α,β-unsaturated ester is prepared. This ester is then transformed to the formula CXIV free acid (X_1 is —COOH) by saponification and this free acid is transformed to the various other formula CXIV compounds as indicated in Chart H (refer to the transformation of the formula XCVI compound to the formula XCVII compound).

Chart K provides the preferred method whereby the formula VI CBA intermediates wherein Z_1 is trans—CH_2—CH=CH— are prepared. With respect to Chart K, the formula CXXI compound is transformed to the formula CXXIII compound by methods analogous to those described in Chart J for the preparation of the formula CXIV compound from the formula CXI compound.

For a detailed description of the methodology employed in Charts J–K, refer to the discussion in British Pat. No. 2,014,143, and references cited therein.

Charts L–O provide methods whereby CBA_2 intermediates and analogs are employed in the synthesis of corresponding CBA_1 intermediates and analogs.

Charts L provides the preferred method for preparing the formula VII CBA_1 intermediates wherein Z_1 is trans—CH_2—CH=CH—. With respect to Chart L the formula CXXXI compound, prepared as the formula CXXII compound of Chart K, is reduced to the formula CXXXII compound by conventional methods. For a discussion of such methods, and general methodologies for transforming CBA_2 intermediates and analogs to corresponding CBA_1 intermediates and analogs, refer to British Published Application No. 2,017,699. For example, catalytic hydrogenation with conventional catalysts under atmospheric pressure is employed.

Thereafter, this formula CXXXII compound is successively transformed to the formula CXXXIII α,β-unsaturated ester and the formula CXXXIV CBA_1 intermediate by methods described in Charts J–K (i.e., the transformation of the formula CXII compound to the corresponding formula CXIV compounds and the transformation of the formula CXXII compound to the formula CXXIII compound).

Otherwise, the formula VII CBA_1 intermediates are prepared according to the method of Chart M, wherein the formula CXLI compound, prepared above, is reduced to the formula CXLII intermediates by techniques described in Chart L and references cited therein.

Chart N describes the preparation of the various CBA_1 analogs from the formula CLI compounds prepared in Charts L and M. Procedures employed in Chart N are those described in Chart F above.

Finally, Chart O provides an alternative method for the preparation of the formula CLXII CBA_1 analogs directly from formula CLXI CBA_2 analogs. This transformation of Chart O proceeds by direct reduction of the formula CLXI compound by methods described in Chart M and references cited therein. Chart O is an especially convenient method for the preparation of CBA_1 analogs wherein Y_1 is —CH_2CH_2—.

The formula XI CBA analogs are prepared according to the methods described in Charts P–U. With respect to Chart P, the formula CLXXI compound is known in the art or prepared by methods known in the art. See U.S. Pat. No. 4,181,789. This compound is conveniently transformed to the corresponding formula CLXXII methylene and formula CLXXIII hydroxymethyl compounds by methods known in the art. Such procedures are particularly and especially described in U.S. Pat. No. 4,012,467 and 4,060,534.

The formula CLXXIII compound thusly prepared is thereafter converted to the formula CLXXIV mesylate by methods known in the art, e.g., reaction with methanesulfonyl chloride in a tertiary amine base. Alternatively, other sulfonated derivatives corresponding to the formula CLXXIV compound are prepared such as those described in connection with formula LIII in Chart D.

Thereafter, the formula CLXXIV mesylate (or other sulfonate) is selectively hydrolyzed to yield the formula CLXXV phenol derivatives. Selective hydrolysis of R_{28} silyl ether groups in the presence of protected R_{18} or M_6 hydroxyl groups is accomplished by methods hereinabove described, i.e., the use of tetra-n-butyl ammonium floride by methods known in the art and hereinabove described. The formula CLXXV phenol derivative is then cyclized to yield the formula CLXXVI compounds. Cyclization proceeds most conveniently by treatment of the formula XVI compound with base at elevated temperatures. For example, n-butyllithium, sodium hydride, or potassium hydride are conveniently employed at reflux temperatures in organic solvent such as tetrahydrofuran or glyme.

The cyclized formula CLXXVI compound is then transformed to the formula CLXXVII compound by ω-carboxyalkylation. Methods known in the art are employed, e.g., methods for preparing 3,7-inter-phenylene-PGFα compounds and corresponding phenolic intermediates. For example, the preparation of the formula CLXXVII compound proceeds by reaction of the formula CLXXVI compound with sodium hydride and the alkyl bromoalkanoate corresponding to the —Z-4—$COOR_1$ group to be introduced into the molecule. Thereafter, the formula CLXXVIII compound is prepared by deprotection, i.e., hydrolysis under mild acidic conditions of the protective groups, followed by transformation to various other C-1 derivatives by methods hereinafter described.

Chart Q provides a method whereby further formula XI CBA analogs in accordance with the present invention are prepared. In particular, formula XI compounds wherein at least one of R_{20}, R_{21}, R_{23}, or R_{24} is not hydrogen are prepared. In accordance with Chart Q, the formula CLXXXI compound, referred to above in the discussion pertaining to Chart P, is oxidized to the corresponding formula CLXXXII aldehyde by methods known in the art. For example, Collins reagent is employed in this oxidation. When conversion of one C-9

stereoisomer of formula CLXXXIII to the other is described, refer to the procedure in Chart R.

Thereafter the formula CLXXXII aldehyde is hydrolyzed to the corresponding formula CLXXXIII phenol derivative by methods described above for the preparation of the formula CLXXV compound from the formula CLXXIV compound of Chart P.

Thereafter, cyclization of the formula CLXXXIII to the corresponding formula CLXXXIV compound is accomplished by heating at reflux in an organic solvent the phenoxide anion of the formula CLXXXIII compound. See for reference Casiraghi, G., et al., J.C.S. Perkin I, 2027 (1979). The C-9 isomers of the formula CLXXXIV compound are conveniently separated by conventional techniques, e.g., column chromatography. Thereafter, the formula CLXXXIV compound is transformed to the formula CLXXXV compound by methods described in Chart P for the preparation of the formula CLXXVII compound from the formula CLXXVI compound. This alcohol is then oxidized to the corresponding formula CLXXXVI ketone (e.g., by methods described above for the preparation of the formula CLXXXII compound from the formula CLXXXI compound) or dehydrated to yield the formula CLXXXVIII compound. Such dehydrations proceed by methods known in the art and include first preparing the mesylate corresponding to the formula CLXXXV compound following by treatment with base.

Thereafter, the formula CLXXXVI or CLXXXVIII compound is transformed, respectively, to the formula CLXXXVII or CLXXIX compound by methods hereinafter described.

Finally, the formula CLXXXIX compound thusly prepared is dehydrogenated to yield the formula CXC compound by conventional means, e.g., catalytic dehydrogenation (palladium-on-carbon catalyst) or treatment with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone).

Chart R provides a method whereby the C-9 epimeric forms of compounds prepared according to the Chart P are prepared. With respect to Chart R, the formula CXCI aldehyde, prepared as the formula CLXXXII compound of Chart Q is isomerized by treatment under basic conditions (i.e., the use of an organic base such as 1,8-diazobicyclo[5.4.0]-undec-7-ene in an organic solvent (e.g., methylene chloride)). Thereafter this 9β-aldehyde is reduced to the corresponding formula CXCIII alcohol by treatment with a suitable reducing agent, such as a borohydride reducing agent. (e.g., sodium, lithium, or potassium borohydride). Thereafter, the formula CXCIII alcohol thusly prepared is transformed to the corresponding 9β-CBA analogs by methods described in Chart P, e.g., the transformation of the formula CLXXIII to the formula CLXXVIII compound.

Optionally, the various formula XI CBA analogs prepared according to Charts P, Q, and R are prepared by the procedure of Chart S. The procedure of Chart S employs the formula CCI starting material described in chart P which is thereafter converted to the formula CCII compound prepared in accordance with methods described for the preparation of the formula CLXXVIII compound from the formula CLXXI compound of Chart P, the formula CLXXXVII, formula CLXXXIX, formula CXC compounds from the formula CLXXXI compound of Chart Q and the formula CXCIV compounds from the formula CXCI compound of Chart R. The formula CCII compound thusly prepared is then transformed to the formula CCIII compounds by methods hereinabove described, e.g., the transformation of the formula LXXI compound to the formula LXXIII compound of Chart F.

Chart T provides a preferred method whereby the 9-deoxo-2',9-metheno-3-oxa-4,5,6-trinor-3,7-(1,3-interphenylene)-PGE$_1$ compounds of formula CCXIII are prepared. In accordance with Chart T the formula CCXI compound, prepared as the formula CLXXXIII compound of Chart Q, is treated with a methyl Grignard reagent, methyl magnesium bromide and heated at reflux in an organic solvent (e.g., glyme).

The formula CCXII thusly prepared is then transformed to the formula CCXIII product by the method described in Chart P for the preparation of the formula CLXXVIII product from the formula CLXXVI phenol intermediate.

Chart U provides a convenient method whereby formula XI compounds wherein Y_1 is trans—CH=CH—, the formula CCXXI compound of Chart U, are transformed to corresponding formula CCXXII aldehyde intermediates. This transformation is accomplished by ozonolysis by methods otherwise known in the art.

The formula CCXXII intermediate is then conveniently transformed to various formula XI products (the Formula CCXXIII compound of Chart U) by methods described above, i.e., reaction of the formula CCXXII compound with the appropriate Wittig reagent followed by reduction and hydrolysis. Accordingly by the procedure described in Chart U the C-12 side chains of the various formula CCXXI compounds is conveniently modified by the formula CCXXII aldehyde intermediates. As discussed above, the processes herein described lead variously to carboxylic acids (X_1 is —COOR$_1$ and R$_1$ is hydrogen) or to esters or primary alcohols (X_1 is —CH$_2$OH).

When the alkyl ester has been obtained and an acid is desired, saponification procedures, as known in the art for PGF-type compounds are employed.

When an acid has been prepared and an alkyl, cycloalkyl, or aralkyl ester is desired, esterification is advantageously accomplished by interaction of the acid with appropriate diazohydrocarbon. For example, when diazomethane is used, the methyl ester is produced. Similar use of diazoethane, diazobutane, and 1-diazo-2-ethylhexane, and diazodecane, for example, gives the ethyl, butyl, and 2-ethylhexyl and decyl esters, respectively. Similarly, diazocyclohexane and phenyldiazomethane yield cyclohexyl and benzyl esters, respectively.

Esterification with diazohydrocarbons is carried out by mixing a solution of the diazohydrocarbon in a suitable inert solvent, preferably diethyl ether, with the acid reactant, advantageously in the same or a different inert diluent. After the esterification reaction is complete the solvent is removed by evaporation, and the ester purified if desired by conventional methods, preferably by chromatography. It is preferred that contact of the acid reactants with the diazohydrocarbon be no longer than necessary to effect the desired esterification, preferably about one to about 10 min, to avoid undesired molecular changes. Diazohydrocarbons are known in the art or can be prepared by methods known in the art. See, for example, Organic Reactions, John Wiley and Sons, Inc., New York, N.Y., Vol. 8, pp. 389-394 (1954).

An alternative method for alkyl, cycloalkyl or aralkyl esterification of the carboxy moiety of the acid compounds comprises transformation of the free acid to the corresponding substituted ammonium salt, followed by interaction of that salt with an alkyl iodide. Examples of suitable iodides are methyl iodide, ethyl iodide, butyl iodide, isobutyl iodide, tert-butyl iodide, cyclopropyl iodide, cyclopentyl iodide, benzyl iodide, phenethyl iodide, and the like.

Various methods are available for preparing phenyl or substituted phenyl esters within the scope of the invention from corresponding aromatic alcohols and the free acid, differing as to yield and purity of product.

With regard to the preparation of the phenyl, particularly p-substituted phenyl esters disclosed herein (i.e., X_1 is —$COOR_1$ and R_1 is p-substituted phenyl), such compounds are prepared by the method described in U.S. Pat. No. 3,890,372. Accordingly, by the preferred method described therein, the p-substituted phenyl ester is prepared first by forming a mixed anhydride, particularly following the procedures described below for preparing such anhydrides as the first step in the preparation of amido and cycloamido derivatives.

This anhydride is then reacted with a solution of the phenol corresponding to the p-substituted phenyl ester to be prepared. This reaction proceeds preferably in the presence of a tertiary amine, such as pyridine. When the conversion is complete, the p-substituted phenyl ester has been recovered by conventional techniques.

A preferred method for substituted phenyl esters is that disclosed in U.S. Pat. No. 3,890,372 in which a mixed anhydride is reacted with an appropriate phenol or naphthol. The anhydride is formed from the acid with isobutylchloroformate in the presence of a tertiary amine.

Phenacyl-type esters are prepared from the acid using a phenacyl bromide, for example p-phenylphenacyl bromide, in the presence of a tertiary amine. See, for example, U.S. Pat. No. 3,984,454, German Offenlegungsschrift No. 2,535,693, and Derwent Farmdoc No. 16828X.

Carboxyamides (X_1 is —COL_4) are prepared by one of several amidation methods known in the prior art. See, for example, U.S. Pat. No. 3,981,868, issued 21 Sept. 1976 for a description of the preparation of the present amido and cycloamido derivatives of prostaglandin-type free acids and U.S. Pat. No. 3,954,741 describing the preparation of carbonylamido and sulfonylamido derivatives of prostaglandin-type free acids.

The preferred method by which the present amido and cycloamido derivatives of the acids are prepared is, first, by transformation of such free acids to corresponding mixed acid anhydrides. By this procedure, the prostaglandin-type free acid is first neutralized with an equivalent of an amide base, and thereafter reacted with a slight stoichiometric excess of a chloroformate corresponding to the mixed anhydride to be prepared.

The amine base preferred for neutralization is triethylamine, although other amines (e.g., pyridine, methyldiethylamine) are likewise employed. Further, a convenient, readily available chloroformate for use in the mixed anhydride production is isobutyl chloroformate.

The mixed anhydride formation proceeds by conventional methods and accordingly the free acid is mixed with both the tertiary amine base and the chloroformate in a suitable solvent (e.g., aqueous tetrahydrofuran), allowing the reaction to proceed at −10° C. to 20° C.

Thereafter, the mixed anhydride is converted to the corresponding amido or cycloamido derivatives by reaction with the amine corresponding to the amide to be prepared. In the case where the simple amide (—NH_2) is to be prepared, the transformation proceeds by the addition of ammonia. Accordingly, the corresponding amine (or ammonia) is mixed with the mixed anhydride at or about −10° to +10° C., until the reaction is shown to be complete.

Thereafter, the novel amido or cycloamido or cycloamido derivative is recovered from the reaction mixture by conventional techniques.

The carbonylamido and sulfonylamido derivative of the presently disclosed PG-type compounds are likewise prepared by known methods. See, for example, U.S. Pat. No. 3,954,741 for description of the methods by which such derivatives are prepared. By this known method the acid is reacted with a carboxyacyl or sulfonyl isocyanate, corresponding to the carbonylamido or sulfonylamido derivative to be prepared.

By another, more preferred method the sulfonylamido derivatives of the present compounds are prepared by first generating the PG-type mixed anhydride, employing the method described above for the preparation of the amido and cycloamido derivatives. Thereafter, the sodium salt of the corresponding sulfonamide is reacted with the mixed anhydride and hexamethylphosphoramide. The pure PG-type sulfonylamido derivative is then obtained from the resulting reaction mixture by conventional techniques.

The sodium salt of the sulfonamide corresponding to the sulfonylamido derivative to be prepared is generated by reacting the sulfonamide with alcoholic sodium methoxide. Thus, by a preferred method methanolic sodium methoxide is reacted with an equal molar amount of the sulfonamide. The sulfonamide salt is then reacted, as described above, with the mixed anhydride, using about four equivalents of the sodium salt per equivalent of anhydride. Reaction temperatures at or about 0° C. are employed.

The compounds of this invention prepared by the processes of this invention, in free acid form, are transformed to pharmacologically acceptable salts by neutralization with appropriate amounts of the corresponding inorganic or organic base, examples of which correspond to the cations and amines listed hereinabove. These transformations are carried out by a variety of procedures known in the art to be generally useful for the preparation of inorganic, i.e., metal or ammonium salts. The choice of procedure depends in part upon the solubility characteristics of the particular salt to be prepared. In the case of the inorganic salts, it is usually suitable to dissolve an acid of this invention in water containing the stoichiometric amount of a hydroxide, carbonate, or bicarbonate corresponding to the inorganic salt desired. For example, such use of sodium hydroxide, sodium carbonate, or sodium bicarbonate gives a solution of the sodium salt. Evaporation of the water or addition of a water-miscible solvent of moderate polarity, for example, a lower alkanol or a lower alkanone, gives the solid inorganic salt if that form is desired.

To produce an amine salt, an acid of this invention is dissolved in a suitable solvent of either moderate or low polarity. Examples of the former are ethanol, acetone, and ethyl acetate. Examples of the latter are diethyl ether and benzene. At least a stoichiometric amount of the amine corresponding to the desired cation is then

added to that solution. If the resulting salt does not precipitate, it is usually obtained in solid form by evaporation. If the amine is relatively volatile, any excess can easily be removed by evaporation. It is preferred to use stoichiometric amounts of the less volatile amines.

Salts wherein the cation is quaternary ammonium are produced by mixing an acid of this invention with the stoichiometric amount of the corresponding quaternary ammonium hydroxide in water solution, followed by evaporation of the water.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is more completely understood by the operation of the following examples:

EXAMPLE 1

3-oxo-7α-tetrahydropyran-2-yloxy-6β[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]-bicyclo[3.3.0]-oct-1-ene

(Formula XXIV: R_{18} is tetrahydropyranyloxy; Y_1 is trans—CH=CH—, M_6 is α-tetrahydropyranyloxy:β-H, L_1 is α-H:β-H, R_{27} is n-butyl; and n is the integer one).

Refer to Chart A.

A. To a stirred solution of 19 ml (170 mmoles) dimethyl methylphosphonate and 600 ml of dry tetrahydrofuran at −78° C. under an argon atmosphere is added dropwise over 5 min 110 ml (172 mmoles) of 1.56 M n-butyllithium in hexane. The resulting solution is stirred for 30 min at −78° C., treated with 25.4 g of 3α,5α-dihydroxy-2β-(3α-hydroxy-trans-1-octenyl)-1α-cyclopentaneacetic acid, lactone, bis(tetrahydropyranyl)ether, in 100 ml of dry tetrahydrofuran dropwise over one hr, and stirred for one hr at −78° C. and four hr at room temperature. The reaction is then quenched by addition of 10 ml glacial acetic acid, diluted with 700 ml of brine, and extracted with diethyl ether (3×700 ml). The combined ethereal layers are washed with 200 ml bicarb and 500 ml brine and dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield 37 g of formula XXII compound as oily white solid: 3-dimethylphosphonomethyl-3-hydroxy-2-oxa-7α-tetrahydropyran-2-yloxy-6β[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]-bicyclo[3.3.0]octane. Crystallization of the crude product from hexane and ether yields 22.1 g of purified formula XXII product. Silica gel TLC R_f is 0.22 in ethyl acetate. The melting range is 89°–93° C. NMR absorptions are observed at 3.72 (doublet, J=11 Hz) and 3.83 (doublet, J=11 Hz)δ. Characteristic infrared absorptions are 3340, 1250, 1185, 1130, 1075, and 1030 cm^{-1}.

B. To a solution of 10.0 g of the product of Part A in 75 ml acetone stirring under a nitrogen atmosphere at −10° C. is added over 30 min 9.0 ml of Jones reagent. The resulting suspension is stirred for 30 min at −10° C. and then quenched with 4 ml 2-propanol. The solvents are decanted away from the green residue and most of the acetone removed at reduced pressure. The acetone concentrate is then taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate and then with brine and dried over anhydrous sodium sulfate. Concentration under reduced pressure yields 8.2 g of formula XXIII product: 2-decarboxy-6-desbutyl-6-dimethylphosphonomethyl-6-keto-PGE_1, 11,15-bis(tetrahydropyranyl ether). Chromatography of formula XXIII product on 600 g silica gel eluting with 20% acetone in methylene chloride yields 4.95 g of pure formula XXIII product. Silica gel TLC R_f (in 20% acetone in methylene chloride) is 0.22. Characteristic NMR absorptions are observed at 3.14 (doublet, J=23 Hz) and 3.80 (doublet, J=11 Hz), 5.4–5.8 (m)δ. Characteristic infrared absorptions are observed at 1745, 1715, 1260, 1200, 1185, 1130, 1030, 970, 870 cm^{-1}.

C. A suspension of 5.37 g of the product of Example 1, Part B, 1.33 g anhydrous potassium carbonate, and 5.37 g 18-Crown-6 ether in 200 ml toluene is heated at 75° C. for six hr under a nitrogen atmosphere, cooled to 0° C., and washed with 200 ml brine, 200 ml of 3:1 water:brine, and 200 ml brine, and dried over anhydrous sodium sulfate. Most of the solvents are removed under reduced pressure and the residue is filtered through 50 g silica gel eluting with 250 ml ethyl acetate to give 3.9 g of formula XXIV product: 3-oxo-7α-tetrahydropyranyl-2-yloxy-6β[(3'S)-3'-tetrahydropyran-2-yl-trans-1'-octenyl]bicyclo[3.3.0]oct-1-ene. The crude product is chromatographed on 300 g silica gel eluting with 60:40 hexane:ethyl acetate to give 2.39 g of pure title product. Silica gel TLC R_f is 0.22 in 60:40 hexane:ethyl acetate. NMR absorptions are observed at 5.18–5.86 (m) and 5.94 (broad singlet)δ. Infrared absorptions are observed at 1710 and 1632 cm^{-1}.

Following the procedure of Example 1, but employing the various 3α,5α-hydroxy-2-substituted-1α-cyclopentaneacetic acid δ-lactones of formula XXI, there are prepared each of the various corresponding formula XXIV products wherein n is one.

Further, following the procedure of Example 1, but employing each of the various 3α,5α-dihydroxy-2-substituted-1α-cyclopentanepriopionic acid, δ-lactones of formula XXI, there are prepared each of the various formula XXIV compounds wherein n is 2.

Further, following the procedure of Example 1, but employing each of the various 5α-hydroxy-2-substituted-1α-cyclopentanealkanoic acid lactones of formula XXI, there are prepared each of the various formula XXIV compounds wherein R_{18} is hydrogen. Finally, following the procedure of Example 1, but employing each of the various 3α-hydroxymethyl-5α-hydroxy-2-substituted-1α-cyclopentanealkanoic acid lactones of formula XXI, there are prepared each of the various formula XXIV compounds wherein R_{18} is $—CH_2OR_{10}$.

EXAMPLE 2

3-oxo-8α-tetrahydropyran-2-yloxy-7β[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]bicyclo[4.3.0]non-1-ene

(Formula XXIV: R_{18}, Y_1, M_6, R_7 are defined in Example 1 and n is the integer 2).

Refer to Chart A.

A. A solution of 2.05 ml (18.9 mmoles) of dimethyl methylphosphonate and 100 ml of dry tetrahydrofuran is stirred at −78° C. under a nitrogen atmosphere and treated dropwise with 11.8 ml (18.9 mmoles) of 1.6 molar n-butyllithium in hexane. After stirring for 30 min at −78° C., the resulting mixture is treated dropwise over 25 min with 4.25 g of 3α,5α-dihydroxy-2β-(3α-hydroxy-trans-1-octenyl) 1α-cyclopentane propionic acid, δ-lactone, 11,15-bis(tetrahydropyranyl ether), in 30 ml of dry tetrahydrofuran. The resulting mixture is then stirred for one hr at 78° C. The solution is then allowed to stir at ambient temperature for 2 hr and is quenched by addition of 1.2 ml of acetic acid. The mixture is then added to 250 ml of brine and 200 ml of diethyl ether. The aqueous and organic layers are then separated and the aqueous layer extracted twice with

diethyl ether. The ethereal extracts are then washed with brine, dried over anhydrous sodium sulfate, and concentrated to yield 5.6 g of crude formula XXII compound, as an oil: 3-(dimethylphosphonomethyl)-3-hydroxy-2-oxo-8α-tetra-hydropyran-2-yl-oxy-7β[(3'S)-3'-tetrahydropyran-2-yloxy-trans1'-octenyl]-bicyclo[4.3.0]nonane. Chromatography on silica gel eluting with 4:1 ethyl acetate:acetone yields 4.1 g of purified formula XXII product. Characteristic NMR absorption is observed at 5.15–5.65 (multiplet)δ. Silica gel TLC R_f is 0.34 in 4:1 ethyl acetone:acetone. Characteristic infrared absorptions are observed at 3350, 1235, and 1030 cm^{-1}.

B. A suspension of 3.42 g of chromium trioxide and 80 ml of methylene chloride is treated with 5.8 ml of pyridine, stirred at ambient temperature under a nitrogen atmosphere for 30 min, and combined with 3 scoops of dry diatomaceous earth. The resulting mixture is then treated with 3.25 g of the reaction product of Part A and 8 ml of dry dichloromethane, stirred for 30 min at ambient temperature under nitrogen, filtered through 30 g of silica gel (eluting with 200 ml of ethylacetate and acetone, 2:1) and concentrated under reduced pressure. Chromatographing the residue (3.73 g) on 120 g of silica gel, eluting with ethyl acetate and acetone (4:1) yields 2.07 g of formula XXIII product: 2-decarboxy-5-despropyl-6-dimethylphosphonom ethyl-5-keto-PFE_1, 11,15-bis(tetrahydropyranyl ether). Characteristic infrared absorptions are observed at 1740 and 1715 cm^{-1}.

Characteristic NMR absorptions are observed at 3.1 (doublet, J=23 Hz) and 3.8 (doublet, J=11 Hz)δ.

C. A suspension of 12 mg of 50% sodium hydride in mineral oil and 3 ml of diglyme is stirred at 0° C. under an argon atmosphere. The suspension is then treated with 150 mg of the product of Part B in 3 ml of diglyme. After 1 hr, the cooling bath is removed and the resulting solution is stirred at ambient temperature under argon. After a total of 20 hr from addition of the formula XXIII reactant, the resulting solution is then added to 30 ml of water and extracted with 90 ml of diethyl ether. The ethereal extract is washed with brine (30 ml), dried over anhydrous sodium sulfate, concentrated under reduced pressure to a brown oil (110 mg) and chromatographed on 10 g of silica gel eluting with hexane and ethyl acetate (1:1). There is accordingly prepared 15 mg of formula XXIV compound: 3-oxo-8α-tetrahydropyran-2-yloxy-7β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]bicyclo[4.3.0]non-1-ene. NMR absorptions are observed at 4.7 (broad singlet) and 5.3–6.0 (multiplet)δ. IR absorption is observed at 1670 cm^{-1}.

Alternatively, the formula XXIV compound above is prepared as follows:

A solution of 150 mg of the product of Part B and 5 ml of dry tetrahydrofuran at 0° C. under an argon atmosphere is treated dropwise with 0.5 ml of 0.52 M potassium hydride and 18-crown-6 ether (Aldrich Chemical Co. Catalog Handbook of Fine Chemicals 1979–1980, Milwaukee, Wisconsin, p. 133; Pedersen, J. C., JACS 92:386 (1970) in tetrahydrofuran (prepared from 800 mg potassium hydride and 1.0 g 18-crown-6 ether in 8.7 ml of dry tetrahydrofuran). After stirring for one hr at 0° C. under argon, the mixture is added to 30 ml of water, extracted with 90 mg of diethyl ether and the ethereal extract is washed with brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure, and chromatographed on 9 g of silica gel eluting with ethyl acetate and hexane. Formula XXIV product (40 mg) is thereby obtained. Silica gel TLC R_f is 0.30 in ethyl acetate and hexane (1:1).

EXAMPLE 3

1β-Methyl-3-oxo-7α-tetrahydropyran-2-yl-oxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]-bicyclo-[3.3.0]octane

(Formula XXV: R_{18}, Y_1, M_6, n, L_1, R_7 are as defined in Example 1, R_{16} is hydrogen and R_{37} is methyl).

Refer to Chart A.

A suspension of 2.70 g of anhydrous copper iodide is stirred in 100 ml of anhydrous diethyl ether at −20° C. under an argon atmosphere and is treated dropwise with 20.0 ml of 1.4 M ethereal methyllithium. The resulting solution is then stirred for 15 min at −20° C. and treated over 2.5 hr at −20° C. with a solution of 2.00 g of the title product of Example 1 in 100 ml of anhydrous diethyl ether. Stirring is continued for an additional 1.5 hr at −20° C. and the resulting mixture added to 200 ml of 1 M aqueous ammonium chloride. The aqueous and organic layers are then separated and the aqueous layer extracted with diethylether (400 ml). The combined organic extracts are then washed with 200 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield 2.4 g of title product as a pale green oil. Chromatography on 25 g of silica gel eluting with hexane in ethyl acetate (3:1) yields 2.0 g of title product as a colorless oil. Characteristic NMR absorptions ($CDCl_3$) are observed at 1.18, 3.20–4.43, 4.70, and 5.2–5.9δ. Characteristic infrared absorptions are observed at 1745, 1665, 1200, 1130, 1110, 1075, 1035, 1020, 980, and 870 cm^{-1}. Silica gel R_f is 0.26 in ethyl acetate and hexane (1:3).

By procedures known in the art, each of the various novel formula XXV intermediates is transformed to a 9β-methyl-CBA_2 or CBA_1 compound by methods examplified hereinafter or known from British Published Specification Nos. 2,013,661, 2,014,143, and 2,017,699.

EXAMPLE 4

5-Carboxypentanol, t-butyldimethylsilyl ether

A solution of 4 g of sodium hydroxide in 100 ml of methanol and water (4:1) is treated with 10 ml of caprolactone and stirred at ambient temperature under a nitrogen atmosphere. After 20 hr, solvent is evaporated following addition of toluene, yielding 15 g of solid, crude 5-carboxypentanol.

The above solid is suspended in 300 ml of dimethylformamide under a nitrogen atmosphere, cooled to 0° C., treated with 35 g of imidazole, stirred for 15 min at 0° C. and 15 min at ambient temperature, cooled to 0° C. and treated with 39 g of t-butyldimethyl silylchloride. The resulting solution is then allowed to warm to ambient temperature under a nitrogen atmosphere. After 26 hr, the resulting solution is treated with 8 g of sodium hydroxide in 40 ml of water and 40 ml of methanol, with stirring maintained under a nitrogen atmosphere. After 13 hr, the suspension is acidified to pH 4 with 500 ml of 1 N aqueous hydrogen chloride, then saturated with sodium chloride and extracted with ethyl acetate. The ethyl acetate extracts are then washed with 1 N aqueous sodium hydroxide. The basic extracts are then acidified to pH 4 with concentrated hydrochloric acid, saturated with brine, and extracted with ethyl acetate. The ethyl acetate extracts are then washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 22.6 g of a yellow liquid, 5-carboxypen-

tanol, t-butyldimethylsilyl ether. Chromatography on 800 g of silica gel eluting with ethyl acetate and hexane (1:9 to 1:1) yields 14.8 g of 5-carboxypentanol, t-butyldimethylsilyl ether. NMR absorptions are observed at 0.05 (singlet) and 0.90 (singlet)δ. Infrared absorptions are observed at 3000 (broad) and 1700 cm^{-1}.

Following the procedure of Example 4, but employing each of the various lactones corresponding to the ω-carboxyalkanol compounds of formula XXXII there are prepared each of the various formula XXXII products.

EXAMPLE 5

2-Decarboxy-2-(t-butyldimethylsilyloxy)methyl-5-carboxy-6-hydroxy-9β-methyl-CBA_1, 11,15-bis(tetrahydropyran)ether

(Formula XXXIII: R_{28} is t-butyldimethylsilyl, Z_2 is $—(CH_2)_3—$, n is 1, and R_{16}, R_{18}, R_{37}, M_6, L_1, and R_4 are as defined in Example 3).

Refer to Chart B.

A solution of 0.58 ml of dry diisopropylamine and 20 ml of dry tetrahydrofuran at 0° C. under an argon atmosphere is treated with 2.6 ml of 1.56 M n-butyllithium in hexane, stirred for 5 to 10 min at 0° C., treated with 0.50 g of the title product of Example 4 in 5 ml of tetrahydrofuran, stirred for 15 min at 0° C. and 1 hr at ambient temperature, cooled to 0° C., treated with 0.91 g of the title product of Example 3 in 5 ml of tetrahydrofuran, and allowed to slowly warm to ambient temperature under an argon atmosphere. Thereafter, 130 ml of water and 20 ml of brine are added and the mixture extracted with diethyl ether. The ethereal extracts are then washed with 4 ml of 1 N aqueous hydrochloric acid and 150 ml of brine and dried over sodium sulfate, and concentrated under reduced pressure to yield title product.

Following the procedure of Example 5, but employing each of the various formula XXXI compounds described following Example 1, there are prepared each of the various formula XXXIII compounds wherein R_{28} t-butyldimethylsilyl and Z_2 is $—(CH_2)_3—$.

EXAMPLE 6

2-Decarboxy-2-(t-butyldimethylsilyloxy)methyl-9β-methyl-CBA_2, 11,15-bis-(tetrahydropyranylether)

(Formula XXXIV: R_{28}, Z_2, n, R_{18}, Y_1, M_6, L_1 and R_7 are as defined for Examples 1 and 5).

The reaction product of Example 5 (1.37 g) and 16 ml of methylene chloride is treated with 2.9 ml of dimethylformamide dineopentyl acetal, stirred for 3 hr at ambient temperature under nitrogen, added to 160 ml of ice water and 40 ml of brine, and extracted with diethyl ether. The ethereal extracts are then washed with 150 ml of sodium bicarbonate and 150 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield crude title product. Chromatography on 100 g of silica gel eluting with 10% ethyl acetate in hexane yields pure title product.

Following the procedure of Example 6, but employing each of the various formula XXXIII compounds described following Example 5, there are prepared each of the various corresponding formula XXXIV products wherein R_{28} is t-butyldimethylsilyl and Z_2 is $—(CH_2)_3—$.

EXAMPLE 7

2-Decarboxy-2-hydroxymethyl-9β-methyl-CBA_2, 11,15-bis(tetrahydropyranyl)ether

(Formula XXXV: Z_2, n, R_{16}, R_{37}, R_{18}, Y_1, M_6, L_1, and R_7 are as defined in Examples 1 and 5).

Refer to Chart B.

A solution of 0.71 g of the title product of Example 6 and 16 ml of dry tetrahydrofuran at 0° C. under a nitrogen atmosphere is treated with 3.2 ml of 0.75 molar tetra-n-butylammoniumfluoride and tetrahydrofuran. After allowing the reaction mixture to slowly warm to ambient temperature overnight with stirring, 150 ml of brine is added and the resulting mixture extracted with ethyl acetate. The ethyl acetate extracts are then washed with 0.5 N aqueous potassium bisulfate, 100 ml of sodium bicarbonate, and 100 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield crude title product. Filtering through 25 g of silica gel with 200 ml of ethyl acetate and hexane yields 0.61 g of further purified product. Chromatography on silica gel eluting with 35% ethyl acetate in hexane yields pure title product.

Following the procedure of Example 7, but employing each of the various formula XXXIV compounds described in and following Example 6, there are prepared each of the various formula XXXV compounds wherein Z_2 is $—(CH_2)_3—$.

Following the procedure of Examples 5, 6, and 7, and employing the various starting materials described in and following these examples and each of the various formula XXXII compounds described in and following Example 4, there are prepared each of the various formula XXXV compounds.

EXAMPLE 8

2-Decarboxy-2-hydroxymethyl-9β-methyl-CBA_2

(Formula XXXVI: X_1 is $—CH_2OH$, Z_2 is $—(CH_2)_3—$, R_8 is hydroxy, Y_1 is trans—CH=CH—, M_1 is α-OH:β-H, L_1 is α-H:β-H and R_7 is n-butyl).

Refer to Chart B.

The title product of Example 7 (0.25 g) is combined with 9 ml of acetic acid, water and tetrahydrofuran (6:3:1) and heated to 37°–40° C. for two hr. Thereafter the resulting mixture is cooled and extracted with diethyl ether. The ethereal extracts are then washed with brine, dried over sodium sulfate and concentrated to yield crude title product. Chromatography on silica gel yields pure title product.

Following the procedure of Example 7, but employing each of the various formula XXXV primary alcohols described in and following Example 7 there are prepared each of the various corresponding formula XXXVI products wherein X_1 is $—CH_3OH$.

EXAMPLE 9

o-(t-Butyldimethylsilyloxyethyl)benzaldehyde

(Formula XLIV: R_{28} is t-butyldimethylsilyloxy and g is one).

Refer to Chart C.

A. To a mixture of 7.6 g of lithium aluminum hydride and 400 ml of dry tetrahydrofuran under a nitrogen atmosphere is added dropwise with stirring 18 g of homophthalic acid (Aldrich Chemical Company) in 250 ml of dry tetrahydrofuran. Dropwise addition rate is adjusted such that mild reflux is maintained during the

course of the exothermic reaction. The resulting mixture is then heated at reflux for 5 hr, cooled to 0° C., and 7.6 g of water in 50 ml of tetrahydrofuran is added dropwise with stirring. Thereafter 27 ml of 10% aqueous sodium hydroxide is added and the resulting mixture is stirred at ambient temperature for 20 min, filtered, and the filter solids washed with 150 ml of tetrahydrofuran. The filtrate and tetrahydrofuran wash are then concentrated under reduced pressure to yield 14.0 g of crude formula XXXII diol, 2-(o-hydroxymethylphenyl)ethanol. Chromatography on 1.2 kg of silica gel, deactivated by addition of 240 ml of ethyl acetate, eluting with ethyl acetate, yields 13.5 g of formula XLII product. Melting range is 41.5°–43° C.

B. To a solution of 13.5 g of the reaction product of Part A in 50 ml of dry tetrahydrofuran under a nitrogen atmosphere is added with stirring 9.05 g of imidazole. The resulting solution is then cooled to −5° C. and 13.9 g of t-butyldimethylsilyl chloride is added. The resulting mixture is then maintained for 20 min and thereafter allowed to warm to ambient temperature. After 1 hr, the resulting mixture is then shaken with 500 ml of hexane and diethylether (2:1) and 250 ml of water and brine (1:1). The organic layer is then washed with water and brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield a crude mixture of mono- and bis-silyl ethers corresponding to the starting material of Part A. This mixture of products is then chromatographed on 2 kg of silica gel, deactivated with 400 ml of ethyl acetate and eluted with 25% ethyl acetate and Skellysolve B to yield 6.82 g of formula XLIII product, o-(t-butyldimethylsilyloxyethyl)phenylmethanol. NMR absorptions are observed at 7.20–7.52, 4.57, 3.91 (t, J G.1), 2.93 (t, J 6.1), 0.82, and −0.08δ. Silica gel TLC R_f is 0.54 in 25% ethyl acetate and hexane.

C. A mixture of 5.0 g of the reaction product of Part B, 100 ml of trichloromethane, and 25 g of activated manganese dioxide (MnO_2) is stirred at ambient temperature for 4 hr. Chloroform (100 ml) is then added and the resulting mixture filtered through diatomaceous earth. After washing filter solids with 200 ml of trichloromethane, the resulting filtrate and wash is then concentrated under reduced pressure to yield a residue containing title product. Chromatography on 400 g of silica gel, deactivated with 80 ml of ethyl acetate and elution with 25% ethyl acetate and hexane yields 2.93 g of pure title product. Silica gel TLC R_f is 0.74 in 25% ethyl acetate and hexane. NMR absorptions are observed at 10.34, 7.25–8.00, 3.89 (t, J 6.0), 3.27 (t, J 6.0), 0.83 and −0.09δ. The mass spectrum exhibits a peak at 265 (M+1) and other peaks of decreasing intensity at m/e 75, 207, 73, 133, 223, 208, 77, 177, 76 and 105.

Following the procedure described in Chart C, but employing each of the various formula XXXI acids, there is prepared each of the various corresponding formula XXXIV aldehydes wherein R_{28} is t-butyldimethylsilyl.

EXAMPLE 10

m-(t-Butyldimethylsilyloxymethyl)benzaldehyde

(Formula XLIV: g is zero and R_{28} is t-butyldimethylsilyl).

Refer to Chart C.

A. To a solution of 10.0 g of m-(hydroxymethyl)-phenylmethanol in 40 ml of dry tetrahydrofuran under a nitrogen atmosphere is added with stirring 7.35 g imidazole. The resulting solution is then cooled to 0° C. and 11.3 g of t-butyldimethylsilyl is added. The resulting mixture is then stirred with cooling for 15 min and thereafter allowed to warm to ambient temperature. After 90 min, the resulting mixture is then shaken in 400 ml of hexane and diethyl ether (2:1) and 200 ml of water and brine (1:1). The organic layer is then washed successively with water and brine (1:1, 300 ml) and brine (150 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a mixture of mono- and bis-t-butyldimethylsilyloxy ether corresponding to the formula XXXII compound. This mixture of products is then chromatographed on 1.4 kg of silica gel, deactivated by addition of 280 ml of ethyl acetate and eluted with 25–40% ethyl acetate in hexane to yield 7.65 kg of pure formula XLIII product, m-(t-butyldimethylsilyloxymethyl)phenylmethanol. Silica gel TLC R_f is 0.46 in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.25, 4.72, 4.60, 2.23, 0.92, and 0.09δ. The mass spectrum exhibits a peak at 251 (M+-1) and other peaks of decreasing intensity at m/e 235, 121, 195, 237, 105, 133, 75, 89, 236, and 119.

B. A mixture of 5.0 g of the reaction product of Part A and 100 ml of trichloromethane and 25 g of activated manganese dioxide (MnO_2) is stirred at ambient temperature for 4 hr. Chloroform (100 ml) is then added and the resulting mixture filtered through diatomaceous earth. The filter solids are washed with 200 ml of trichloromethane and the filtrate and trichloromethane wash are then concentrated under reduced pressure to yield 5.2 g of crude title product. Chromatography on 400 g of silica gel, deactivated with 80 ml of ethyl acetate and elution with ethyl acetate and hexane (1:3) yields 3.65 g of pure title product. Silica gel TLC R_f is 0.46 in 10% ethyl acetate and hexane. NMR absorptions are observed at 10.00, 7.26–7.86, 4.81, 0.95, and 0.11δ.

EXAMPLE 11

3-Phenylsulfonyl-7α-tetrahydropyran-2-yloxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]-bicyclo-[3.3.0]octane

(Formula LV: n is the integer one, R_{18} is tetrahydropyranyloxy, Y_1 is trans—CH=CH—, M_6 is α-tetrahydropyranyloxy:β-hydrogen, L_1 is α-hydrogen:β-hydrogen, R_{16} and R_{17} are both hydrogen, and R_{27} is n-butyl).

Refer to Chart D.

A. Sodium borohydride (0.38 g) is added with stirring to a solution of 2.90 g of 3-oxo-7α-tetrahydropyran-2-yloxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]-bicyclo[3.3.0]octane in 25 ml of 95% aqueous ethanol. The resulting mixture is then stirred at ambient temperature for 20 min. Thereafter the resulting mixture is shaken in 100 ml of brine and 200 ml of ethyl acetate. The organic layer is then immediately washed in brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield 2.94 g of formula LII alcohol: (3RS)-3-hydroxy-7α-tetrahydropyran-2-yloxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]bicyclo[3.3.0]-octane. Infrared absorptions are observed at 3600 and 3450 cm^{-1} and no carbonyl absorption. Silica gel TLC R_f is 0.63 and 0.67 in ethyl acetate and hexane (1:1).

B. To a solution of 2.9 g of the reaction product of Part A in 25 ml of dry dichloromethane and 1.4 ml (1.02 g) of triethylamine at 0° C. is added with stirring 0.57 ml of (0.848 g) of methanesulfonyl chloride over 5 min. The resulting is then stirred an additional 20 min and

shaken with 160 ml of diethyl ether and 80 ml of cold (0° C.) dilute aqueous hydrochloric acid. The organic layer is then washed successively in brine, dilute aqueous potassium bicarbonate, and brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 3.5 g of crude formula LIII compound: (3RS)-3-hydroxy-7α-tetrahydropyran-2-yloxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl]bicyclo[3.3.-0]octane, 3-methylsulfonate.

C. Thiophenol (1.13 ml, 1.21 g) is added to a mixture of 1.12 g of potassium t-butoxide in 15 ml of dry dimethylsulfoxide (DMSO) under a nitrogen atmosphere. To the solution of potassium thiophenoxide thus prepared is added 3.5 g of the reaction product of Part B in 8 ml of dimethylsulfoxide. The resulting mixture is then stirred at ambient temperature for 16 hr, whereupon additional potassium t-butoxide is added so as to transform the solution to a distinct yellow color. The resulting mixture is then stirred an additional 4 hr at ambient temperature, diluted with 100 ml of diethyl ether and 100 ml of hexane, washed with 5% aqueous potassium hydroxide (200 ml) and brine (200 ml), dried over magnesium sulfate, and concentrated under reduced pressure to yield 5 g of a residue of crude formula LIV compound: 3-phenylthio-7-α-tetrahydropyran-2-yloxy-6β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-ocenyl]-bicyclo[3.3.0]octane. Chromatography on 300 g of silica gel, deactivated with 40 ml of diethyl ether and 40 ml of trichloromethane and eluted with 5% diethyl ether in trichloromethane yields 3.1 g of pure product. Silica gel TLC R_f is 0.75 in 10% ethyl acetate in dichloromethane.

D. To a solution of 3.1 g of the reaction product of Part C and 50 ml of dichloromethane at 0° C. is added with stirring over 10 min 2.43 g of 85% m-chloroperbenzoic acid. The resulting mixture is then stirred at 0° C. for 30 min. diluted with 150 ml of dry ethyl ether, washed with ice cold dilute aqueous potassium hydroxide and brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield 3.4 g of crude title product. Chromatography on 350 g of silica gel, deactivated with 70 ml of ethyl acetate and elution with 500 ml of 30–50% ethyl acetate in hexane yields 2.90 g of pure title product as a mixture of C-6 isomers. Silica gel TLC R_f's are 0.41, 0.45 and 0.48 in 30% ethyl acetate in hexane (stereoisomers). NMR absorptions are observed at 7.52–8.02, 5.30–5.67, 4.70, and 3.30–4.13δ.

Following the procedure of Example 11, each of the formula LI compounds is transformed to the corresponding formula LV 3-phenylsulfonyl compound.

EXAMPLE 12

(5E)-2,5-inter-o-phenylene-3,4-dinor-CBA$_2$

(Formula LX: X_1 is —COOH, g is one, n is one, R_{16} and R_{17} are hydrogen, R_8 is hydroxy, Y_1 is trans—CH=CH—, M_1 is α-OH:β-H, L_1 is α-H:b-H, and R_7 is n-butyl), its methyl ester and the corresponding (5Z) isomers thereof.

Refer to Chart C.

A. To a solution of 1.26 g of the title product of Example 11 in 15 ml of dry tetrahydrofuran at −78° C. under a nitrogen atmosphere is added dropwise with stirring 1.48 ml of 1.6 M n-butyllithium in hexane over 1 min. After 10 min 0.66 g of title product of Example 4 in 5 ml of dry tetrahydrofuran is added. After 45 min 0.26 ml of distilled acetic anhydride is added. Stirring is then continued at −78° C. for 3 hr and at ambient temperature for an additional 2 hr. The resulting mixture is then shaken with 120 ml of diethyl ether and 80 ml of saturated aqueous ammonium chloride. The organic layer is then washed with 15 ml of brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield 2.21 g of formula LVI product as a mixture of isomers: 3-[α-acetoxy-o-(t-butyldimethylsilyloxyethyl)-α-tolyl]-3-phenylsulfonyl-7α-(tetrahydropyran-2-yl)oxy-6β-[(3'S)-3'-(tetrahydropyran-2-yl)oxy-trans-1'-ocentyl]bicyclo[3.3.0]-octane. R_{28}, g, R_{17}, n, R_{18}, Y_1, M_6, L_1, and R_{27} are defined in Examples 9 and 11. Silica gel TLC R_f range is 0.30–0.53 (8 spots) (stereoisomers) in 25% ethyl acetate and hexane.

B. The mixture of isomeric products of Part A (2.21 g) and 40 ml of methanol and 20 ml of ethyl acetate is stirred at −20° C. with chips of 5.6% sodium amalgam for 60 min. After decanting liquid, excess amalgam and solids are rinsed by decantation employing 200 ml of diethyl ether. The organic solutions are then combined, washed with brine, dried, and concentrated under reduced pressure to yield 1.8 g of crude 2-decarboxy-2-(t-butylidmethylsilyloxymethyl)-2,5-inter-o-phenylene-3,4-dinor-CBA$_2$, 11,15-bis(tetrahydropyranyl ether). Chromatography on 250 g of silica gel, deactivated with 50 ml of diethyl ether and eluted with 30% diethyl ether in hexane yields 1.06 g of pure product. Silica gel TLC R_f's are 0.49, 0.56, and 0.62 (stereoisomers) in 30% diethyl ether and hexane. NMR absorptions are observed at 7.20, 6.54, 5.22–5.80, 4.72, 3.38–4.16 and 2.74–3.00δ.

C. A solution of 1.06 g of the reaction product of Part B in 10 ml of dry tetrahydrofuran is treated with 3.2 ml of 0.75 N tetra-n-butylammonium fluoride in tetrahydrofuran at ambient temperature for 40 min. The resulting mixture is then diluted with 125 ml of diethyl ether. The resulting solution is then washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield a residue of isomeric formula LVIII products: (5E)- and (5Z)-2-decarboxy-2-hydroxymethyl-2,5-inter-o-phenylene-3,4-dinor-CBA$_2$, 11,15-bis-(tetrahydropyranyl ether). Chromatography on 100 g of silica gel, deactivated with 20 ml of ethyl acetate and eluted with 25–50% ethyl acetate in hexane yields 0.40 g of (5Z) isomer and 0.51 g of (5E) isomer. For the (5Z) isomer silica gel TLC R_f's and 0.31 and 0.35 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.20, 6.51, 5.10–5.72, 4.69, 3.32–4.16, and 2.76–3.00δ. For the (5E) isomer silica gel TLC R_f's are 0.20 and 0.24 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.19, 6.50, 5.10–5.64, 4.70, 3.32–4.10, and 2.88–3.01δ.

D. To a solution of 400 mg of the (5Z) reaction product of Part C in 20 ml of dry acetone at −50° C. is added with stirring 1.0 ml of Jones reagent (prepared as follows: 26.72 g of chromium trioxide in 23 ml of concentrated sulfuric acid diluted with water to a volume of 100 ml). The resulting mixture is then allowed to warm to −20° C. over a 20 min period and stirred at −20° C. for 30 min. Excess Jones reagent is then destroyed by addition of 0.5 ml of isopropanol. After 5 min the reaction mixture is then shaken in 100 ml of ethyl acetate and 80 ml of brine containing 0.5 ml of concentrated hydrochloric acid. The organic layer is then washed twice in 50 ml of water containing a trace (10 drops) of concentrated hydrochloric acid, twice in 50 ml of water and in brine. The organic layer is then dried over magnesium sulfate and concentrated under reduced pressure to yield 360 mg of crude (5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA$_2$, 11,15-bis(tetrahy-

dropyranyl ether), a formula LIX compound. Crude formula LIX compound is then taken up in 30 ml of diethyl ether and extracted in the mixture of 15 ml of water and 5 ml of methanol containing a trace amount (10 drops) of 45% aqueous potassium hydroxide. The extraction is repeated 6 times, until the acid is completely extracted from the ethereal solution. The aqueous extracts are then acidified to pH2 and extracted with ethyl acetate. The organic extract is then washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield a residue of pure title product. Silica gel TLC is a streak to about R_f 0.50 in ethyl acetate and hexane (1:1). Purified acid is then converted to the corresponding ethyl ester by treatment with excess ethereal diazomethane for 10 min. Following esterification, the resulting reaction mixture is treated with ethyl acetate and washed with dilute aqueous potassium hydroxide and brine. After drying and concentrating to a residue, chromatography on 20 g of silica gel deactivated with 4 ml of ethyl acetate and elution with 10% ethyl acetate in trichloromethane yields 210 mg of (5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2, methyl ester, 11,15-bis(tetrahydropyranyl ether). Silica gel TLC R_f's are 0.52, 0.56, and 0.60 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.20, 6.45, 5.34–5.78, 4.70, 3.68, and 3.30–4.28δ.

E. A mixture of 200 mg of methyl ester of Part D, 5 ml of acetic acid, 2.5 ml of water, and 1 ml of tetrahydrofuran is heated to 40° C. and stirred for 4 hr. The resulting mixture is then diluted with 100 ml of ethyl acetate and washed with a mixture of 6 g of 85% aqueous potassium hydroxide in 20 ml of water and 30 g of ice, washed with brine (40 ml), dried over magnesium sulfate, and concentrated under reduced pressure to yield 180 mg of crude (5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2, methyl ester. Chromatography on 20 g of silica gel deactivated with 4 ml of ethyl acetate and elution with 100 ml of 50% ethyl acetate in trichloromethane and 100 ml of 50% acetone in trichloromethane yields 105 mg of pure product. Silica gel TLC R_f is 0.57 in 40% acetone and trichloromethane and 0.52 in ethyl acetate. NMR absorptions are observed at 7.20, 6.43, 5.45–5.59, 3.65, 3.40–4.20, and 3.18δ. The mass spectrum of the bis TMS derivative exhibits peaks of decreasing intensity at m/e 73, 75, 74, 147, 43, 129, 41, 45, 167, 59, and an $M^+—C_5H_{11}$ peak at 485.2513.

F. To a solution of 105 mg of the reaction product of Part E in 5 ml of methanol and 2.5 ml of water under a nitrogen atmosphere is added 0.33 g of potassium carbonate. The resulting mixture is stirred at ambient temperature for 20 hr whereupon a small quantity (5 drops) of 45% aqueous potassium hydroxide is added. The resulting mixture is stirred for an additional 4 hr at ambient temperature. Thereupon the mixture is shaken with 100 ml of ethyl acetate and excess cold dilute aqueous hydrochloric acid. The organic layer is then washed with brine, dried, and concentrated under reduced pressure to yield 100 mg of pure (5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2. Silica gel TLC R_f is 0.56 in the A-IX solvent system (the organic phase of an equilibrated mixture of ethyl acetate, acetic acid, cyclohexane, and water, 9:2:9:10). The mass spectrum of the tris TMS derivative exhibits peak of decreasing intensity at m/e 73, 75, 129, 167, 74, 55, 69, 57, 147, and 45 and an $M^+—CH_3$ peak at 599.3418.

G. Following the procedure of Part D, 510 mg of the (5E) reaction product of Part C is transformed to 310 mg of (5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2, 11,15-bis(tetrahydropyranyl ether). Silica gel TLC R_f is 0.41 in 25% ethyl acetate and hexane containing 1% acetic acid, and 220 mg of (5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2, 11,15-bis(tetrahydropyranyl ether)-methyl ester . Silica gel TLC R_f's are 0.48, 0.51, and 0.56 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.20, 6.43, 5.26–5.64, 4.70, 3.65, and 3.30–4.10δ.

H. Following the procedure of Part E, the reaction product of Part G (210 mg) is transformed to 110 mg of (5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2, methyl ester. Silica gel TLC R_f is 0.57 in 40% acetone and trichloromethane and 0.46 in ether acetate. NMR absorptions are observed at 7.22, 6.44, 5.32–5.47, 3.68, 3.50–4.08, and 3.10δ. The mass spectrum of the bis TMS derivative exhibits peaks of decreasing intensity at m/e 73, 75, 129, 227, 167, 55, 57, 173, 74, 466 and an $M^+—CH_3$ peak at 541.3198.

I. Following the procedure of Part F, the reaction product of Part H (110 mg) is transformed to 102 mg of (5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2. Silica gel TLC Rf is 0.50 in the A-IX solvent system. The mass spectrum of the tris TMS derivative exhibits peaks of decreasing intensity at m/e 73, 75, 167, 129, 524, 453, 285, 147, 434, 213, and an $M^+—CH_3$ peak at 599.3424.

EXAMPLE 13

(5E)-1,5-inter-m-phenylene-2,3,4-trinor -CBA_2 its methyl ester, and the corresponding (5Z) isomers.

Refer to Chart D.

A. Following the procedure of Example 12, Part A, a solution of 1.26 g of the title product of Example 6 and 0.62 g of the title product of Example 5 are transformed to 2.3 g of formula LVI compound. Silica gel TLC R_f range is 0.37–0.56 (7 spots) (stereoisomers) in 25% ethyl acetate in hexane.

B. Following the procedure of Example 12, Part B, the reaction product of Part A (2.3 g) is transformed to 1.0 g of isomeric formula LVII compounds: (5E)- and (5Z)-2-decarboxy-2-(t-butyldimethysilyloxymethyl)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, 11,15-bis(tetrahydropyranyl ether). Silica gel TLC R_f's are 0.47, 0.54 and 0.58 (stereoisomers) in 30% diethyl ether and hexane.

C. Following the procedure of Example 12, Part C, 1.0 g of the isomerically mixed reaction product of Part B in transformed to 0.51 g of (5Z)-2-decarboxy-2-hydroxymethyl-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, 11,15-bis(tetrahydropyranyl ether) and 0.40 g of (5E)-2-decarboxy-2-hydroxymethyl-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, 11,15-bis(tetrahydropyranyl ether). For the (5Z)-isomer, silica gel TLC R_f's are 0.31 and 0.35 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.18, 6.36, 5.19–5.65, 4.63, 4.58, 3.31–4.08, and 2.92δ. For the (5E)-isomer, silica gel TLC R_f's are 0.23 and 0.27 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.19, 6.37, 5.29–5.72, 4.67, 4.60, 3.30–4.17, and 2.78δ.

D. Following the proceduce of Example 12, Part D, 510 mg of the (5Z) reaction product of Part C is transformed to 310 mg of (5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, 11,15-bis(tetrahydropyranyl ether) and 240 mg of (5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, methyl ester, 11,15-bis(tetrahydropyranyl ether). For the acid, silica gel TLC streak to about R_f

0.54 in 50% ethyl acetate and hexane. For the methyl ester, silica gel TLC R_f's are 0.58, 0.63, and 0.68 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.28–8.00, 6.40, 5.13–5.73, 4.71, 3.89, and 3.28–4.08δ.

E. Following the procedure of Example 12, Part E, 240 mg of the methyl ester product of Part D is transformed to 140 mg of (5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, methyl ester. Silica gel TLC R_f is 0.49 in ethyl acetate. NMR absorptions are observed at 7.28–7.93, 6.40, 5.34–5.48, 3.88, and 3.32δ. The mass spectrum of the bis TMS derivative exhibits peaks of decreasing intensity at m/e 83, 85, 73, 47, 213, 75, 129, 48, 87, 77, and an M^+-CH_3 peak at 527.2996.

F. To a solution of 140 mg of the reaction product of Part E in 6 ml of methanol under a nitrogen atmosphere is added a solution of 0.20 g of 85% potassium hydroxide in 2 ml of water. The resulting mixture is then stirred at ambient temperature for 7 hr, shaken with 200 ml of ethyl acetate and excess cold dilute aqueous hydrochloric acid. The organic layer is then washed with brine, dried over magnesium sulfate, concentrated under reduced pressure to yield 110 g of pure (5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2. Silica gel TLC R_f is 0.60 in the A-IX solvent system. The mass spectrum of the tris TMS derivative exhibits peaks of decreasing intensity at m/e 73, 271, 394, 129, 420, 510, 75, 147, 32, 74, and an M^+-CH_3 peak at 585.3234.

G. Following the procedure of Example 12, Part D, 400 mg of the (5E) reaction product of Part C is transformed to 260 mg of (5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, 11,15-bis(tetrahydropyranyl ether) and 190 mg of (5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, methyl ester, 11,15-bis(tetrahydropyranyl ether). For the acid silica gel TLC streak to about R_f 0.36 in 50% ethyl acetate and hexane. For the methyl ester, silica gel TLC R_f's are 0.50, 0.53, and 0.57 (stereoisomers) in 25% ethyl acetate and hexane. NMR absorptions are observed at 7.38–7.95, 6.42, 5.13–5.75, 4.68, 3.89, and 3.30–4.09δ.

H. Following the procedure of Example 12, Part E, 190 mg of the reaction product of Part G is transformed to 81 mg of (5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2, methyl ester. Silica gel TLC R_f is 0.51 in ethyl acetate. NMR absorptions are observed at 7.30–7.93, 6.43, 5.45–5.59, 3.89, 3.50–4.14, and 3.09δ. The mass spectrum of the bis TMS derivative exhibits peaks of decreasing intensity at m/e 73, 213, 129, 75, 83, 452, 173, 85, 262, 362, and an M^+-CH_3 peak at 527.2996.

I. Following the procedure of Example 13, Part F, 81 mg of the reaction product of Part H is transformed to 65 mg of (5E)-1,5-inter-m-phenylene-2,3,4-trino-CBA_2. Silica gel TLC R_f is 0.60 in the A-IX solvent system. The mass spectrum of the tris TMS derivative exhibits peaks of decreasing intensity at m/e 73, 271, 394, 75, 510, 129, 420, 147, 173, 395, and an M^+-CH_3 peak at 585.3227.

Following the procedure of Examples 12–13, but employing each of the various formula LV compounds described in and following Example 11 in each of the various formula XLIV described in and following Examples 9 and 10, there are prepared each of the various formula L compounds in free acid or methyl ester form.

EXAMPLE 14

9β-methyl-CBA_2, methyl ester, 11,15-bis(tetrahydropyranyl ether)

(Formula LXXXIV: R_{16} is hydrogen, R_{37} is methyl, Z_2 is $-(CH_2)_3-$ and R_{18}, Y_1, M_6, L_1, and R_7 are as defined in Example 3) and the corresponding (5E) and (5Z) free acids (Formula LXXXIII).

Refer to Chart G.

A. A suspension of 57% sodium hydride in mineral oil (1.90 g) is washed with hexane and treated with 130 ml of dry dimethyl sulfoxide (DMSO). The resulting suspension is heated at 65° C. for 1 hr under a nitrogen atmosphere and the resulting solution cooled to 15° C. and treated dropwise over 15 min with 10.0 g of 4-carboxybutyltriphenylphosphonium bromide. The resulting orange solution is stirred for 15 min at 10° C. and then treated dropwise over 15 min with a solution of 2.12 g of the title product of Example 3 in 20 ml of dry DMSO. The resulting solution is then stirred at ambient temperature under a nitrogen atmosphere for 60 hr, treated with 15 ml of water, stirred for 30 min at ambient temperature, added to 200 ml of ice water and 100 ml of brine, acidified with 1 N aqueous hydrochloric acid, and extracted with 900 ml of diethyl ether. The ethereal extracts are then washed with 1 l of water and 200 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 4.8 g of a yellow oil, the formula LXXXIII carboxylic acid.

B. The formula LXXXIII product and 42 ml of diisopropylethylamine in 120 ml of acetonitrile at 10° C. under a nitrogen atmosphere is treated with 15 ml of methyl iodide and allowed to warm slowly to ambient temperature. The resulting suspension is then stirred for 16 hr, treated with 3.0 ml of methyl iodide, stirred for an additional 2 hr, added to 500 ml of brine, and extracted with 1 l of ethyl acetate. The organic extracts are then washed with 250 ml of 0.5 N potassium bisulfate, 250 ml of saturated aqueous sodium bicarbonate, 250 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield a solid residue. The residue is then chromatographed on 500 g silica gel, eluting with 8% acetone in hexane to yield 2.25 g of title formula LXXXIV product. NMR absorptions ($CDCl_3$) are observed at 0.9, 1.05, 1.08, 3.66, 3.02–4.35, 4.70, and 4.95δ. Infrared absorptions are observed at 1730, 1670, 1645, 1200, 1165, 1135, 1080, 1035, 1020, 980, and 870 cm^{-1}. Silica gel TLC R_f is 0.46 in ethyl acetate and hexane (1:3) and 0.26 in ethyl acetate and hexane (1:6).

C. Alternatively the isomeric formula LXXXIII reaction products of Part A are separated into the (5E) and (5Z) title free acid products by chromatography on acid washed silica gel eluting with 10–30% ethyl acetate in hexane.

Following the procedure of Example 9, but employing each of the various formula LXXXI ketones in place of the Example 3 product, there are prepared each of the various formula LXXXIV methyl esters wherein Z_2 is $-(CH_2)_3-$.

Further following the procedure of Example 14, but employing a formula LXXXII ω-carboxytriphenylphosphonium compound wherein Z_2 is other than $-(CH_2)_3-$, each of the various formula LXXXI ketones is transformed to corresponding formula LXXXIV ester wherein Z_2 is other than $-(CH_2)_3-$.

EXAMPLE 15

(5Z)-2-Decarboxy-2-hydroxymethyl-9β-methyl-CBA$_2$, 11,15-bis(tetrahydropyranyl ether)

(Formula LXXXVI: R_{16}, R_{37}, Z_2, R_{18}, M_6, L_1, and R_7 are as defined in Example 14) and its (5E) isomer (formula LXXXVII).

Refer to Chart G.

A suspension of 0.16 g of lithium aluminum hydride in 45 ml of dry tetrahydrofuran at 0° C. under a nitrogen atmosphere is treated dropwise with 1.98 g of the title product of Example 14 in 15 ml of dry tetrahydrofuran. The resulting suspension is stirred for 1 hr at 0° C. and thereafter for 1 hr at ambient temperature. The resulting mixture is then cooled to 0° C., quenched by addition of 0.16 ml of water, 0.16 ml of 15% aqueous sodium hydroxide. After stirring for 1 hr at ambient temperature, treatment with magnesium sulfate and filtration with diatomaceous earth, rinsing with diethyl ether, yields a mixture which is concentrated under reduced pressure. The resulting product, 0.25 g, is chromatographed on 180 g of silica gel, eluting with 30% ethyl acetate in hexane to yield 1.03 g of formula LXXXVII product and 1.06 g of formula LXXXVI product. For the formula LXXXVI product NMR absorptions ($CDCl_3$) are observed at 0.90, 1.09, 3.2–4.4, 4.72, 5.0–5.9δ. Infrared absorptions are observed at 3470, 1760, 1200, 1135, 1120, 1075, 1035, 1020, and 980 cm^{-1}. Silica gel TLC R_f is 0.29 in ethyl acetate and hexane (35:65). For the formula LXXXVII product NMR absorptions ($CDCl_3$) are observed at 0.90, 1.05, 3.2–4.4, 4.6–4.95, 5.05–5.97δ. Infrared absorptions are observed at 3470, 1670, 1200, 1125, 1110, 1080, 1035, 1020, and 985 cm^{-1}. Silica gel TLC R_f is 0.36 in ethyl acetate and hexane (35:65).

Following the procedure of Example 15, but employing each of the various formula LXXXIV esters described following Example 14, there are prepared each of the respective formula LXXXVI and formula LXXXVII primary alcohols.

EXAMPLE 16

(5Z)-9β-methyl-CBA$_2$, methyl ester

(Formula LXXXVIII: X_1 is —$COOCH_3$, R_8 is hydroxy, M_1 is α-OH:β-H, and R_{16}, R_{17}, L_1, R_7, Y_1, and Z_2 are as defined in Example 15).

Refer to Chart G.

A. A solution of the formula LXXXVI title product of Example 15 in 38 ml of acetone at −20° C. under a nitrogen atmosphere is treated over 5 min with 1.9 ml of Jones reagent (prepared by dissolving 133.6 g of chromium trioxide in 115 ml concentrated sulfuric acid and diluting with water to a volume of 500 ml), stirred for 2 hr at −20° C., quenched by addition of 2.3 ml of isopropanol, stirred for 40 min at −20° C., diluted with 200 ml of brine, extracted with 400 ml of ethyl acetate, washed with 600 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 1.01 g carboxylic acid corresponding to the formula LXXXVI primary alcohol as a pale green oil.

B. A solution of the product of Part A in 11 ml of acetonitrile at 15° C. under a nitrogen atmosphere is treated with 4.1 ml of diisopropylethylamine and 1.5 ml of methyl iodide. The resulting suspension is then stirred at ambient temperature for 17 hr, treated with 0.3 ml of methyl iodide, stirred for 2 hr at ambient temperature, diluted with 50 ml of brine, extracted with 100 ml of ethyl acetate, washed with 50 ml of 0.5 M potassium bisulfate, 50 ml of aqueous sodium bicarbonate and 50 ml of brine, dried over anhydrius sodium sulfate, and concentrated under reduced pressure to yield 1.02 g of the methyl ester corresponding to the carboxylic acid product of Part A.

C. A solution of the product of Part B in 56 ml of a mixture of tetrahydrofuran, water, and acetic acid (1:2:4) is heated to 45° C. under a nitrogen atmosphere for 3 hr, cooled, diluted with 200 ml of brine, and extracted with 400 ml of diethyl acetate. The organic extracts are then washed with 600 ml of saturated acqueous sodium bicarbonate and 400 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 0.9 g of crude title product as a yellow oil. Chromatographing on 100 g of silica gel, eluting with hexane and ethyl acetate (3:7) yields 0.39 g of pure title product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.89, 1.08, 3.5–4.35, 3.66, 5.0–5.7δ. Infrared absorptions are observed at 3360, 1740, 1670, 1455, 1435, 1370, 1240, 1225, 1195, 1170, 1075, 1020, and 970 cm^{-1}. Silica gel TLC R_f is 0.22 in ethyl acetate and hexane (7:3).

Following the procedure of Example 16, but employing each of the various formula LXXXVI compounds described following Example 15, there are prepared each of the various formula LXXXVIII 9β-methyl-CBA$_2$ compounds wherein X_1 is —$COOR_1$.

EXAMPLE 17

(5E)-9β-methyl-CBA$_2$, methyl ester

(Formula LXXXIX: R_{16}, R_{17}, X_1, Z_2, R_8, R_1, M_1, L_1, and R_7 are as defined in Example 16).

Refer to Chart G.

A. Following the procedure of Example 16, Part A, 0.60 g of the formula LXXXVII product of Example 15 is transformed to the carboxylic acid corresponding to the formula LXXXVII primary alcohol, yielding 0.66 g of a green oil.

B. Following the procedure of Example 16, Part B, the product of Part A above (0.66 g) is transformed to the methyl ester corresponding to the carboxylic acid product of Part A, yielding 0.58 g of a yellow oil.

C. Following the procedure of Example 16, Part C, the product of Part B above (0.58 g) is transformed to 0.25 g of title product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.90, 1.05, 3.30, 3.66, 3.75–4.25, 5.0–5.7δ. Infrared absorptions are observed at 3360, 1740, 1670, 1455, 1435, 1250, 1225, 1195, 1170, 1075, 1020, and 970 cm^{-1}. Silica gel TLC R_f is 0.22 in ethyl acetate and hexane (3:7).

Following the procedure of Example 17, but employing each of the various formula LXXXVII compounds described following Example 15, there are prepared each of the various formula LXXXIX products wherein X_1 is —$COOCH_3$.

EXAMPLE 18

(5Z)-9β-methyl-CBA$_2$

A solution of 0.28 g of the title product of Example 16 in 8 ml of methanol is stirred at ambient temperature under a nitrogen atmosphere and treated with 1 ml of 8 M aqueous sodium hydroxide. The resulting yellow solution is then stirred for 5 hr at ambient temperature under a nitrogen atmosphere, diluted with 90 ml of ice and brine, acidified to pH2 with 1 N hydrochloric acid, extracted with 360 ml of ethyl acetate, washed with 120 ml of brine, dried over anhydrous sodium sulfate, and

concentrated under reduced pressure to yield 0.25 g of crude title product. Chromatography on 30 g of silica gel, eluting with the A–IX solvent system (the organic phase of an equillibrated mixture of ethyl acetate, acetic acid, cyclohexane, and water, 9:2:5:10), yields 0.235 g of pure title product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.89, 1.08, 3.5–4.35, 5.0–5.7, 6.05δ. Infrared absorptions are observed at 3340, 2660, 1710, 1240, 1205, 1175, 1130, 1075, 1055, 1020, and 970 cm^{-1}. Silica gel TLC R_f is 0.25 in the A–IX solvent system.

Following the procedure of Example 18 each of the various methyl esters prepared following Example 16 is transformed to the corresponding carboxylic acid.

EXAMPLE 19

(5E)-9β-methyl-CBA$_2$

Following the procedure of Example 18, 0.25 g of the title product of Example 17 is transformed to 0.21 g of title product as a colorless oil. NMR absorptions ($CDCl_2$) are observed at 0.90, 1.06, 3.5–4.3, 5.0–5.7, and 5.93δ. Infrared absorptions are observed at 3340, 2660, 1710, 1300, 1240, 1175, 1130, 1075, 1055, 1020, and 970 cm^{-1}. Silica gel TLC R_f is 0.27 in the A–IX solvent system.

Each of the various carboxylic acids corresponding to LXXXVIII and LXXXIX wherein X_1 is —COOH— can be prepared from the corresponding formula LXXXIII reaction products by acid hydrolysis of the tetrahydropyranyl ether protecting groups of C-11 and C-15. [The (5Z) LXXXIII reaction products from Example 14, Part C go to formula LXXXVIII products; and the (5E) LXXXIII reaction products from Example 14, Part C go to formula LXXXIX products.]

Following the procedure of Example 19, but employing each of the various formula LXXXIX methyl esters described following Example 17, there are prepared each of the various corresponding carboxylic acids.

EXAMPLE 20

2β-(t-butyldimethylsilyloxymethyl)-5β-methyl-7-oxo-3α-tetrahydropyran-2-yl-oxy-bicyclo[3.3.0]octane

(Formula LXII: n is the integer one, R_{31} is t-butyldimethylsilyl, and R_{38} is tetrahydropyranyloxy).

Refer to Chart E.

A. A solution of 40.6 g of 3α-benzoyloxy-5α-hydroxy-2β-hydroxymethyl-1α-cyclopentaneacetic acid, ω-lactone in 250 ml of dimethylformamide, stirring at 0° C. under a nitrogen atmosphere, is treated with 25 g of imidazole in 28 g of t-butyldimethylsilyl chloride. The resulting solution is then stirred for 67 hr at ambient temperature, added to 500 ml of water, extracted with three 500 ml portions of diethyl ether, washed with 500 ml of 10% aqueous potassium bisulfate, 500 ml of aqueous sodium bicarbonate and 500 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 59.9 g of 3α-benzoyloxy-5α-hydroxy-2β-(t-butyldimethylsilyloxymethyl)-1α-cyclopentaneacetic acid, ω lactone as a white solid. NMR absorptions ($CDCl_3$) are observed at 0.06, 0.91, 2.1–3.12, 3.74, 4.94–5.54, 7.24–7.67, and 7.9–8.2δ. Infrared absorptions are observed at 1780, 1720, 1600, 1585, 1490, 1270, 1255, 1180, 1115, 1100, 1070, 1050, 830, 790, and 710 cm^{-1}. Silica gel TLC R_f is 0.20 in ethyl acetate and hexane (1:4).

B. A solution of 59.1 g of the reaction product of Part A and 500 ml of absolute methanol, stirring at ambient temperature under a nitrogen atmosphere, is treated with 35 ml of a 25% solution of sodium methoxide and methanol. The resulting reaction mixture is then stirred for 90 min at ambient temperature and quenched by addition of 9.5 ml of glacial acetic acid. Methanol is removed under reduced pressure and the resulting residue diluted with 500 ml of saturated aqueous sodium bicarbonate. The resulting mixture is then extracted with two 500 ml portions of ethyl acetate, washed with 300 ml of saturated aqueous sodium bicarbonate in 200 ml of brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 58 g of an oily solid, crude 3α,5α-dihydroxy-2β-(t-butyldimethylsilyloxymethyl)-1α-cyclopentaneacetic acid, ω lactone. This crude product is then chromatographed in 800 g of silica gel, eluting with 20–75% ethyl acetate in hexane to yield pure title product as a white crystal solid. Melting range is 60.5° C. to 62° C. NMR absorptions ($CDCl_3$) are observed at 0.06, 0.90, 1.7–3.0, 3.67, 3.9–4.4, and 4.7–5.13δ. Silica gel TLC R_f is 0.3 in 50% ethyl acetate in hexane.

C. A solution of 37.3 g of reaction product of Part B in 400 ml of methylene chloride, stirring at 0° C. under a nitrogen atmosphere, is treated with 18 ml of dihydropyran and 0.14 g of pyridine hydrochloride. The resulting solution is stirred at ambient temperature for 13 hr, treated with an additional 3 ml of dihydropyran and 30 mg of pyridine hydrochloride, stirred for an additional 4 hr, washed with two 400 ml portions of saturated aqueous sodium bicarbonate and 400 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 49 g of a pale yellow oil, crude 5α-hydroxy-3α-tetrahydropyran-2-yloxy-2β-(t-butyldimethylsilyloxymethyl)-1α-cyclopentaneacetic acid, ω lactone. Chromatography on 800 g of silica gel, eluting with 0–75% ethyl acetate in hexane yields 37 g of pure product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.05, 0.90, 1.62, 2.0–3.0, 3.6, 3.2–4.4, 4.67, and 4.8–5.2δ. Infrared absorptions are observed at 1780, 1255, 1175, 1160, 1116, 1080, 1035, 1020, 1005, 975, 835, and 775 cm^{-1}. Silica gel TLC R_f is 0.25 in hexane and ethyl acetate (2:1).

D. A solution of 28 ml of dimethyl methylphosphonate in 800 ml of dry tetrahydrofuran at −70° C. under a nitrogen atmosphere is treated with 160 ml of 1.56 M n-butyllithium in hexane, stirred for 30 min at −70° C. The resulting mixture, maintained at −70° C., is then treated dropwise over 30 min with 41.7 g of reaction product of Part C in 200 ml of tetrahydrofuran. The resulting solution is then stirred at −70° C. for 1 hr, allowed to warm, stirred for an additional 2.5 hr at ambient temperature, quenched by addition of 14 ml of glacial acetic acid, added to 1 l of brine, extracted with three 700 ml portions of diethyl ether, washed with 500 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 63 g of a yellow oil, crude 6β-(t-butyldimethylsilyloxymethyl)-3-dimethylphosphonomethyl-3-hydroxy-2-oxa-7α-tetrahydropyranyloxy-bicyclo[3.3.0]octane. Chromatography on 800 g of silica gel eluting with 50–75% ethyl acetate in hexane yields 44.2 g of pure title product as a colorless oil. NMR absorptions ($CDCl^3$) are observed at 0.05, 0.89, 1.23–3.02, 2.2–4.37, 4.70, and 4.99δ. Infrared absorptions are observed at 3380, 1255, 2235, 1120, 1050, 1035, 835, and 775 cm^{-1}. Silica gel TLC R_f is 0.25 in ethyl acetate.

E. A suspension of 29.2 g of chromium trioxide in 700 ml of methylene chloride, stirring at ambient tempera-

ture under a nitrogen atmosphere, is treated rapidly with 50 ml of pyridine, treated with dry diatomaceous earth, stirred for 5 min, and then treated with 23.8 g of title product of Part D in 60 ml of methylene chloride. The resulting suspension is then stirred for 45 min at ambient temperature under a nitrogen atmosphere and filtered through 300 g of silica gel, eluting with 2 l of ethyl acetate in acetone (2:1). Concentration under reduced pressure yields 24 g of a brown yellow oil, crude 3β-(t-butyldimethylsilyloxymethyl)-2α-(2'-dimethylphosphonomethyl-2'-oxoethyl)-4α-tetrahydropyranyloxy-pentanone. High pressure liquid chromatography of 12 g of the crude product on silica gel eluting with 20% acetone in methylene chloride yields 4.54 g of pure product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.05, 0.88, 2.8–4.5, 3.77, and 4.86δ. Infrared absorptions are observed at 1745, 1715, 1255, 1130, 1115, 1060, 1025, 835, 810, and 775 cm^{-1}. Silica gel TLC R_f is 0.27 in 20% acetone in methylene chloride and 0.3 in ethyl acetate.

F. A degassed suspension of 0.52 g reaction product of Part E, 0.15 g anhydrous potassium carbonate, and 0.59 g 18-crown-6 ether in 20 ml toluene are stirred at 75° C. for 6 hr under a nitrogen atmosphere and thereafter cooled to 0° C. The resulting solution is then washed successively with 20 ml brine, a solution of 15 ml water and 5 ml brine, and 20 ml brine, dried over anhydrous sodium sulfate, and concentrated to yield a brown residue crude 6β-t-butyldimethylsilyloxymethy-7α-tetrahydropyran-2-yl-oxybicyclo[3.3.0]oct-1-en-2-one, filtering through 7 g of silica gel and eluting with hexane and ethyl acetate (70 ml, 1:1) yields 0.31 g of product as an oil. High pressure liquid chromatography (10 ml fractions, 3.8 ml/minute flow rate) on silica gel, eluting with hexane and ethyl acetate (3:1) yields 0.20 g of pure product as a colorless oil. NMR absorption ($CDCl_3$) of the trimethylsilyl derivative are observed at 0.06, 0.90, 1.20–3.20, 3.20–4.85, and 5.85–6.0δ. Infrared absorptions are observed at 1710, 1630, 1250, 1130, 1115, 1075, 1030, 965, 870, 835, 810, 775 cm^{-1}. Silica gel TLC R_f is 0.34 in hexane and ethyl acetate (2:1).

G. A suspension of 0.35 g of anhydrous copper iodide in 12 Ml of anhydrous diethyl ether at −20° C. under an argon atmosphere is treated dropwise with 2.0 ml of 1.4 M methyllithium. The resulting solution is then stirred at −20° C. for 15 min, treated at −20° C. dropwise over 1.5 hr with a solution of 0.22 g of the reaction product of Part F in 12 ml of anhydrous diethyl ether. The resulting suspension is then stirred at −20° C. for 2 hr, added to 50 ml of 1 M aqueous ammonium chloride, extracted with 150 ml of diethyl ether, washed with 50 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 0.23 g of crude title product as a pale yellow oil. Chromatography on 30 g of silica gel, eluting with ethyl acetate and hexane (1:4) yields 0.22 g of pure title product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.05, 0.90, 1.16, 1.3–2.9, 3.3–4.4, and 4.63δ. Infrared absorptions are observed at 1745, 1255, 1135, 1110, 1095, 1075, 1035, 1020, 835, and 775 cm^{-1}. Silica gel TLC R_f is 0.32 in ethyl acetate and hexane (1:4).

EXAMPLE 21

N-methyl-(1-fluoro-5-tetrahydropyranyloxypentyl)-phenylsulfoximine

(Formula XCII: Z_2 is $—(CH_2)_{32}—$ and R_{10} is tetrahydropyranyl.

Refer to Chart H.

Diisopropylamine (0.59 g) is dissolved in 21 ml of tetrahydrofuran and the resulting mixture cooled to −78° C. with stirring under an argon atmosphere. Thereafter triphenylmethane is added, for use as an indicator, and a solution of n-butyllithium and hexane is added dropwise until the resulting mixture attains a pink color. After stirring for an additional 75 min, the resulting mixture is treated with 1.50 g of N-methyl-(5-tetrahydropyranyloxypentyl)-phenylsulfoximine dissolved in 6 ml of dry tetrahydrofuran. The resulting mixture is then stirred for an additional 30 min at −78° C. Thereafter excess perchloryl fluoride ($FClO_3$) is bubbled through the solution for 4–5 min, during which time a stream of argon is also bubbled through the mixture for safety reasons. The resulting mixture is then stirred at additional 90 min at −78° C. and then the reaction is quenched by addition of 5% aqueous sodium bicarbonate. After equilibration of the reaction mixture to ambient temperature, the mixture is diluted with additional 5% aqueous sodium bicarbonate and extracted with methylene chloride. The organic extracts are then washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield 1.64 g of a yellow oil. Chromatography on silica gel columns in a series, eluting with ethyl acetate and hexane (1:1) yields 0.18 g of the formula XCII title product as a mixture of diastereomers. Silica gel TLC R_f in ethyl acetate and hexane (1:1) are 0.54 (less polar isomer) and 0.45 (more polar isomer). NMR absorptions ($CDCl_3$) for the less polar isomer are 1.2–2.15, 3.65, 3.68, 3.1–4.1, 4.4–4.8, 5.5, and 7.4–8.1δ. NMR absorptions ($CDCl_3$) for the more polar isomer are 1.15–2.20, 3.63, 3.1–4.1, 4.45–4.65, 5.27, and 7.4–8.1δ.

Following the procedure of Example 21, but employing each of the various formula XCI phenylsulfoxamines, there are prepared each of the various corresponding formula XCII fluorinated phenylsulfoxamines.

EXAMPLE 22

5-Fluoro-2-decarboxy-2-hydroxymethyl-CBA_2, 1,11,15-tris(yetrahydropyranyl ether)

(Formula XCIV: R_{16} and R_{17} are both hydrogen, R_{10} is tetrahydropyranyl, Z_2 is $—(CH_2)_3—$, n is the integer one, R_{18} is tetrahydropyranyloxy, Y_1 is trans—CH=CH—, M_6 is α-tetrahydropyranyloxy:β-hydrogen, R_3 and R_4 of the L_1 moiety are both hydrogen, and R_7 is n-butyl).

Refer to Chart H.

Diisopropylamine (164 mg) and triphenylmethane (1.5 mg) are dissolved in 4 ml of dry tetrahydrofuran and the resulting solution is cooled to −78° C. under a nitrogen atmosphere. A solution of n-butyllithium and hexane is added until a faint pink color is attained. This solution is then stirred an additional 80 min. Thereafter, 0.488 g of the title product of Example 21 in 4 ml of dry tetrahydrofuran is added dropwise. Thereafter 608 mg of 7-oxo-3α-tetrahydropyran-2-yl-oxy-2β-[(3'S)-3'-tetrahydropyran-2-yloxy-trans-1'-octenyl] bicyclo-[3.3.0]octane (Formula XCIII: R_{16}, R_{17}, n, R_{18}, Y_1, M_6, L_1, and R_7 are as defined for the title product) in 4 ml of tetrahydrofuran is added to the reaction mixture. After 4 min, the resulting mixture is quenched by addition of saturated aqueous ammonium chloride and ethyl acetate is thereafter added to the reaction mixture, which is maintained at −78° C. The resulting mixture is then allowed to warm until solids separate. Thereupon addi-

tional ethyl acetate is added, the reaction extracted with brine. The ethyl acetate layer is then dried over sodium sulfate and concentrated under reduced pressure.

An aluminum amalgam is then prepared by reacting 0.31 g of 20 mesh aluminum with 2.5 ml of aqueous mercuric chloride followed by washing with ethyl acetate and diethyl ether. The residue from the ethyl acetate layer (described in the preceeding paragraph) is dissolved in 5 ml of tetrahydrofuran and the solution cooled to 0° C. This cooled solution is then treated with aluminum amalgam, 2 ml of water, and 1 ml of glacial acetic acid. The resulting mixture is then stirred for 2 hr at 0° C. and 16 hr at 20° C. The reaction is then diluted with ethyl acetate and filtered with diatomaceous earth. The ethyl acetate layer is then washed with 5% aqueous sodium bicarbanate and saturated brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 0.96 g as an oily residue. Chromatgraphing over 100 g of silica gel and eluting with 500 ml of 15% ethyl acetate in mixed hexanes, 500 ml of 25% ethyl acetate in mixed hexanes, 300 ml of 50% ethyl acetate in mixed hexanes, and 800 ml of 50% acetone in methylene chloride, taking 20 ml fractions, yields a less polar isomer in fractions 22–26 (80 mg) and a more polar isomer in fractions 30–36 (74 mg). These isomers represent the C-5 diastereomers of the formula XCIV product. For the less polar isomer, NMR absorptions ($CDCl_3$) are observed at 0.65–2.65, 3.15–4.15, 4.35–4.75, and 5.25–5.75δ. For the more polar isomer, NMR absorptions ($CDCl_3$) are observed at 0.6–2.65, 3.10–4.15, 4.40–4.7, and 5.2–5.7δ. Silica gel TLC R_f for the less polar isomer is 0.66 and for the more polar isomer is 0.57 in ethyl acetate and mixed hexanes (3:7).

Following the procedure of Example 22, but employing each of the various formula XCIII ketones, there are obtained each of the various formula XCIV intermediates wherein Z_2 is $—(CH_2)_3—$.

Further following the procedure of Example 22, but substituting each of the various fluorinated phenylsulfoximines described following Example 21, there are prepared from the various formula XCIII ketones each of the various formula XCIV products wherein Z_2 is other than $—(CH_2)_3—$.

EXAMPLE 23

5-Fluoro-2-Decarboxy-2-hydroxymethyl-CBA_2 (more polar isomer)

(Formula XCV: R_{16}, R_{17}, Z_2, n, R_8, M_1, L_1, and R_7 are as defined in Example 17).

Refer to Chart H.

The title product of Example 22 (74 mg) is dissolved in 2 ml of a mixture of tetrahydrofuran, water, and glacial acetic acid (2:2:1) and the resulting mixture stirred under a nitrogen atmosphere. The reaction mixture is maintained at ambient temperature for 17 hr, thereafter at 40° C. for 7 hr, and finally at 23° C. for an additional 24 hr. The resulting mixture is then diluted with ethyl acetate, washed with 5% aqueous sodium bicarbonate and saturated brine, dried over sodium sulfate, and concentrated under reduced pressure to yield 52 mg of crude title product. Chromatography over silica gel, eluting with acetone and methylene chloride (60:40) yields 19 mg of pure title product. NMR absorptions ($CDCl_3$) are observed at 0.6–2.60, 2.60–3.30, 3.30–4.15, 5.1–5.9δ. ^{13}C-NMR absorptions ($CDCl_3$) are observed at 135.8, 133.0, 117.5 (d J=18 Hz), 77.4, 73.3, 62.6, 57.6, 46.4, 41.1, 38.0, 37.2, 36.2 (d J=5 Hz), 31.9, 31.8, 31.2, 29.5 (d J=29 Hz), 25.2, 22.5, 14.0δ. Silica gel TLC R_f is 0.280 in acetone and methylene chloride (1:1).

EXAMPLE 24

5-Fluoro-2-decarboxy-2-hydroxymethyl-CBA_2 (less polar isomer)

Following the procedure of Example 23, 85 mg of less polar title product of Example 22 are transformed to 25 mg of pure title product. NMR absorptions ($CDCl_3$) are observed at 0.5–2.5, 3.1–4.75, and 5.05–5.8δ. ^{13}C-NMR absorptions ($CDCl_3$) are observed at 137.0, 132.6, 77.0, 73.6, 62.3, 57.4, 45.5, 41.6, 36.9, 36.5, 34.4 (d J=3.1 Hz), 32.5 (d J=5.4 Hz), 31.8, 31.7, 29.2 (d J=28.9 Hz), 25.4, 22.6, 22.4, and 14.0δ. Silica gel TLC R_f is 0.33 in acetone and methylene chloride.

Following the procedure of Examples 23 and 24, but employing the various diastereomeric products described following Example 22, there are prepared each of the various diastereomers corresponding to formula XCV.

EXAMPLE 25

5-fluoro-CBA_2 (more polar isomer)

(Formula LXXVI: Z_2, n, R_8, Y_1, M_1, L_1, and R_7 are as defined in Example 23).

Refer to Chart H.

The platinum oxide catalyst is prepared by suspending 46 mg of 85% platinum oxide in 9 ml of water and hydrogenating the resulting mixture at ambient temperature and pressure for 34 min. To this suspension is added 58 mg of sodium bicarbonate and 18 mg of the title product of Example 23 dissolved in 2 ml of acetone. The resulting mixture is then warmed to 60° C. and oxygen bubbled therethrough for 80 min. The reaction mixture is then filtered through diatomaceous earth and the filter cake washed in water. The filtrate is then acidified to pH4 with 5% aqueous sodium hydrogen sulfate and extracted with ethyl acetate. The organic extracts are then dried over magnesium sulfate and concentrated under reduced pressure to yield 21 mg of pure title product. NMR absorptions ($CDCl_3$) are observed at 0.6–2.8, 3.0–4.2, and 4.65–5.8δ. ^{13}C-NMR absorptions ($CDCl_3$) are observed at 176.9, 135.5, 133.2, 118.5 (d J=17.5 Hz), 77.7, 73.5, 57.3, 46.5, 41.0, 38.2, 37.0, 36.2 (d J=4.8 Hz), 32.3, 31.7, 31.1 (d J=13.5 Hz), 28.5 (d J=28.3 Hz), 25.2, 22.6, 21.0, and 14.0δ. Silica gel TLC R_f is 0.39 in the A–IX solvent system.

EXAMPLE 26

5-Fluoro-CBA_2 (less polar isomer)

Following the procedure of Example 25, 24 mg of the title product of Example 24 yields 23 mg of pure title product. NMR absorptions ($CDCl_3$) are observed at 0.6–2.9, 3.3–4.2, 5.0–6.0δ. ^{13}C-NMR absorptions ($CDCl_3$) are observed at 176.8, 135.4, 132.9, 118.3 (d J=18.2 Hz), 77.6, 73.4, 57.2, 46.3, 41.2, 37.8, 36.8, 34.6 (d J=2.7 Hz), 32.8, 32.4, 31.7, 28.7 (d J=28.4 Hz), 25.2, 22.6, 21.1, and 14.0δ. TLC R_f is 0.50 in the A–IX solvent system.

The reaction products of Example 25–26 are obtained as diastereomeric mixtures of (5E) and (5Z) geometric isomers. These geometric isomers are characterized herein as "less polar" and "more polar" isomers based on TLC motilities. The isomers of these 5-fluoro-CBA_2 compounds correspond to the (5E) and (5Z) geometric isomers of CBA_2 itself. On the basis of relative biologi-

cal activities, the more polar 5-fluoro-CBA_2 isomer yields more potent pharmacological effects and on this basis could be assigned the (5Z) structure based on pharmacological considerations alone. However, the ^{13}C-NMR data suggests the more polar isomer corresponds to the (5E) structure of the 5-fluoro-CBA_2 compound.

Following the procedure of Examples 25–26, there are prepared each of the various formula XCVI 5-fluoro-CBA_2 diastereomers from the starting materials described following Example 24.

Further following the procedures known in the art, each of the various 5-fluoro-CBA_2 compounds described in and following Examples 24–25 is transformed to the corresponding formula XCVII 5-fluoro-CBA_2 analogs.

EXAMPLE 27

(5Z)-9β-methyl-CBA_2 adamantylamine salt

The title product of Example 18 (54 mg), (5Z)-9β-methyl-CBA_2 in 6 ml of diethyl ether is combined with 23 mg of adamantylamine. After 10 min the precipitate forms which is thereafter stirred for 12 hr, decanted, and concentrated under reduced pressure to yield 68 mg of a solid, pure title product. Melting range is 110°–114° C.

EXAMPLE 28

(5Z)-9β-methyl-CBA_2, calcium salt hydrate

The title product of Example 18 (0.95 g), 9β-methyl-(5Z)-CBA_2, calcium oxide (0.064 g), freshly boiled water (9.2 ml), and distilled tetrahydrofuran (6 ml), are combined by heating to 50° C. under a nitrogen atmosphere with stirring for 20 min. The resulting mixture is then filtered, washed with tetrahydrofuran, and concentrated under reduced pressure to yield a residue. The residue is then dissolved in tetrahydrofuran (10 ml) and concentrated 8 times to yield a cream-colored foam. This foam is then dissolved in 6 ml of tetrahydrofuran which is dripped into anhydrous diethyl ether (95 ml). The resulting suspension is then stirred for 15 min at ambient temperature under a nitrogen atmosphere and filtered. The filter cake is then washed with anhydrous diethyl ether and dried for 20 hr under reduced pressure at ambient temperature to yield 0.686 g of title product. Melting range is 101°–108° C. Following atmospheric equillibration melting range is 80°–117° C. Infrared absorptions are observed at 3330, 1670, 1555, 1455, 1345, 1310, 1270, 1075, 1020, 970 cm^{-1}.

EXAMPLE 29

8α-hydroxy-7β-(3α-hydroxy-trans-1-octenyl)-tricyclo-[4.3.1]nonan-4-one, 8,3′-bis(tetrahydropryanyl ether)

(Formula XXV: R_{18}, Y_1, M_6, L_1, R_{27}, and n are as defined in Example 1, R_{16} and R_{37} taken together are $—CH_2—$).

Refer to Chart A.

A. The formula XXIV title product of Example 1 (4.0 g) and benzophenone (2 g) in one liter of methanol is photolyzed (3500 A lamp) for 3 hr while argon is bubbled through the solution. The methanol is then removed by concentration under reduced pressure and the residue chromatographed on 600 g of silica gel eluting with a mixture ranging from ethyl acetate in hexane (1:3) to 100% ethyl acetate. Compound XXVI, 1β-hydroxymethyl-7α-hydroxy-6β-(6′α-hydroxy-trans-1′-octenyl)bicyclo[3.3.0]octan-3-one, 7,3′-bis(tetrahydropyranyl ether) is obtained as a white solid (3.45 g). Crystallization from ethyl acetate in hexane yields a white solid with melting range 65°–70° C. NMR absorptions ($CDCl_3$) are observed at 0.89, 1.17–2.90, 2.92–4.40, 4.69, and 5.24–5.77δ. Infrared absorptions are observed at 3420, 1730, 1200, 1125, 1110, 1070, 1040, 1020, and 970 cm^{-1}. Silica gel TLC R_f is 0.29 in hexane and ethyl acetate (1:4).

B. A solution of 0.6 g of the reaction product of Part A and 0.49 g of p-toluenesulfonyl chloride in 30 ml of pyridine is cooled to 0° C. under argon for 70 hr, added to 100 ml of ice, diluted with 300 ml of water, and extracted with diethyl ether (800 ml). The ethereal extracts are then washed with brine, dried over magnesium sulfate, concentrated under reduced pressure, and chromatographed eluting with 50% to 80% hexane in ethyl acetate to yield 0.49 g of formula XXVII compound, 3-oxo-7α-tetrahydropyran-2-yloxy-6β-[(3′s)-3′-tetrahydropyran-2-yloxy-trans-1′-octenyl]-1β-(p-toluenesolfonyl)-oxymethylbicyclo[3.3.0]octane, as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.88, 1.06–2.9, 2.45, 3.17–4.35, 4.52–4.83, 5.2–5.8, 7.37, and 7.81 δ. Infrared absorptions are observed at 1740, 1600, 1360, 1200, 1190, 1175, 1130, 1110, 1075, 1035, 1020, 970, and 820 cm^{-1}. Silica gel TLC R_f is 0.45 or 0.26 in ethyl acetate and hexane (1:1 or 1:2).

C. A degassed solution of 0.49 g of the reaction product of Part B and 1 ml of t-butanol in 50 ml of dry tetrahydrofuran at 0° C. under an argon atmosphere is treated with 0.8 ml of 1.7 M potassium t-butoxide in tetrahydrofuran. After 5 min the reaction is allowed to warm and the resulting brown solution stirred for 3 hr at ambient temperature. Thereafter 90 ml of brine is added and the mixture is extracted with 270 ml of ethyl acetate. The ethyl acetate extracts are then washed with 100 ml of saturated aqueous sodium bicarbonate, 100 ml of brine, dried over anhydrous magnesium sulfate, concentrated under reduced pressure, yielding 0.37 g of a brown oil, and chromatographed on 40 g of silica gel eluting with hexane and ethyl acetate (2:1) to yield 0.32 g of pure formula XXV title product as a colorless oil.

D. Alternatively, a suspension of 207 mg of 57% sodium hydride in mineral oil and 1.08 g of trimethyloxosulfonium iodide is treated dropwise under a nitrogen atmosphere with 6 ml of dimethylsulfoxide. The resulting grey slurry is then stirred at ambient temperature for 20 min, treated with 2.03 g of the title product of Example 1 in 4 ml of dry dimethylsulfoxide and stirred for 2 hr at ambient temperature. Thereafter stirring is continued for 1 hr at 50° C., the reaction mixture is cooled and diluted with 200 ml of water and thereafter extracted with three 100 ml portions of diethyl ether. The combined ethereal extracts are then washed with 200 ml of water, washed with 100 ml of brine, dried over anhydrous magnesium sulfate, concentrated under reduced pressure, yielding a brown oil, and chromatographed on 250 g of silica gel eluting with ethyl acetate and hexane (1:2) to yield 453 mg of pure title product.

E. For title product prepared according to Part C or Part D above, NMR absorptions ($CDCl_3$) are observed at 0.25–2.75, 3.15–4.39, 4.68, and 5.2–5.8δ. Infrared absorptions are observed at 1725, 1665, 1135, 1080, 1040, 1020, 980 cm^{-1}.

The mass spectrum exhibits a molecular ion at 446 and silica gel TLC R_f is 0.30 in ethyl acetate and hexane.

EXAMPLE 30

(5Z) and (5E)-6αβ,9β-methano-CBA$_2$

(Formula X: X_1 is —COOH, Z_1 is —$(CH_2)_3$—, R_{15} is hydrogen, R_{16} and R_{17} taken together are methano, n is one, R_8 is hydroxy, Y_1 is trans-—CH=CH—, M_1 is α-OH:β-H, L_1 is α-H:β-H, R_7 is n-butyl, and the C-5, C-6 positions are unsaturated).

Refer to Chart G.

A. A suspension of 452 mg of 57% sodium hydride in mineral oil and 30 ml of dimethylsulfoxide is heated to 65° C. for 1 hr under a nitrogen atmosphere, cooled to 17° C. and thereafter treated over 15 min with 2.39 g of 4-carboxybuthyltriphenylphosphonium bromide. The resulting red solution is then stirred for 15 min at 17°–20° C., treated with a solution of 716 mg of the title product of Example 29, 6 ml of dry dimethylsulfoxide, stirred for 43 hr at 40° C., cooled to 0° C., treated with 3.5 ml of water, stirred for 30 min at 0° C., added to 75 ml of water and brine (2:1), acidified with one N aqueous hydrochloric acid, and extracted with 225 ml of diethyl ether. The ethereal extracts are then washed with 375 ml of water and 75 ml of brine, dried over magnesium sulfate, concentrated under reduced pressure, and chromatographed on 150 g of acid-washed silica gel eluting with 10–25% ethyl acetate in hexane to yield 290 mg of (5Z)-6αβ,9β-methano-CBA$_2$, 11,15-bis(tetrahydropyranyl ether), 70 mg of (5E)-6αβ,9β-methano-CBA$_2$, 11,15-bis(tetrahydropyranyl ether), and 400 mg of a mixture of (5E) and (5Z) formula LXXXIII isomers. Rechromatographing the isomeric mixture on 150 g of acid-washed silica gel yields an additional 50 mg of (5E) isomer and 180 mg of (5Z) isomer.

For the (5Z) isomer NMR absorptions ($CDCl_3$) are observed at 0.5–2.85, 3.22–4.4, 4.70, 4.9–5.75, and 10.1 δ. Infrared absorptions are observed at 3600–3000 (a broad band), 1740, 1710, 1240, 1210, 1135, 1080, 1035, 1020, 980, and 870 cm^{-1}. Silica gel TLC R_f is 0.27 in hexane, ethyl acetate, and acetic acid (65:34:1). For the (5E) isomer NMR absorptions are observed at 0.40–2.70, 3.2–4.4, 4.70, 5.0–5.8, and 8.82δ. Infrared absorptions are observed at 3600–3000, 1740, 1710, 1460, 1445, 1200, 1135, 1075, 1035, 1020, and 980 and cm^{-1}. Silica gel TLC R_f is 0.32 in hexane, ethyl acetate, and acetic acid (65:34:1).

B. A solution of 446 mg of the (5Z) reaction product of Part A in 44 ml of acetic acid, water, and tetrahydrofuran (6:3:2) is heated at 45° C. under a nitrogen atmosphere for 3 hr, cooled, added to 200 ml of brine, extracted with 160 ml of ethyl acetate in hexane (3:2), washed with 500 ml of brine, extracted with 120 ml of ethyl acetate and hexane (3:2) dried over sodium sulfate, concentrated under reduced pressure, yielding 0.38 g of a yellow oil and chromatographed on 60 g of acid washed silica gel eluting with 70% ethyl acetate in hexane to yield 170 mg of pure (5Z) title product as a colorless oil. NMR absorptions are observed at 0.5–2.90, 0.89, 4.05, 4.85–5.8, and 6.13δ. Infrared absorptions are observed at 3360, 2260, 1710, 1245, 1240, 1075, 1025, and 970 cm^{-1}. The mass spectrum for the tris-trimethylsilyl derivative exhibits a high resolution peak at 578.3653. Silica gel TLC R_f is 0.30 in the A–IX solvent system (the organic phase of an equilibrated mixture of ethyl acetate, acetic acid, cyclohexane, and water; 9:2:5:10).

C. Following the procedure of Part B above 90 mg of the (5E) reaction product of Part A is converted to 46 mg of (5E) title product as a colorless oil. NMR absorptions are observed at 4.40–2.8, 0.89, 4.06, and 5.0–5.85 δ. Infrared absorptions are observed at 3340, 2630, 1710, 1070, 970 cm^{-1}. The mass spectrum exhibits a high resolution peak at 578.3664. Silica gel TLC R_f is 0.32 in the A–IX solvent system.

Following the procedure of Examples 27–29, each of the various formula X products is prepared wherein R_{16} and R_{17} are methano from the corresponding formula LXXXI reactants of Chart G.

Accordingly, the above examples provide methods for preparing each of the various formula X CBA analogs of the present invention.

EXAMPLE 31

9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF$_1$α

(Formula XI: X_1 is COOH, R_{20}, R_{21}, R_{23}, and R_{24} are all hydrogen, Z_4 is —CH_2—, R_{22} is β-hydrogen, R_8, Y_1, M_1, L_1, and R_7 are as defined in Example 8) and its corresponding methyl ester (X_1 is —$COOCH_3$).

Refer to Chart P.

A. A solution of methyl phenyl-N-methyl sulfoximine (3.39 g) in dry tetrahydrofuran (60 ml), is alternately degassed and flushed with nitrogen, cooled to −78° C. and treated dropwise over 7 min with 2.8 M methyl magnesium chloride (7.16 ml). The resulting solution is stirred at −78° C. for 30 min, then at 0° C. for 15 min. The reaction is cooled to −78° C. and treated with a solution of 3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGE$_1$, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether) (6.05 g), a formula CLXXI compound, in dry tetrahydrofuran (35 ml). The resulting mixture is stirred for 1.75 hr while the temperature permitted to go from −78° C. to 0° C. and then stirred for one hr at 0° C. The reaction mixture is then diluted with brine (170 ml) and extracted with diethyl ether. The ethereal extracts are then washed successively with brine (170 ml), 0.5 M aqueous potassium bisulfate (170 ml), saturated aqueous sodium bicarbonate (170 ml) and brine (170 ml), dried over magnesium sulfate, filtered and concentrated to a yellow oil (8.0 g), 9-[(N-methyl)-phenylsulfoximinoethyl]-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF$_1$, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). A degassed solution of 9-[(N-methyl)phenylsulfoximinomethyl]-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF$_1$, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether) (8.0 g) in tetrahydrofuran (150 ml) is cooled to 0° C., treated with 50% acetic acid/water (45 ml) then immediately with aluminum amalgam under nitrogen. (The aluminum amalgam is prepared by washing 20 mesh aluminum, 8.00 g, with diethyl ether, 170 ml, methanol, 340 ml, mercuric chloride, 8.03 g, in water, 275 ml, methanol, 170 ml, and diethyl ether, 170 ml).

The resulting black suspension is stirred for 1.75 hr during which the reaction temperature is permitted to go from 0° to 15° C. (slowly) then cooled to 0°, treated with ethyl acetate (210 ml) and stirred for an additional 30 min at 0° C. The suspension is filtered through diatomaceous earth and the filter cake washed with ethyl acetate. The combined filtrate is then washed with brine (300 ml), 0.5 M aqueous potassium bisulfate (300 ml), saturated aqueous sodium bicarbonate (300 ml) and brine (300 ml), dried, filtered, and concentrated to a yellow oil, crude formula CLXXII compound (6.03 g), 9-deoxy-9-methylene-3-oxa-1,2,3,4,5,6-pentanor-3,7-

inter-m-phenylene-P GF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). The crude product is combined with that from a repeat preparation to yield 10.1 g of formula CLXXII product which is chromatographed on silica gel eluting with 5% ethyl acetate in Skellysolve B (SSB, isomeric hexanes) to yield 6.93 g of 9-deoxy-9-methylene-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 4.52–5.12 and 6.53–7.30δ. Infrared absorptions are observed at 1600 and 1655 cm^{-1}. Silica gel TLC R_f is 0.39 in 10% ethyl acetate in hexane.

B. A degassed solution of 9-deoxy-9-methylene-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether), the reaction product of Part A, (1.33 g) in dry tetrahydrofuran (70 ml) is cooled to 0° C. and treated under nitrogen with 0.5 M 9-borabicyclo[3.3.1-]nonane (14 ml), dropwise over 5 min. The colorless solution is stirred for 4.5 hr at 0° and treated with 30% hydrogen peroxide (6 ml) followed by 3 N potassium hydroxide (6 ml). The resulting suspension is stirred for an additional 30 min at 0° C. and for 75 min while warming to room temperature. The reaction mixture is transferred to a separatory funnel, diluted with brine (300 ml) and ethyl acetate (300 ml). The layers are separated, and the aqueous layer extracted with ethyl acetate (600 ml). The organic extracts are washed with brine (6 ml), dried, filtered, and contrated to formula CLXXIII product, a colorless oil (3.3 g), 9-deoxy-9α-(hydroxymethyl)-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis-(tetrahydropyranyl ether). The crude formula CLXXIII product is chromatographed on silica gel (300 g) in 35% ethyl acetate in hexane to yield 1.26 g of 9-deoxy-9α-(hydroxymethyl)-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether) as a colorless oil. NMR absorptions are observed at 4.73, 5.12–5.70, 6.52–7.23δ. Infrared absorptions are observed at 3480 and 1670 cm^{-1}. Silica gel TLC R_f is 0.21 in 35% ethyl acetate in hexane.

C. A degassed solution of 9-deoxy-9α-hydroxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether) (2.01 g), reaction product of Part B, in dry methylene chloride (45 ml) is cooled to −5° C. under nitrogen and treated with triethylamine (0.72 ml), then with methanesulfonyl chloride (0.76 ml). The resulting solution is stirred at −5° C. for 5 min then for 75 min while warming to ambient temperature. The reaction solution is poured over ice, and the resulting mixture swirled for a few minutes then transferred to a separatory funnel and partitioned between diethyl ether and brine. The layers are separated, and the aqueous layer extracted with ether (400 ml). The organic layer is washed with brine (200 ml) and saturated aqueous sodium bicarbonate (400 ml), dried, filtered, and concentrated to a formula CLXXIV product, a colorless oil (2.69 g), 9-deoxy-9α-mesyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). This product (2.69 g) is chromatographed on silica gel (185 g) eluting with 25% ethyl acetate in Skellysolve B to yield 1.99 g of 9-deoxy-9α'-mesyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1), 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 2.95, 4.70, 5.20–5.70, and 6.52–7.22δ. Silica gel TLC R_f is 0.30 in 35% ethyl acetate in hexane.

D. A degassed solution of 9-deoxy-9α-mesyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether) (0.971 g), the reaction product of Part C, in dry tetrahydrofuran (35 ml) is cooled to 0° C. and treated under nitrogen with 0.75 M tetrabutylammonium fluoride (2.6 ml). The resulting amber solution is stirred for 2.5 hr at 0°–5° C. and is partitioned between ethyl acetate (150 ml) and brine (150 ml). The layers are separated, and the aqueous layer extracted with ethyl acetate (300 ml). The organic layer is then washed with 0.5 M aqueous ammonium chloride (150 ml), saturated aqueous sodium bicarbonate (300 ml) and brine (150 ml), dried, filtered and concentrated to give 0.82 g of formula CLXXV product, 9-deoxy-9α-mexyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 11,15-bis(tetrahydropyranyl ether. Infrared absorptions are observed at 3330 cm^{-1}. Silica gel TLC R_f is 0.37 in 50% ethyl acetate in hexane.

E. A degassed solution of 9-deoxy-9α-mesyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 11,15-bis(tetrahydropyranyl ether) (0.82 g), reaction product of Part D, is cooled to −40° C. under argon and treated with 57% sodium hydride (0.67 g). The resulting suspension is then stirred for 40 min at −40° C. then 15 min at 0° C. The suspension is stirred for an additional 20 min while warming to room temperature and then stirred for 2.5 hr at reflux. The reaction is then cooled to 10° C., diluted with ice cold brine (200 ml) and extracted with ethyl acetate (450 ml). The ethyl acetate extracts are then washed with brine (300 ml), dried, filtered and concentrated to give 0.72 g of the formula CLXXVI crude product. The crude product is chromatographed in silica gel (175 g) in 25% ethyl acetate in Skellysolve B to yield 0.49 g of 9-deoxy-2',9α-methano-3-oxa-1,2,4,5,6-pentanor-3,7-(1',3'-interphenylene)-PGF_1, 11,15-bis-(tetrahydropyranyl ether). NMR absorptions are observed at 4.77, 5.32–6.03, and 6.52–7.22δ. Infrared absorptions are observed at 3340 and 1670 cm^{-1}. Silica gel TLC R_f is 0.56 in 35% ethyl acetate in hexane.

F. A degassed solution of 9-deoxy-2',9α-methano-3-oxa-1,2,4,5,6-pentanor-3,7-(1',3'-inter-phenylene)-PGF_1, 11,15-bis(tetrahydropyranyl ether) (0.47 g), reaction product of Part E, in dry glyme (15 ml) is cooled to 0° C. and treated under nitrogen withmethyl bromoacetate (0.26 ml) followed by 57% sodium hydride suspension (0.136 g). Following vigorous effervescence, a white precipitate is formed. The resulting suspension is stirred for 2.5 hr at 0°–5° C., diluted with ice cold brine (200 ml) and extracted with ethyl acetate (450 ml). The ethyl acetate extracts are washed with brine (300 ml), dried over magnesium sulfate, filtered and concentrated to a pale yellow oil (0.62 g), formula CLXXVII compound, 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-PGF_1, methyl ester, 11,15-bis(tetrahydropyranyl ether). Infrared absorptions are observed at 1765 and 1740 cm^{-1}.

G. A solution of 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1, methyl ester, 11,15-bis(tetrahydropyranyl ether) (0.62 g), reaction product of Part F, in acetic acid (15 ml), water (7.5 ml) and tetrahydrofuran (5 ml) is reacted at 45° C. under nitrogen for 2.75 hr, cooled and diluted with ice cold brine (200 ml). The resulting suspension is extracted with ethyl acetate (400 ml), and the organic extracts

washed with brine (400 ml), saturated aqueous sodium bicarbonate (600 ml) and brine (200 ml). The ethyl acetate extracts are then dried over magnesium sulfate, filtered and concentrated to give 0.44 g of pale yellow oil.

This crude product is chromatographed on silica gel (60 g) in 50% ethyl acetate in Skellysolve B to yield 0.37 g of product which was crystallized to yield 0.216 g of title product, 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1, methyl ester. Melting range is 82°–84° C. NMR absorptions are observed at 3.77, 4.62, 5.42–5.63, and 6.53–7.25δ. Infrared absorptions are observed at 3520, 3400, and 1735 cm^{-1}. Silica gel TLC R_f is 0.30 in 35% acetone in methylene chloride.

H. A solution of 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1, methyl ester (0.15 g), reaction product of Part G, in 5% potassium hydroxide in 9:1 methanol-water (5.5 ml) is stirred at 0° C. under nitrogen. The solution is turbid initially and a precipitate forms within 5 min. The reaction is then stirred for one hr at 0° C., diluted with ice cold brine (90 ml), acidified with 1 N hydrochloric acid, and extracted with ethyl acetate (180 ml). The ethyl acetate extract is then washed with brine (270 ml), dried over magnesium sulfate, and concentrated under reduced pressure to yield a waxy, semi-solid (0.131 g), which is crystallized to yield 0.105 g of title product, 9-deoxy-2', 9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)PGF_1. Melting range is 131°–133° C. NMR absorptions are observed at 4.68, 5.48–5.72, 6.68–7.22. Infrared absorptions are observed at 3460, 3280, 1735, 1720, and 1700 cm^{-1}.

I. The dosage at which the title compounds should be administered to achieve their effect, chiefly anti-platelet aggregation or blood pressure lowering, will vary according to the potency of the particular compound under study. When given orally, the compounds will show a desired effect in man at a dose from about 0.05 to about 50 mg/kg orally, preferably from about 0.1 to about 5 mg/kg. The compounds 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-PGF_1, methyl ester, given to a rat orally at a dose of 1 mg/kg lowered blood pressure 44 mmHg. After 52 min the blood pressure was still lower 14 mm. Intravenous dosages for the desired effect are from about 1 to about 500 ng/kg/min in man, preferably from about 10 to about 100 ng/kg/min.

EXAMPLE 32

9-Deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-16,16-difluoro-PGF_1

(Formula XI: X_1 is —COOH, L_1 is α-fluoro:β-fluoro, R_{20}, R_{20}, R_{21}, R_{23}, and R_{24} are all hydrogen, Z_4 is $—CH_2—$, R_{22} is β-hydrogen, R_8, Y_1, M_1, and R_7 are as defined in Example 8) and its corresponding methyl ester (X_1 is $—COOCH_3$).

Refer to Chart P.

A. Diethyl ether (55 ml) tri-n-butylphosphine (2.28 g) and cuprous iodide (2.13 g) are combined with stirring with the resulting mixture being alternately degassed and flushed with nitrogen at 25° C. for 1 hr. The resulting solution is then cooled to −78° C. and is hereafter referred to as solution 32-I. Thereafter 60 ml of anhydrous diethyl ether and 6.47 g of m-bromo-phenol, t-butyldimethylsilyl ether are combined and the resulting solution alternately degassed and flushed with nitrogen and cooled to −78° C. After cooling, the resulting mixture is treated with 44.16 ml of a 1.02 M solution of t-butyllithium in n-pentane. This reaction mixture is then stirred at −78° C. for 1 hr and hereinafter referred to as solution 32-II. Solution 32-II is then transferred with stirring over 15 min to solution 32-I under a nitrogen atmosphere. The resulting solution changed in color from clear to yellow to an orange-brown to tan. The resulting mixture is then stirred at −78° C. for 30 min and labelled solution 32-III. Thereafter 4α-hydroxy-3β-(4',4'-difluoro-3'α-hydroxy-trans-1'-octenyl)-2-methylene-cyclopentanone, 4,3'-bis(tetrahydropyran-2-yl ether), 4 g, Example 25 of U.S. Pat. No. 4,181,798, and 38 ml of anhydrous dry ethyl ether are combined with stirring and the resulting mixture alternately degassed and flushed with nitrogen and thereafter cooled to −78° C. The resulting solution is referred to herein as solution 32-IV. Solution 32-IV is then added to solution 32-III with vigorous stirring over 25 min at −78° C. under a nitrogen atmosphere. The reaction mixture is then stirred at −78° C. for 30 min and thereafer transferred to 100 ml of 8% glacial acetic acid in diethyl ether (−40° C.) with vigorous stirring under a nitrogen atmosphere. The resulting mixture is then diluted with brine and extracted with diethyl ether. The ethereal extracts are then washed with aqueous sodium bicarbonate in brine, dried over sodium sulfate, concentrated under reduced pressure, and chromatographed on silica gel eluting with 20% ethyl acetate in Skellysolve B to yield 5.56 g of pure formula CLXXI compound: 16,16-difluoro-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGE_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyran-2-yl ether). NMR absorptions ($CDCl_3$) are observed at 0.18, 3.1–5.0, 5.67, 6.52–6.88, and 6.88–7.2δ. Infrared absorptions are observed at 1745, 1600, 1585, 1490, 1275, 1260, 1200, 1155, 1125, 1075, 1035, 1025, 975, 840, and 780 cm^{-1}. Silica gel TLC R_f is 0.36 and 0.41 in 25% ethyl acetate in Skellysolve B. Silica gel TLC R_f is 0.5 in 5% acetone in methylene chloride.

B. Following the procedure of Example 31, Part A, 3.47 g of the reaction product of Part A of this example is converted to 2.98 g of formula CLXXII product as a colorless oil, 9-deoxy-9-methylene-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-16,16-difluoro-PGF_1, 3-(t-butylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 0.17, 0.97, 1.0–3.2, 3.2–4.4, 4.4–5.0, 5.3–6.0, and 6.4–7.3δ. Infrared absorptions are observed at 1655, 1605, 1585, 1485, 1275, 1260, 1200, 1144, 1125, 1080, 1025, 970, 870, and 780 cm^{-1}. Silica gel TLC R_f is 0.31 and at 0.36 in 10% ethyl acetate in hexane.

C. Following the procedure of Example 31, Part B, 2.83 g of the reaction product of Part B of this example is converted to 2.5 g of formula CLXXIII product as a colorless oil, 9-deoxy-9α-(hydroxymethyl)-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-16,16-difluoro-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions ($CDCl_3$) are observed at 0.18, 0.98, 1.15–3.0, 3.0–4.5, 4.5–5.0, 5.3–5.9, and 6.4–7.3δ. Infrared absorptions are observed at 3460, 1670, 1600, 1585, 1485, 1275, 1260, 1160, 1135, 1125, 1075, 1025, 975, 840, and 780 cm^{-1}. Silica gel TLC R_f is 0.28 in 35% ethyl acetate in hexane.

D. Following the procedure of Example 31, Part C, the reaction product of Part C of this example (2.29 g) is converted to 1.83 g of formula CLXXIV product as a colorless oil, 9-deoxy-9α-mesyloxymethyl-3-oxa-

1,2,4,5,6-pentanor-3,7-inter-m-phenylene-16,16-difluoro-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 0.18, 0.98, 1.15–2.85, 2.95, 3.11–4.5, 4.5–5.0, 5.2–5.9, and 6.5–7.4δ. Infrared absorptions are observed at 2930, 2860, 1605, 1590, 1490, 1465, 1440, 1360, 1275, 1200, 1175, 1120, 1025, 975, and 840 cm^{-1}. Silica gel TLC R_f is 0.28 in 30% ethyl acetate and hexane.

E. Following the procedure of Example 31, Part D, 1.7 g of the reaction product of Part D of this example is converted to 1.6 g of formula CLXXV product as a yellow oil, 9-deoxy-9α-mesyloxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-16,16-difluoro-PGF_1, 11,15-bis(tetrahydropyranyl ether). Silica gel TLC R_f is 0.34 in ethyl acetate and hexane (1:1).

F. Following the procedure of Example 31, Part E, 1.52 g of the reaction product of Part D of this example is converted to 0.83 g of formula CLXXVI product as a white foam, 9-deoxy-2',9α-methano-3-oxa-1,2,4,5,6-pentanor-3,7-(1',3'-inter-phenylene)-16,16-difluoro-PGF_1, 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 0.95, 1.05–2.95, 3.5–5.0, 5.3–6.0, and 6.5–7.2δ. Infrared absorptions are observed at 3350, 2930, 1670, 1615, 1590, 1465, 1280, 1200, 1120, 1070, and 975 cm^{-1}. The mass spectrum exhibits peaks at 534, 451, 446, 402, and 348. Silica gel TLC R_f is 0.26 in ethyl acetate and hexane (1:3) and 0.40 in acetone and methylene chloride (1:19).

G. Following the procedure of Example 31, Part F, 0.80 g of the reaction product of Part F of this example is converted to 1.06 g of formula CLXXVII product as a colorless oil, 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-16,16-difluoro-PGF_1, methyl ester, 11,15-bis(tetrahydropyranyl ether). Silica gel TLC R_f is 0.44 in 5% acetone and methylene chloride.

H. Following the procedure of Example 31, Part G, 1.0 g of the reaction product of Part G of this example is converted to 0.62 g of crystalline methyl ester title product, a Formula CLXXVIII white solid. Recrystallization from hexane in diethyl ether yields a material with melting range 93°–95° C. NMR absorptions are observed at 0.95, 1.10–2.90, 2.90–4.8, 5.4–5.8, and 6.4–7.3. Infrared absorptions are observed at 3560, 3400, 1765, 1750, 1735, 1720, 1675, 1605, 1585, 1270, 1215, 1205, 1120, 1105, 1080, 1010, 970, and 770 cm^{-1}. The mass spectrum for the bis-trimethylsilyl derivative exhibits a high resolution peak at 582.2997. Silica gel TLC R_f is 0.35 in hexane and ethyl acetate (1:4).

Following the procedure of Example 31, Part H, the reaction product of Part H of this example (0.25 g) is converted to the carboxylic acid title product (158 mg) as a crystalline solid. Melting range is 128°–130° C. NMR absorptions ($COCD_3$) are observed at 0.9, 1.3–3.0, 3.0–4.6, 4.68, 4.8–5.5, 6.5–6.9, 5.5–5.9, and 6.6–7.3δ. Infrared absorptions are observed at 3570, 3480, 3370, 3220, 2800, 1740, 1720, 1605, 1585, 1235, 1210, 1125, 1105, 1080, 1000, and 970 cm^{-1}. The mass spectrum for the tris-trimethylsilyl derivative exhibits a high resolution peak at 640.3232. Silica gel TLC R_f is 0.18 in the A–IX solvent system.

Following the procedure of Examples 31 and 32, there are prepared each of the various formula CLXXVIII products in free acid or ester form from corresponding formula CLXXI reactants.

Formula CLXXVIII compounds wherein Y_1 is unsaturated (trans- or cis—CH=CH—) are transformed to corresponding formula CLXXVIII compounds wherein Y is saturated (—CH_2CH_2—) by hydrogenation, as exemplified below:

EXAMPLE 33

9-Deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-13,14-dihydro-PGF_1

(Formula XI: X_1 is COOH, Y_1 is —CH_2CH_2—, R_{20}, R_{21}, R_{23}, and R_{24} are all hydrogen, Z_4 is —CH_2—, R_{22} is β-hydrogen, R_8, M_1, L_1, and R_7 are as defined in Example 8) and its corresponding methyl ester (X_1 is —$COOCH_3$).

A. A solution of the methyl ester title product of Example 31 (0.341 g) in ethyl acetate (35 ml) is treated at ambient temperature with 5% palladium-on-charcoal and hydrogenated at atmospheric pressure. The resulting suspension is then stirred for 70 minutes with a hydrogen uptake of 20 ml (atmospheric pressure). The resulting suspension is then filtered through diatomaceous earth and the filter cake washed with ethyl acetate. The combined filtrate is then concentrated under reduced pressure to yield a colorless oil which is chromatographed on silica gel eluting with ethyl acetate in Skellysolve B to yield 0.306 g of title product (methyl ester), a colorless oil. NMR absorptions ($CDCl_3$) are observed at 0.9, 0. 1.07–1.23, 3.3–4.03, 3.77, 4.62, 6.52, and 7.27δ. Infrared absorptions are observed at 3350, 2930, 2855, 1760, 1740, 1605, 1585, 1467, 1435, 1275, 1205, 1120, 1080, 1025, and 775 cm^{-1}. Silica gel TLC R_f is 0.54 in ethyl acetate.

B. Following the procedure of Example 31, Part H, the title product of Part A of this example (0.177 g) is converted to 0.23 g of title product (free acid) as a solid. Recrystallization from ethyl acetate in hexane yields 0.096 g with melting range 121°–123° C. The mass spectrum for the tris-trimethylsilyl derivatives exhibits a high resolution peak at 606.3553 and other peaks at 591–535, 516, 427, 426, 275, 274, 173, and 157. Silica gel TLC R_f is 0.27 in A–IX.

EXAMPLE 34

9-Deoxy-2',9β-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1

(Formula XI: X_1 is COOH, R_{20}, R_{21}, R_{22}, and R_{24} are all hydrogen, Z_4 is —CH_2—, R_{22} is α-hydrogen, R_8, Y_1, M_1, L_1, and R_7 are as defined in, Example 8) and its corresponding methyl ester (X_1 is —$COOCH_3$).

Refer to Charts Q and R.

A. A solution of 0.82 g of the reaction product of Example 31, Part B, in 16 ml of methylene chloride is stirred at ambient temperature under nitrogen atmosphere and treated with diatomaceous earth followed by 26 ml of Collins reagent prepared from 2.5 ml of pyridine and 1.55 g of chromium trioxide in 50 ml of methylene chloride). The resulting suspension is then stirred for 35 min at ambient temperature under a nitrogen atmosphere and filtered through 30 g of silica gel, eluting with 150 ml of ethyl acetate. Concentration under reduced pressure yields 0.90 g of a pale yellow oil. Chromatographing on 85 g of silica gel eluting with 20% ethyl acetate in Skellysolve B yields 0.644 g of pure formula CLXXXII aldehyde as a colorless oil, 9-deoxo-9α-formyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGE_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions are observed at 0.18, 0.88, 0.98, 1.13–3.08, 3.23–4.35, 4.73, 5.25–5.75, 6.57–7.37, and 9.88δ. Infrared absorp-

tions are observed at 2730, 1720, 1600, 1585, 1485, 1275, 1260, 1075, 1035, 1030, 1020, 975, and 840 cm^{-1}. Silica gel TLC R_f is 0.47 in ethyl acetate and hexane (1:3).

B. A degassed solution of 1.5 g of the reaction product of Part A and 0.36 ml of 1,8-diazobicyclo[5.4.0]undec-7-ene in 150 ml of methylene chloride is stirred for 40 hr at ambient temperature under a nitrogen atmosphere, washed with 100 ml of ice cold 0.15 M aqueous potassium bisulfate, 100 ml of saturated aqueous sodium carbonate, and 100 ml of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield 1.5 g of formula CXCII product as a yellow oil, 9-deoxy-9β-formyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-bis(tetrahydropyranyl ether). NMR absorptions ($CDCl_3$) are observed at 0.18, 0.89, 0.98, 1.1–3.2, 3.2–4.4, 4.68, 5.2–5.8, 6.58–7.4, and 9.22δ. Infrared absorptions are observed at 1725, 1600, 1585, 1485, 1440, 1275, 1260, 1200, 1160, 1130, 1075, 1035, 1020, 975, 870, and 840 cm^{-1}. Silica gel TLC R_f is 0.24 in ethyl acetate and hexane (1:3).

C. A solution of 1.5 g of the reaction product of Part B in 40 ml of methanol is treated with stirring at 20° C. under a nitrogen atmosphere over several minutes with 400 mg of sodium borohydride, stirred for 20 min at 20° C. The resulting mixture is then added to a cold solution of 200 ml of brine and 32 ml of 0.1 M aqueous potassium sulfate, extracted with 600 ml of ethyl acetate, washed with 200 ml of saturated aqueous sodium bicarbonate in 200 ml of brine, dried over anhydrous magnesium sulfate, concentrated under reduced pressure, and chromatographed on 200 g of silica gel eluting with 35% ethyl acetate in hexane to yield 1.37 g of formula CLCIII product as a colorless oil, 9-deoxy-9β-hydroxymethyl-3-oxa-1,2,4,5,6-pentanor-3,7-inter-m-phenylene-PGF_1, 3-(t-butyldimethylsilyl ether), 11,15-(tetrahydropyranyl ether). NMR absorptions ($CDCl_3$) are observed at 0.17, 0.88, 0.99, 1.1–3.0, 3.0–4.35, 4.7, 5.25–5.85, and 6.5–7.4δ. Infrared absorptions are observed at 3460, 1665, 1605, 1685, 1490, 1275, 1260, 1200, 1160, 1135, 1115, 1075, 1020, 1005, 975, 840, and 780 cm^{-1}. Silica gel TLC R_f is 0.20 in 35% ethyl acetate in hexane.

D. A degassed solution of 1.32 g of the reaction product of Part B in 0.47 ml of triethyl amine and 30 ml of methylene chloride at 20° C. under a nitrogen atmosphere is treated with 0.5 ml of methanesulfonyl chloride, stirred for 5 min at 0° C., warmed to 20° C. over 90 min, added to 50 g of ice, diluted with 150 ml of brine, extracted with 450 ml of diethyl ether, washed with 150 ml of brine and 300 ml of saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, concentrated under reduced pressure to yield an oil, and filtered through 70 g of silica gel eluting with 30% ethyl acetate in hexane to yield 1.47 g of mesylate corresponding to the starting material, i.e., the 9β analog of formula CLXXIV. Silica gel TLC R_f is 0.23 in 30% ethyl acetate in hexane.

E. A degassed solution of 1.47 g of the reaction product of Part D and 50 ml of dry tetrahydrofuran at 0° C. under a nitrogen atmosphere is treated with 3.9 ml of 0.45 M tetra-n-butylammonium fluoride. The resulting solution is then stirred at 0° C. for 4 hr, treated with another 0.5 ml of tetra-n-butylammonium fluoride, stirred for 30 min at 0° C., diluted with 150 ml of brine, extracted with 450 ml of ethyl acetate, washed successively with 150 ml of 0.5 M aqueous ammonium chloride, 300 ml of saturated aqueous sodium bicarbonate, and 150 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 1.3 g of a yellow oil, the phenol corresponding to the starting material, i.e., the 9β isomer of the formula CLXXV compound. Silica gel TLC R_f is 0.11 in 35% ethyl acetate in hexane.

F. A degassed solution of 1.3 g of the reaction product of Part E in 75 ml of dry glyme at −40° C. under a nitrogen atmosphere is treated with 90 mg of 57% sodium hydride dispersion in mineral oil, stirred at −40° to −30° C. for 40 min, stirred at 0° C. for 15 min, stirred at ambient temperature for 15 min, heated and refluxed for 5 hr, cooled to ambient temperature, added to 200 ml of ice cold glyme, extracted with 450 ml of ethyl acetate, washed with 300 ml of brine, dried over anhydrous on 175 g of silica gel eluting with 25% ethyl acetate in hexane to yield 0.61 g of the 9β isomer corresponding to the formula CLXXVI compound as a viscous oil. NMR absorptions are observed at 0.90, 1.07–3.1, 3.1–4.4, 4.75, 5.33–6.16, and 6.5–7.2δ. Infrared absorptions are observed at 3340, 1665, 1610, 1585, 1500, 1465, 1135, 1110, 1075, 1020, and 980 cm^{-1}. Silica gel TLC R_f is 0.26 in 25% ethyl acetate in hexane and 0.23 in 5% acetone in methylene chloride.

G. A solution of 0.50 g of the reaction product of Part F in 28 ml of methyl bromoacetate in 16 ml of dry glyme at 0° C. under an argon atmosphere is treated with 0.14 g of a 57% mineral oil dispersion of sodium hydride. The resulting suspension is then stirred for 2.5 hr at 0° C., quenched with 200 ml of cold brine, extracted with 460 ml of ethyl acetate, washed with 300 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to yield 0.68 g of an oil, the 9β isomer corresponding to the formula CLXXVII compound.

H. A solution of the reaction product of Part G (0.68 g) in 5 ml of tetrahydrofuran, 7.5 ml of water, and 15 ml of acetic acid is heated for 2.5 hr at 45° C., cooled, diluted with 200 ml of brine, extracted with 400 ml of ethyl acetate, washed with 400 ml of brine, washed with 200 ml of saturated aqueous sodium bicarbonate, and 200 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield an oil, chromatographed on 75 g of silica gel eluting with 30% hexane in ethyl acetate to 100% ethyl acetate to yield 0.32 g of title methyl ester as a white foam. Crystallization from hot diethyl ether in hexane yields 0.23 g of pure ester product as a white solid. Melting range is 85°–87° C. NMR absorptions ($CDCl_3$) are observed at 0.90, 1.07–2.9, 2.9–4.5, 4.61, 5.4–5.8. and 6.38–7.34δ. Infrared absorptions are observed at 3520, 3420, 1735, 1720, 1605, 1580, 1300, 1240, 1210, 1110, 1085, 1050, 1010, 970, 760, 720, and 710 cm^{-1}. The mass spectrum of the bis-trimethylsilyl derivative exhibits a high resolution peak at 546.3182. Silica gel TLC R_f is 0.14 in 30% ethyl acetate in hexane.

I. Following the procedure of Example 31, Part H, the title product of Part H (158 mg) is transformed to the title free acid (129 mg) as a white solid. Melting range is 150°–154° C. NMR absorptions are observed at 0.90, 1.07–3.5, 3.85–4.35, 4.70, 5.09–5.9, and 6.5–7.3δ. Infrared absorptions are observed at 3380, 2640, 2560, 1730, 1605, 1580, 1260, 1230, 1115, 1050, 1025, 970, and 770 cm^{-1}.

Following the procedure of Example 34, each of the various formula XI compounds are prepared wherein R_{22} is α-hydrogen. Further following the procedure of Example 33, the various 9β-methano isomers of Exam-

ple 34 and corresponding formula XI compounds wherein Y_1 is cis- or trans—CH=CH— are hydrogenated to corresponding 13,14-dihydro-PGF_1 compounds.

EXAMPLE 35

9-Deoxo-2',9-metheno-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGE_1

(Formula XI: X_1 is COOH, R_{20}, R_{21}, R_{23}, and R_{24} are all hydrogen, Z_4 is $—CH_2—$, R_{21} and R_{22} taken together form a valence bond, R_8, Y_1, M_1, L_1, and R_7 are as defined in Example 8) and its corresponding methyl ester (X_1 is $—COOCH_3$).

Refer to Chart T.

A. A degassed solution of the reaction product of Example 34, Part A, (1.68 g) in dry tetrahydrofuran (50 ml) is cooled to 0° C. and treated under a nitrogen atmosphere with 0.75 M tetrabutylammonium fluoride (4.37 ml). The resulting solution is then stirred at 0° C. for 2 hr, diluted with brine (300 ml), extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to yield 2.3 g of an oil. The oil is chromatographed on silica gel (160 g) in 25% ethyl acetate in Skellysolve B yielding 1.21 g of formula CCXI compound, 9-deoxo-9α-formyl-1,2,4.5,6-pentanor-3,7-inter-m-phenylene-PGE_1, 11,15-bis(tetrahydropyranyl ether). NMR absorptions ($CDCl_3$) are observed at 0.88, 1.13–3.15, 3.27–4.47, 4.71, 6.10, 6.53–7.41, 9.27δ. Infrared absorptions are observed at 3345, 2930, 2860, 2720, 1735, 1715, 1605, 1595, 1585, 1485, 1450, 1370, 1350, 1255, 1235, and 970 cm^{-1}. Silica gel TLC R_f is 0.12 in 25% ethyl acetate and hexane and 0.39 in 50% ethyl acetate in hexane.

B. A degassed solution of 0.28 g of the reaction product of Part A in 33 ml of glyme is cooled to −40° C. under argon and treated with 2.95 N methylmagnesium chloride in tetrahydrofuran (0.2 ml). The reaction mixture is stirred at −40° C. for 15 min, stirred at 0° C. for 15 min, permitted to warm to ambient temperature, stirred at reflux for 115 hr under an argon atmosphere, cooled, diluted with ice cold brine (150 ml), extracted with ethyl acetate (300 ml), washed with brine (300 ml), dried over magnesium sulfate, filtered, concentrated under reduced pressure to yield 0.31 g of an oil, and chromatographed on silica gel eluting with 25% ethyl acetate in Skellysolve B to yield 0.16 g of the formula CCXII compound, 9-deoxo-2',9-metheno-3-oxa-1,2,4,5,6-pentanor-3,7-(1',3'-inter-phenylene)-PGE_1, 11,15-bis(tetrahydropyranyl ether). The mass spectrum of the trimethylsilyl derivative exhibits a molecular peak at 568 and other peaks at 466, 382, 364, 314, 297, 267, 255, 243, 230, 270, 153, and 85. Silica gel TLC R_f is 0.25 in 25% ethyl acetate in hexane and 0.58 in 50% ethyl acetate in hexane.

C. A degassed solution of the reaction product of Part C (0.16 g) in dry glyme (5 ml) is cooled at −5° C. and treated with methylbromo acetate (0.04 ml) under a nitrogen atmosphere. The resulting solution is then treated with 50% sodium hydride dispersion in mineral oil (0.16 g). Precipitate forms in 5 min in the resulting suspension is stirred for 1.5 hr at 0° C., diluted with brine (100 ml), extracted with ethyl acetate (240 ml), washed with brine (100 ml), dried over magnesium sulfate, filtered, concentrated to yield a brown residue which solidifies on refrigeration, and chromatographed on 25 g of silica gel eluting with 20% ethyl acetate in Skellysolve B to yield 0.136 g of the bis(tetrahydropyranyl ether) of a formula CCXIII compound: 9-deoxy-2',9-metheno-3-oxa-4,5,6-trinor-3,7-(1,3-inter-phenylene)-PGE_1, methyl ester, 11,15-bis(tetrahydropyranyl ether). Melting range is 81°–83° C. The mass spectrum exhibits peaks at 366, 384, 364, 279, 247, 230, 215, 149, and 85. Silica gel TLC R_f is 0.45 in 5% acetone in methylene chloride.

D. A solution of the reaction product of Part C (0.12 g) in tetrahydrofuran (1 ml), water (2 ml) and acetic acid (4 ml) is heated at 45° C. under a nitrogen atmosphere for 2.25 hr, cooled, and partitioned between brine (100 ml) in ethyl acetate (90 ml). The layers are separated and the aqueous layer extracted with ethyl acetate (160 ml). The organic layers are then washed successively with brine (100 ml), water (100 ml), saturated aqueous sodium bicarbonate (300 ml) and brine (200 ml), dried over magnesium sultate, filtered, concentrated to yield 0.97 g of a solid, and chromatographed on 30 g of silica gel, eluting with 85% ethyl acetate in hexane to yield 0.083 g of white crystalline formula CCXIII title product in methyl ester form. Recrystallization from diethyl ether in hexane yields 0.056 g of pure methyl ester title product. Melting range is 96°–98° C. NMR absorptions ($CDCl_3$) are observed at 0.94, 3.86, 3.92–4.28, 4.72, 5.58–5.86, and 6.62–7.18δ. Infrared absorptions are observed at 3420, 1765, 1665, 1600, 1575. 1465. 1440, 1275, 1215, 1190, 1105, 1085, 970, and 770 cm^{-1}. The mass spectrum for the trimethylsilyl derivative exhibits a molecular ion at 554 and other peaks at 454, 383, 365, 364, 230, 229, 225. Silica gel TLC R_f is 0.41 in ethyl acetate.

E. Following the procedure of Example 31, Part H, the reaction product of Part D (0.19 g) is converted to 76 mg of crystalline title product in free acid form. Melting range is 150°–152° C. NMR absorptions ($CDCl_3$) are observed at 0.91, 1.2–3.48, 3.88–4.15, 4.70, 5.62–4.66, and 6.63–7.11. The mass spectrum for the trimethylsilyl derivative exhibits a high resolution peak at 602.3251 and other peaks at 512, 422, 287, 225, 174, and 173. Silica gel TLC R_f is 0.23 in the A–IX solvent system.

EXAMPLE 36

9-Deoxy-2',9α-methano-3-oxa-4,5,6,13,14,15,16,17,18,19,20-undecanor-3,7-(1',3'-inter-phenylene)-12-formyl-PGF_1, methyl ester

(formula CCXXII: X_1 is $—COOCH_3$, Z_4 is $—CH_2—$, R_{20}, R_{21}, and R_{23} are hydrogen, R_{22} is β-hydrogen, and R_{18} is tetrahydropyran-2-yl-oxy).

Refer to Chart U.

Ozone is bubbled through a solution of 0.72 g of the reaction product of Example 31, Part F, in 50 ml of absolute methanol at −78° C. for 5 min. Thereafter oxygen is bubbled through the resulting solution for 5 min and the solution is treated with 16 ml of dimethyl sulfide. After standing at 16 hr for 0° C. under a nitrogen atmosphere and 2½ hr at ambient temperature, the solution is diluted with 200 ml of ethyl acetate, washed successively with 100 ml of brine, 100 ml of saturated aqueous sodium bicarbonate and 100 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure, and chromatographed on 175 g of silica gel eluting with 35% ethyl acetate in hexane to yield 367 mg of title product as a colorless oil. NMR absorptions ($CDCl_3$) are observed at 1.0–3.0, 3.1–4.5, 3.63, 6.45–7.34, and 9.77δ. The mass spectrum exhibits peaks at 388 and 304. Silica gel TLC R_f is 0.19 and 0.22 in 25% and 30% ethyl acetate in hexane.

EXAMPLE 37

9-Deoxy-2',9α-methano-20-methyl-3-oxa-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1

(Formula XI: X_1, Z_4, R_8, R_{20}, R_{21}, R_{22}, R_{23}, R_{24}, Y_1, M_1, and L_1 are as defined in Example 31 and R_7 is n-pentyl) its methyl ester (Z_1 is —$COOCH_3$), its 15-epimer (M_1 is α-H:β-OH), and 15-epimer methyl ester (M_1 is α-H:β-OH and Z_1 is —$COOCH_3$).

Refer to Chart U.

A. A suspension of 56 mg of a 57% sodium hydride dispersion in mineral oil and 4 ml of tetrahydrofuran at 0° C. under a nitrogen atmosphere is treated with a solution of 286 mg of dimethyl-2-octylphosphonate in 4 ml of tetrahydrofuran, stirred for 5 min at 0° C., stirred for 1 hr at ambient temperature, cooled to 0° C., treated with a solution of 0.39 g of title product of Example 36 and 4 ml of tetrahydrofuran, stirred for 2½ hr at ambient temperature, cooled in 0° C., added to a solution of 40 ml of ethyl acetate containing several drops of acetic acid), extracted with 120 ml of ethyl acetate, washed with 30 ml of saturated aqueous sodium bicarbonate, washed with 30 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield an oil, and chromatographed on 60 g of silica gel eluting with 25% ethyl acetate in hexane to yield 0.42 g of a colorless oil, 9,15-dideoxy-15-keto-2',9α-methano-20-methyl-4,5,6-trinor-3,7-(1',3'-inter-phenylene)-PGF_1, methyl ester, 11-tetrahydropyranyl ether. NMR absorptions are observed at 0.89, 1.05–3.0, 3.5–4.37, 4.62, and 5.97–7.30δ. The mass spectrum exhibits peaks at 414, 396, 323, 311, and 301. Silica gel TLC R_f is 0.26 in 25% ethyl acetate in hexane.

B. A degassed solution of 42 mg of sodium borohydride and 4 ml of absolute methanol at −30° C. under a nitrogen atmosphere is treated dropwise with a solution of 391 mg of the title reaction product of Part A in 0.3 ml of methylene chloride and 3 ml of methanol, stirred for 1½ hr at −30° C., quenched by careful addition of 0.2 ml of glacial acetic acid, diluted with 70 ml of brine, extracted with 210 ml of ethyl acetate, washed with 70 ml of saturated aqueous sodium bicarbonate, washed with 70 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield 0.42 g of a colorless oil, and chromatographed on 60 g of silica gel eluting with 40% ethyl acetate in hexane to yield 0.36 g of an epimeric mixture of C-15 alcohols. Silica gel TLC R_f is 0.20 in 40% ethyl acetate in hexane.

C. A solution of the reaction products of Part B above in 3 ml of tetrahydrofuran, 4.5 ml of water, and 9 ml of acetic acid is heated to 45° C. under a nitrogen atmosphere for 2.5 hrs, cooled, diluted washed with 100 ml of brine, extracted with 200 ml of ethyl acetate, washed with 100 ml of brine, washed with 300 ml of satureated aqueous sodium bicarbonate and 100 ml of brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to a yellow oil, and chromatographed on 60 g of silica gel eluting with 20% ethyl acetate in methylene chloride to yield 96 mg of 9-deoxy-2',9α-methano-20-methyl-3-oxa-4,5,6-trinor-3,7-(1,3-inter-phenylene)-15-epi-PGF_1, methyl ester as a colorless oil and 159 mg of 9-deoxy-2',9α-methano-20-methyl-3-oxa-4,5,6-trinor-3,7-(1,3-inter-phenylene)-PGF_1, methyl ester as a white solid. Recrystallization of the 15α-hydroxy compound from hot hexane in diethyl ether yields 140 mg as a white solid. Melting range is 79°–82° C. For the title product methyl ester, NMR absorptions are observed at 0.92, 1.08–3.0, 3.38–4.5, 4.64, 5.33–5.70, and 6.5–7.4. The mass spectrum of the trimethylsilyl derivative exhibits a high resolution peak at 560.3375. Silica gel TLC R_f is 0.19 in 20% ethyl acetate in methylene chloride and 0.31 in 20% hexane in ethyl acetate. For the 15-epi compound, NMR absorptions ($CDCl_3$) are observed at 0.89, 1.07–3.0, 3.7–4.33, 4.63, 5.5–5.8, and 6.55–7.37δ. Infrared absorptions are observed at 3360, 1765, 1750, 1735, 1605, 1585, 1470, 1440, 1205, 1120, 1080, 970, and 770 cm^{-1}. The mass spectrum for the trimethylsilyl derivative exhibits a high resolution peak at 560.3385. Silica gel TLC R_f is 0.35 in 20% acetone and methylene chloride and 0.45 in 20% hexane and ethyl acetate.

D. Following the procedure of Example 31, Part H, the 15α-hydroxy title product of Part C (94 mg) is transformed to 9-deoxy-2',9α-methano-20-methyl-3-oxa-4,5,6-trinor-3,7-(1,3-inter-phenylene)-PGF_1, title free acid, as a white solid, 81 mg. Melting range is 144°–146° C. NMR absorptions (CD_3COCD_3) are observed at 0.8, 1.05–2.9, 3.2–4.5, 4.65, 5.38–5.56, and 6.6–7.2δ. The mass spectrum of the trimethylsilyl derivative exhibits a high resolution peak at 618.3576. Silica gel TLC R_f is 0.14 in the A–IX solvent system.

E. Further following the procedure of Example 31, Part H, the 15-epi title product of Part C (93 mg) is converted to 9-deoxy-2',9α-methano-20-methyl-3-oxa-4,5,6-trinor-3,7-(1,3-inter-phenylene)-15-epi-PGF_1, a white solid, 72 mg. Melting range is 105°–108° C. MMR absorptions (CD_3COCD_3) are observed at 0.90, 1.05–2.9, 3.2–4.3, 4.71, 5.0–5.84, and 6.5–7.34δ. Silica gel TLC R_f is 0.19 in the A–IX solvent system.

Following the procedures of Examples 36 and 37, there are substituted C-12 side chains according to the procedure of Chart U for each of the various formula XI compounds.

Thus, according to procedures described above, there are prepared

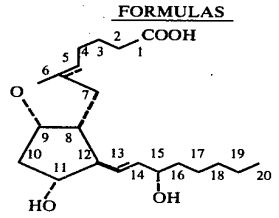
(5E)-9β-methyl-CBA_2 compounds,
(5Z)-9β-methyl-CBA_2 compounds,
(5E)-5-fluoro-9β-methyl-CBA_2 compounds,
(5Z)-5-fluoro-9β-methyl-CBA_2 compounds,
(5E)-5-fluoro-CBA_2 compounds,
(5Z)-5-fluoro-CBA_2 compounds,
(5E)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
(5Z)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
(5E)-9β-methyl-1,5-inter-o-phenylene-2,3,4-trinor-CBA_2 compounds,
(5E)-9β-methyl-1,5-inter-o-phenylene-3,4,5-trinor-CBA_2 compounds,
(5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
(5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
(5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
(5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
2,2-difluoro-(5E)-9β-methyl-CBA_2 compounds,
2,2-difluoro-(5Z)-9β-methyl-CBA_2 compounds,
2,2,5-trifluoro-(5E)-9β-methyl-CBA_2 compounds,
2,2,5-trifluoro-(5Z)-9β-methyl-CBA_2 compounds,
2,2,5-trifluoro-(5E)-CBA_2 compounds,
2,2,5-trifluoro-(5Z)-CBA_2 compounds,
2,2-difluoro-(5E)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,

2,2-difluoro-(5Z)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
2,2-difluoro-(5E)-9β-methyl-1,5-inter-o-phenylene-2,3,4-trinor-CBA_2 compounds,
2,2-difluoro-(5E)-9β-methyl-1,5-inter-o-phenylene-3,4,5-trinor-CBA_2 compounds,
2,2-difluoro-(5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
2,2-difluoro-(5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
2,2-difluoro-(5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
2,2-difluoro-(5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
trans-2,3-didehydro-(5E)-9β-methyl-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-9β-methyl-CBA_2 compounds,
trans-2,3-didehydro-(5E)-5-fluoro-9β-methyl-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-5-fluoro-9β-methyl-CBA_2 compounds,
trans-2,3-didehydro-(5E)-5-fluoro-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-5-fluoro-CBA_2 compounds,
trans-2,3-didehydro-(5E)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-9β-methyl-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
trans-2,3-didehydro-(5E)-9β-methyl-1,5-inter-o-phenylene-2,3,4-trinor-CBA_2 compounds,
trans-2,3-didehydro-(5E)-9β-methyl-1,5-inter-o-phenylene-3,4,5-trinor-CBA_2 compounds,
trans-2,3-didehydro-(5E)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-2,5-inter-o-phenylene-3,4-dinor-CBA_2 compounds,
trans-2,3-didehydro-(5E)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
trans-2,3-didehydro-(5Z)-1,5-inter-m-phenylene-2,3,4-trinor-CBA_2 compounds,
9-deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 compounds,
9-deoxy-2′,9β-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 compounds,
9-deoxo-2′,9-metheno-3-oxa-3,4,5-trinor-3,7-(1′,3′-inter-phenylene)-7,8-didehydro-PGE_1 compounds,
9-deoxo-2′,9-metheno-3-oxa-3,4,5-trinor-3,7-(1′,3′-inter-phenylene)-PGE_1 compounds,
6a-oxo-9-deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 compounds,
6a-oxo-9-deoxy-2′,9β-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene-PGF_1 compounds,
6aα-hydroxy-9-deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 compounds,
6aα-hydroxy-9-deoxy-2′,9β-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)PGF_1 compounds,
6aβ-hydroxy-9-deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene-PGF_1, and
6aβ-hydroxy-9-deoxy-2′,9β-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 compounds,

in free acid or methyl ester form which exhibit the following side chain substituents:
15-cyclohexyl-16,17,18,19,20-pentanor-;
17-(2-furyl)-18,19,20-trinor-;
16-(3-thienyl)oxy-17,18,19,20-tetranor-;
17-(3-thienyl)-18,19,20-trinor-;
15-methyl-;
16-methyl-;
15,16-dimethyl-;
16,16-dimethyl-;
17,20-dimethyl;
16-fluoro-;
15-methyl-16-fluoro-;
16,16-difluoro-;
15-methyl-16,16-difluoro-;
17-phenyl-18,19,20-trinor-;
17-(m-trifluoromethylphenyl)-18,19,20-trinor-;
17-(m-chlorophenyl)-18,19,20-trinor-;
17-(p-fluorophenyl)-18,19,20-trinor-;
15-methyl-17-phenyl-18,19,20-trinor-;
16-methyl-17-phenyl-18,19,20-trinor-;
16,16-dimethyl-17-phenyl-18,19,20-trinor-;
16-fluoro-17-phenyl-18,19,20-trinor-;
16,16-difluoro-17-phenyl-18,19,20-trinor-;
16-phenyl-17,18,19,20-tetranor-;
15-methyl-16-phenyl-17,18,19,20-tetranor-;
16-(m-trifluoromethylphenyl)-17,18,19,20-tetranor-;
16-(m-chlorophenyl)-17,18,19,20-tetranor-;
16-(p-fluorophenyl)-17,18,19,20-tetranor-;
16-phenyl-18,19,20-trinor-;
15-methyl-16-phenyl-18,19,20-trinor-;
16-methyl-16-phenyl-18,19,20-trinor-;
15,16-dimethyl-16-phenyl-18,19,20-trinor-;
16-phenoxy-17,18,19,20-tetranor-;
15-methyl-16-phenoxy-17,18,19,20-tetranor-;
16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-;
16-(m-chlorophenoxy)-17,18,19,20-tetranor-;
16-(p-fluorophenoxy)-17,18,19,20-tetranor-;
16-phenoxy-18,19,20-trinor-;
15-methyl-16-phenoxy-18,19,20-trinor-;
16-methyl-16-phenoxy-18,19,20-trinor-;
15,16-dimethyl-16-phenoxy-18,19,20-trinor-;
13,14-didehydro-;
15-cyclohexyl-16,17,18,19,20-pentanor-13,14-didehydro-;
17-(2-furyl)-18,19,20-trinor-13,14-didehydro-;
16-(3-thienyl)oxy-17,18,19,20-tetranor-13,14-didehydro-;
17-(3-thienyl)-18,19,20-trinor-13,14-didehydro-;
15-methyl-13,14-didehydro-;
16-methyl-13,14-didehydro-;
15,16-dimethyl-13,14-didehydro-;
16,16-dimethyl-13,14-didehydro-;
17,20-dimethyl-13,14-didehydro-;
16-fluoro-13,14-didehydro-;
15-methyl-16-fluoro-13,14-didehydro-;
16,16-difluoro-13,14-didehydro-;
15-methyl-16,16-difluoro-13,14-didehydro-;
17-phenyl-18,19,20-trinor-13,14-didehydro-;
17-(m-trifluoromethylphenyl)-18,19,20-trinor-13,14-didehydro-;
17-(m-chlorophenyl)-18,19,20-trinor-13,14-didehydro-;
17-(p-fluorophenyl)-18,19,20-trinor-13,14-didehydro-;
15-methyl-17-phenyl-18,19,20-trinor-13,14-didehydro-;
16-methyl-17-phenyl-18,19,20-trinor-13,14-didehydro-;
16,16-dimethyl-17-phenyl-18,19,20-trinor-13,14-didehydro-;
16-fluoro-17-phenyl-18,19,20-trinor-13,14-didehydro-;
16,16-difluoro-17-phenyl-18,19,20-trinor-13,14-didehydro-;
16-phenyl-17,18,19,20-tetranor-13,14-didehydro-;
15-methyl-16-phenyl-17,18,19,20-tetranor-13,14-didehydro-;
16-(m-trifluoromethylphenyl)-17,18,19,20-tetranor-13,14-didehydro-;
16-(m-chlorophenyl)-17,18,19,20-tetranor-13,14-didehydro-;

16-(p-fluorophenyl)-17,18,19,20-tetranor-13,14-didehydro-;
16-phenyl-18,19,20-trinor-13,14-didehydro-;
15-methyl-16-phenyl-18,19,20-trinor-13,14-didehydro-;
16-methyl-16-phenyl-18,19,20-trinor-13,14-didehydro-;
15,16-dimethyl-16-phenyl-18,19,20-trinor-13,14-didehydro-;
16-phenoxy-17,18,19,20-tetranor-13,14-didehydro-;
15-methyl-16-phenoxy-17,18,19,20-tetranor-13,14-didehydro-;
16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-13,14-didehydro-;
16-(m-chlorophenoxy)-17,18,19,20-tetranor-13,14-didehydro-;
16-(p-fluorophenoxy)-17,18,19,20-tetranor-13,14-didehydro-;
16-phenoxy-18,19,20-trinor-13,14-didehydro-;
15-methyl-16-phenoxy-18,19,20-trinor-13,14-didehydro-;
16-methyl-16-phenoxy-18,19,20-trinor-13,14-didehydro-;
15,16-dimethyl-16-phenoxy-18,19,20-trinor-13,14-didehydro-;
13,14-dihydro-;
15-cyclohexyl-16,17,18,19,20-pentanor-13,14-dihydro-;
17-(2-furyl)-18,19,20-trinor-13,14-dihydro-;
16-(3-thienyl)oxy-17,18,19,20-tetranor-13,14-dihydro-;
17-(3-thienyl)-18,19,20-trinor-13,14-dihydro-;
15-methyl-13,14-dihydro-;
16-methyl-13,14-dihydro-;
15,16-dimethyl-13,14-dihydro-;
16,16-dimethyl-13,14-dihydro-;
17,20-dimethyl-13,14-dihydro-;
16-fluoro-13,14-dihydro-;
15-methyl-16-fluoro-13,14-dihydro-;
16,16-difluoro-13,14-dihydro-;
15-methyl-16,16-difluoro-13,14-dihydro-;
17-phenyl-18,19,20-trinor-13,14-dihydro-;
17-(m-trifluoromethylphenyl)-18,19,20-trinor-13,14-dihydro-;
17-(m-chlorophenyl)-18,19,20-trinor-13,14-dihydro-;
17-(p-fluorophenyl)-18,19,20-trinor-13,14-dihydro-;
15-methyl-17-phenyl-18,19,20-trinor-13,14-dihydro-;
16-methyl-17-phenyl-18,19,20-trinor-13,14-dihydro-;
16,16-dimethyl-17-phenyl-18,19,20-trinor-13,14-dihydro-;
16-fluoro-17-phenyl-18,19,20-trinor-13,14-dihydro-;
16,16-difluoro-17-phenyl-18,19,20-trinor-13,14-dihydro-;
16-phenyl-17,18,19,20-tetranor-13,14-dihydro-;
15-methyl-16-phenyl-17,18,19,20-tetranor-13,14-dihydro-;
16-(m-trifluoromethylphenyl)-17,18,19,20-tetranor-13,14-dihydro-;
16-(m-chlorophenyl)-17,18,19,20-tetranor-13,14-dihydro-;
16-(p-fluorophenyl)-17,18,19,20-tetranor-13,14-dihydro-;
16-phenyl-18,19,20-trinor-13,14-dihydro-;
15-methyl-16-phenyl-18,19,20-trinor-13,14-dihydro-;
16-methyl-16-phenyl-18,19,20-trinor-13,14-dihydro-;
15,16-dimethyl-16-phenyl-18,19,20-trinor-13,14-dihydro-;
16-phenoxy-17,18,19,20-tetranor-13,14-dihydro-;
15-methyl-16-phenoxy-17,18,19,20-tetranor-13,14-dihydro-;
16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-13,14-dihydro-;
16-(m-chlorophenoxy)-17,18,19,20-tetranor-13,14-dihydro-;
16-(p-fluorophenoxy)-17,18,19,20-tetranor-13,14-dihydro-;
16-phenoxy-18,19,20-trinor-13,14-dihydro-;
15-methyl-16-phenoxy-18,19,20-trinor-13,14-dihydro-;
16-methyl-16-phenoxy-18,19,20-trinor-13,14-dihydro-;
15,16-dimethyl-16-phenoxy-18,19,20-trinor-13,14-dihydro-;
13-cis-;
15-cyclohexyl-16,17,18,19,20-pentanor-13-cis-;
17-(2-furyl)-18,19,20-trinor-13-cis-;
16-(3-thienyl)oxy-17,18,19,20-tetranor-13-cis-;
17-(3-thienyl)-18,19,20-trinor-13-cis-;
15-methyl-13-cis-;
16-methyl-13-cis-;
15,16-dimethyl-13-cis-;
16,16-dimethyl-13-cis-;
17,20-dimethyl-13-cis-;
16-fluoro-13-cis-;
15-methyl-16-fluoro-13-cis-;
16,16-difluoro-13-cis-;
15-methyl-16,16-difluoro-13-cis-;
17-phenyl-18,19,20-trinor-13-cis-;
17-(m-trifluoromethylphenyl)-18,19,20-trinor-13-cis-;
17-(m-chlorophenyl)-18,19,20-trinor-13-cis-;
17-(p-fluorophenyl)-18,19,20-trinor-13-cis-;
15-methyl-17-phenyl-18,19,20-trinor-13-cis-;
16-methyl-17-phenyl-18,19,20-trinor-13-cis-;
16,16-dimethyl-17-phenyl-18,19,20-trinor-13-cis-;
16-fluoro-17-phenyl-18,19,20-trinor-13-cis-;
16,16-difluoro-17-phenyl-18,19,20-trinor-13-cis-;
16-phenyl-17,18,19,20-tetranor-13-cis-;
15-methyl-16-phenyl-17,18,19,20-tetranor-13-cis-;
16-(m-trifluoromethylphenyl)-17,18,19,20-tetranor-13-cis-;
16-(m-chlorophenyl)-17,18,19,20-tetranor-13-cis-;
16-(p-fluorophenyl)-17,18,19,20-tetranor-13-cis-;
16-phenyl-18,19,20-trinor-13-cis-;
15-methyl-16-phenyl-18,19,20-trinor-13-cis-;
16-methyl-16-phenyl-18,19,20-trinor-13-cis-;
15,16-dimethyl-16-phenyl-18,19,20-trinor-13-cis-;
16-phenoxy-17,18,19,20-tetranor-13-cis-;
15-methyl-16-phenoxy-17,18,19,20-tetranor-13-cis-;
16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-13-cis-;
16-(m-chlorophenoxy)-17,18,19,20-tetranor-13-cis-;
16-(p-fluorophenoxy)-17,18,19,20-tetranor-13-cis-;
16-phenoxy-18,19,20-trinor-13-cis-;
15-methyl-16-phenoxy-18,19,20-trinor-13-cis-;
16-methyl-16-phenoxy-18,19,20-trinor-13-cis-; and
15,16-dimethyl-16-phenoxy-18,19,20-trinor-13-cis-.

FORMULAS

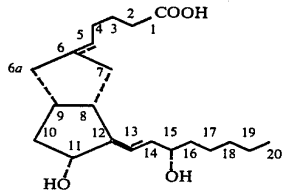


I

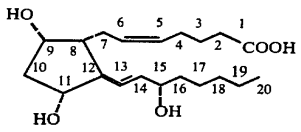
-continued

FORMULAS

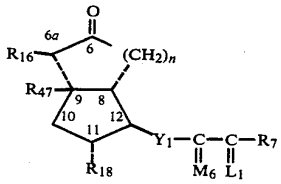
II



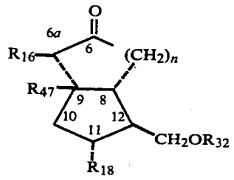
III



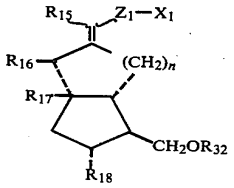
IV



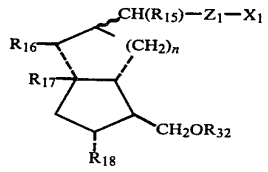
V



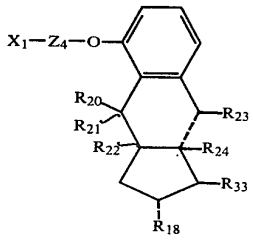
VI



VII



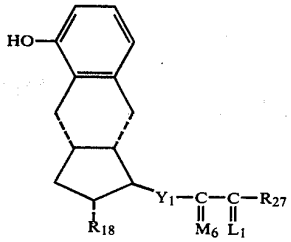
VIII



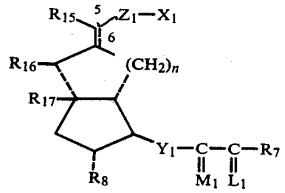
-continued

FORMULAS

IX



X



XI

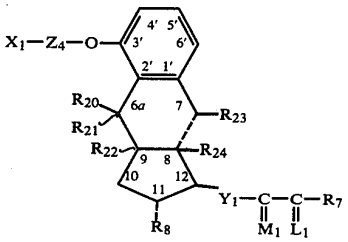
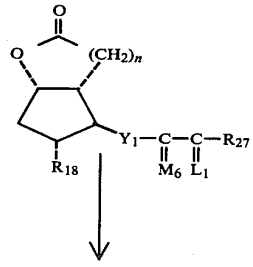
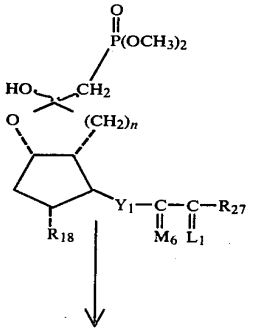


CHART A

XXI



XXII



-continued

CHART A

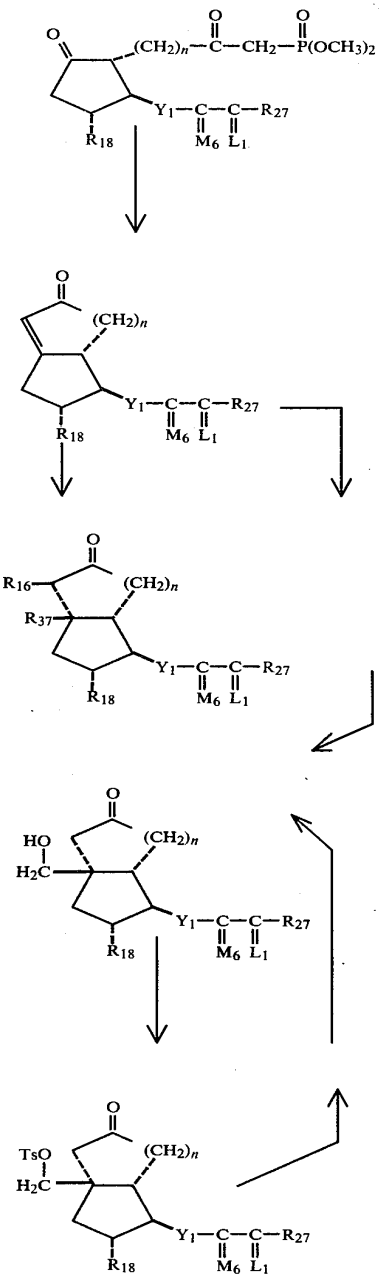


CHART B

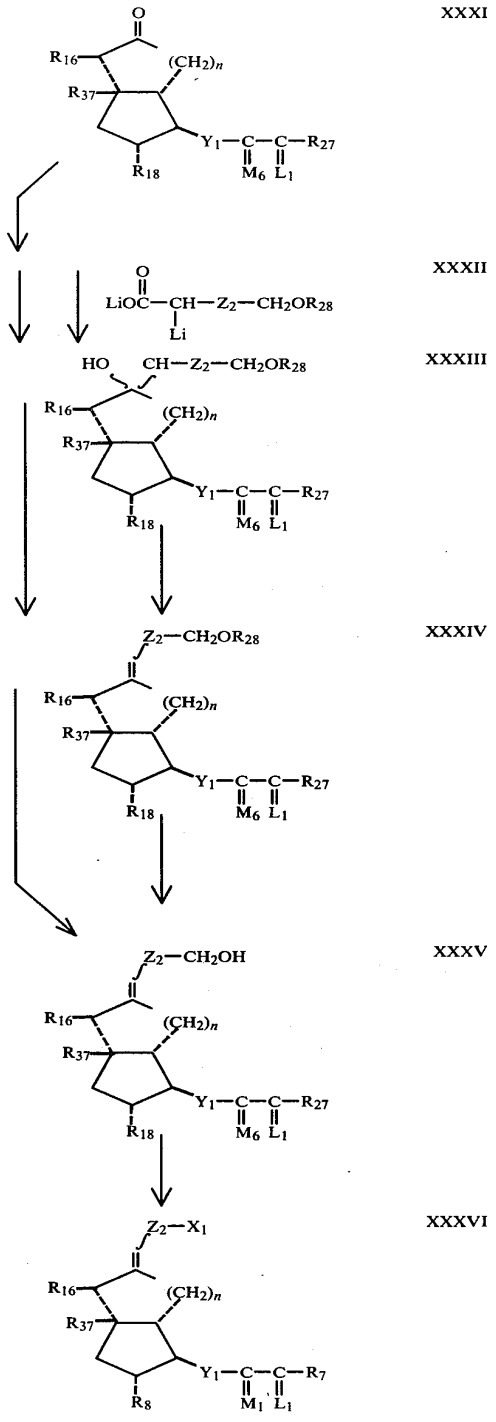


CHART C

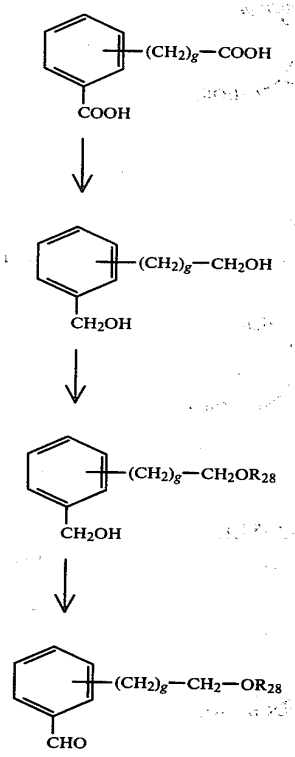
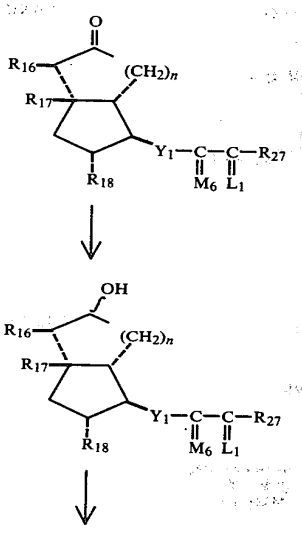
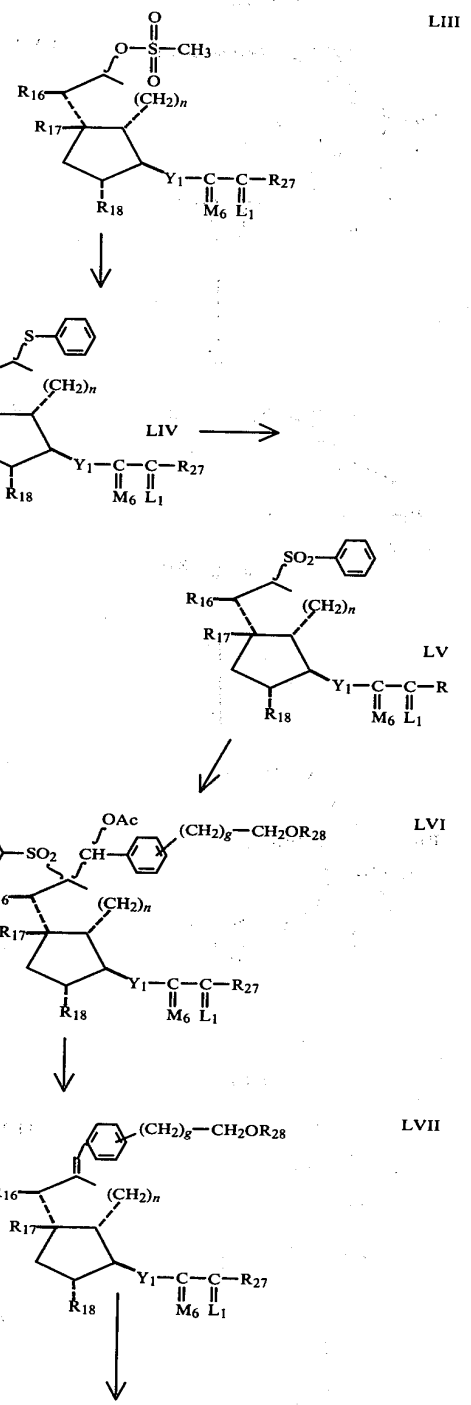


CHART D



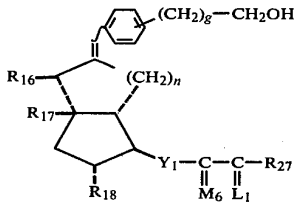
-continued

CHART D

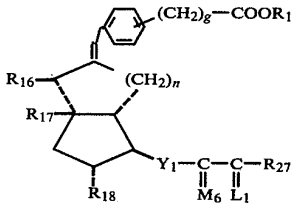


-continued

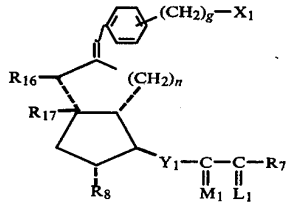
CHART D



LVIII

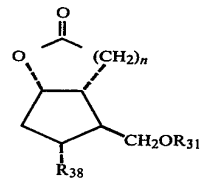


LIX



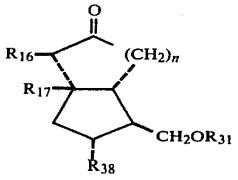
LX

CHART E

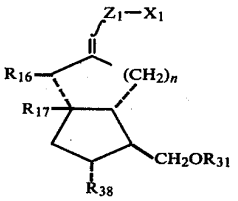


LXI

-continued

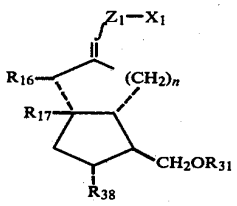


LXII

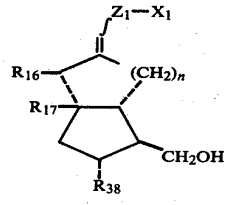


LXIII

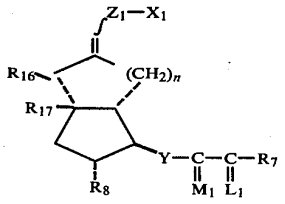
CHART F



LXXI

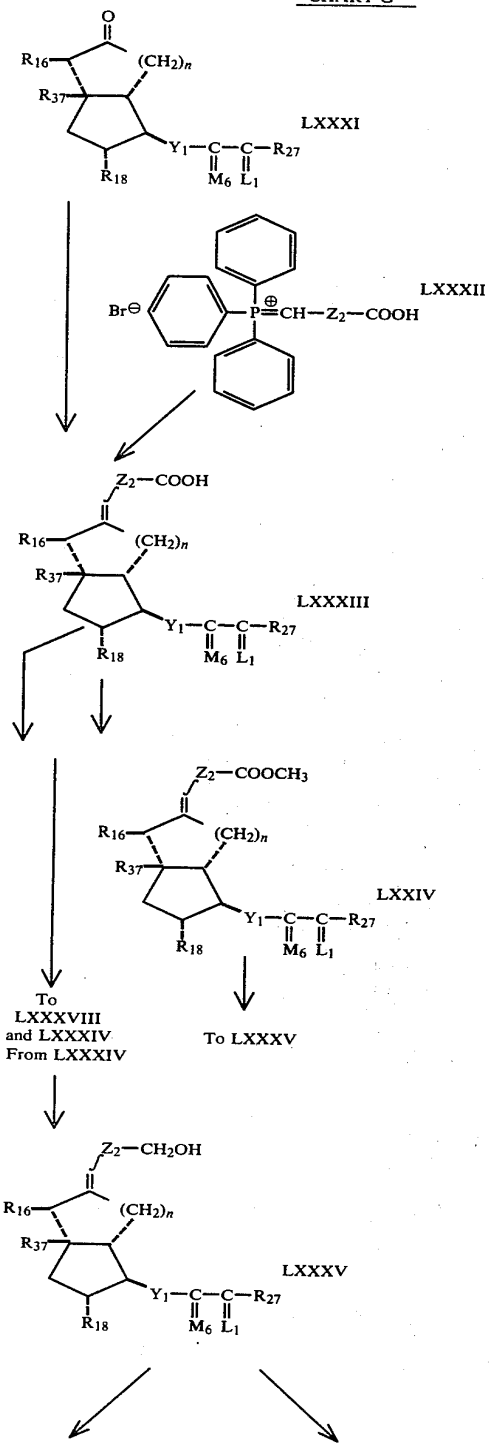


LXXII



LXXIII

CHART G



-continued

CHART G

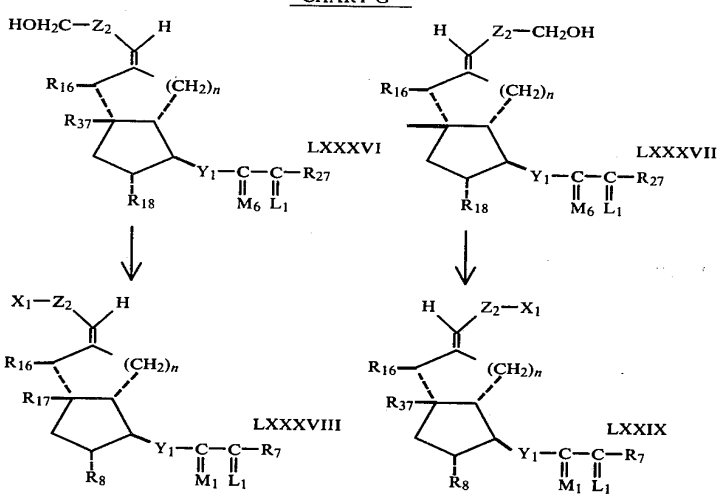
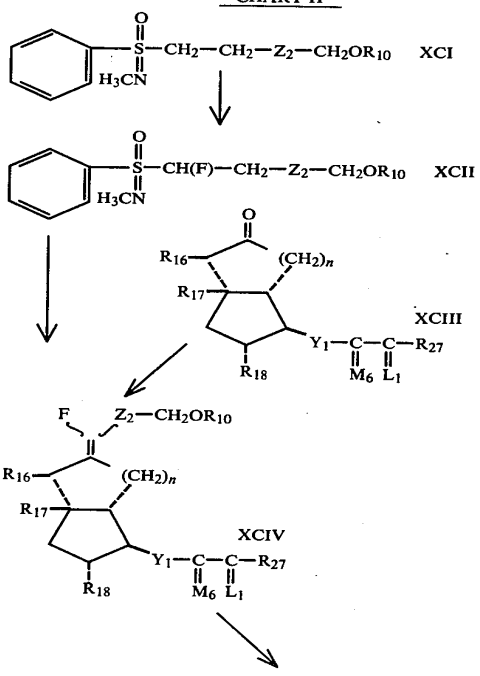


CHART H



-continued

CHART H

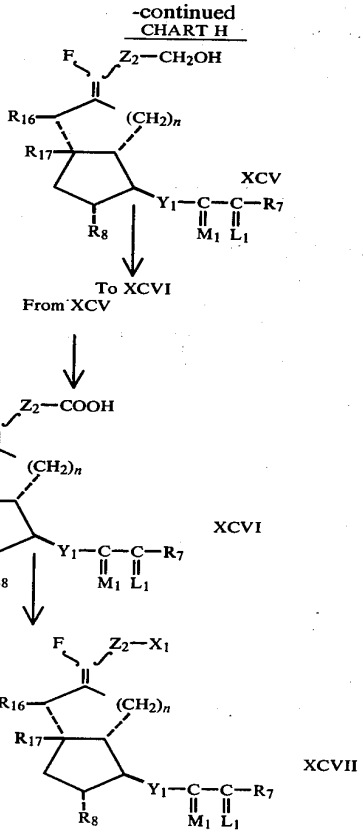
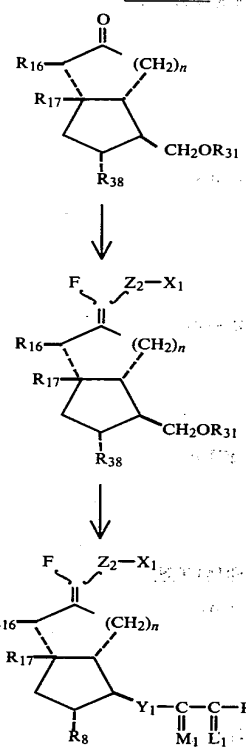
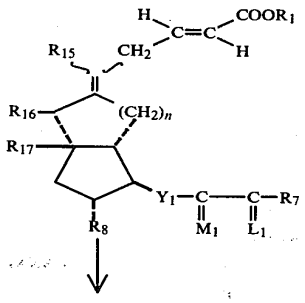


CHART I



-continued

CHART J



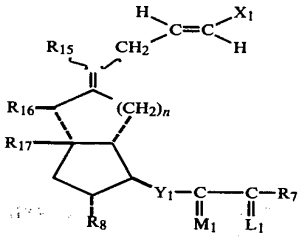
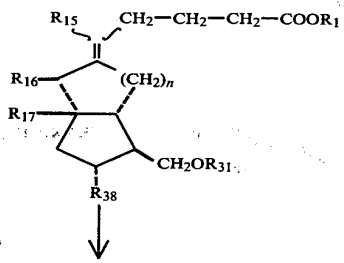


CHART K



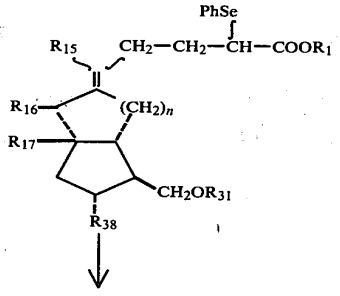
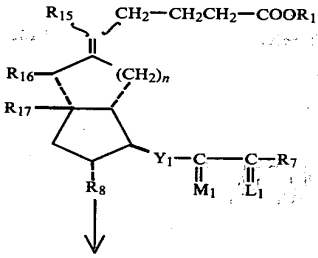
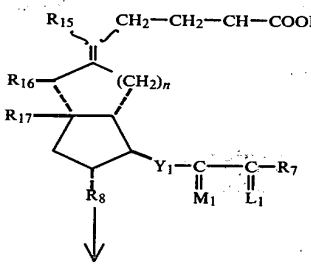


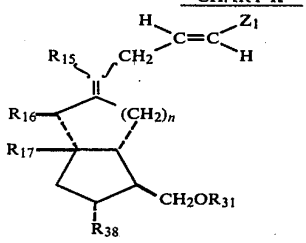
CHART J





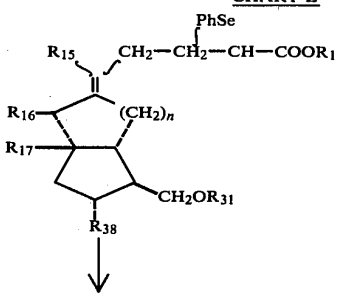
-continued

CHART K

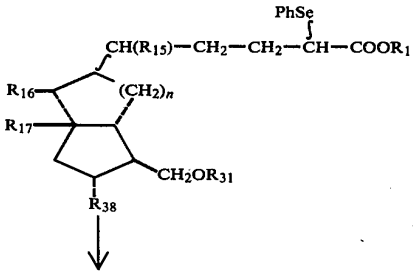


CXXIII

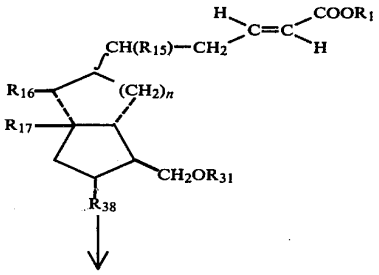
CHART L



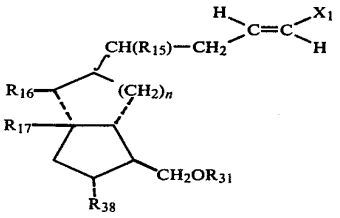
CXXXI



CXXXII

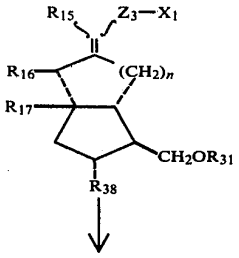


CXXXIII

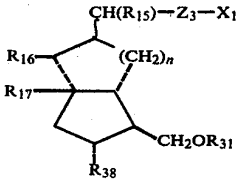


CXXXIV

CHART M

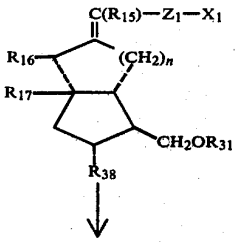


CXLI

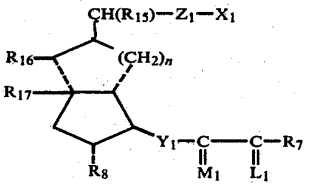


CXLII

CHART N

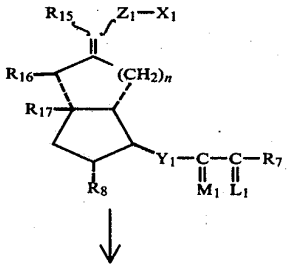


CLI



CLII

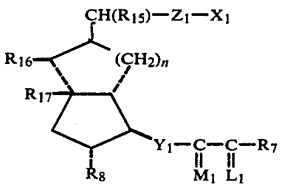
CHART O



CLXI

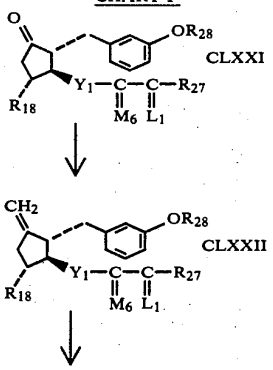
-continued

CHART O



CLXII

CHART P



-continued

CHART P

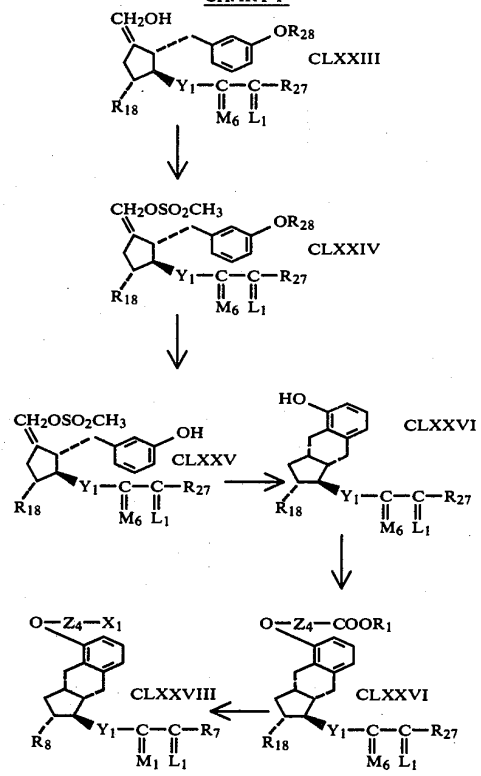
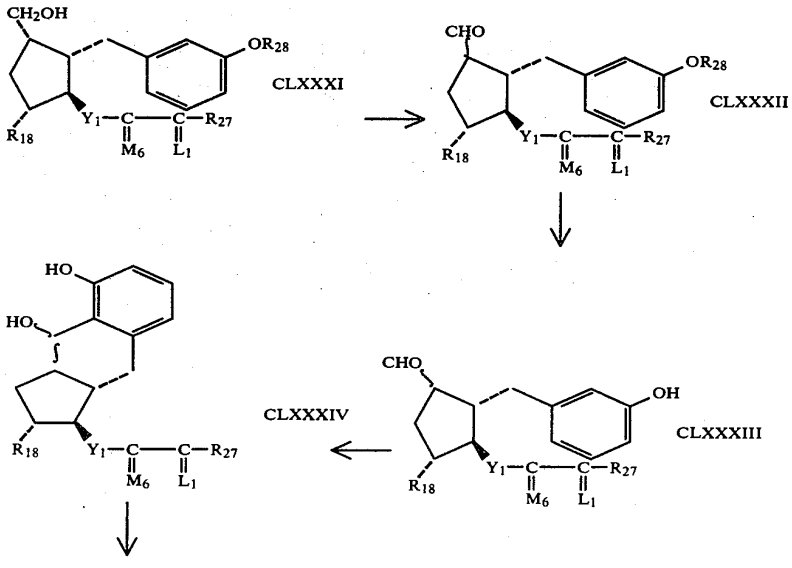


CHART Q



-continued

CHART Q

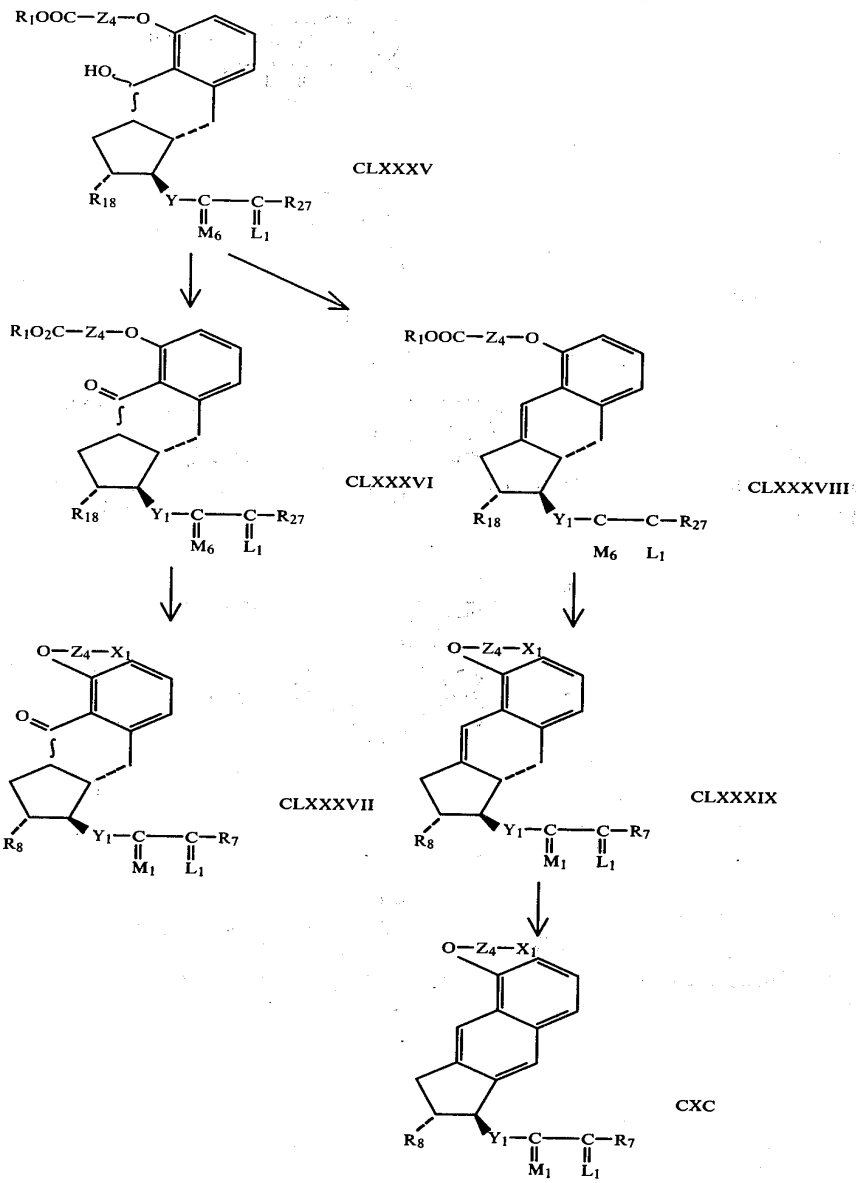
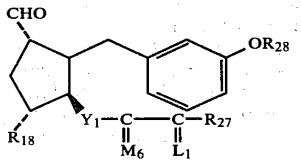
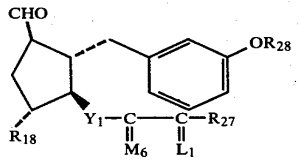


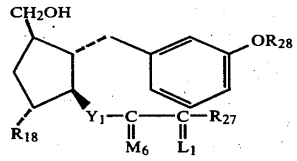
CHART R



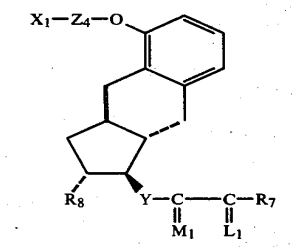
CXCI



CXCII

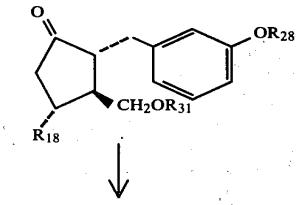


CXCIII



CXCIV

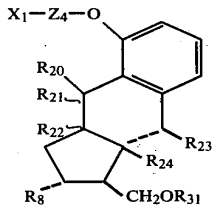
CHART S



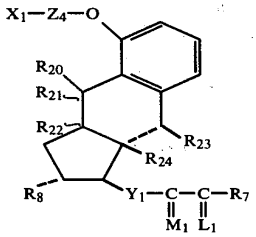
CCI

-continued

CHART S

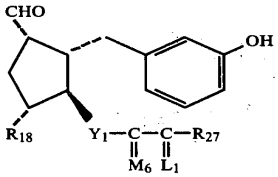


CCII

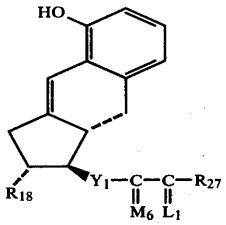


CCIII

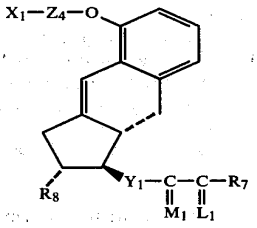
CHART T



CCXI

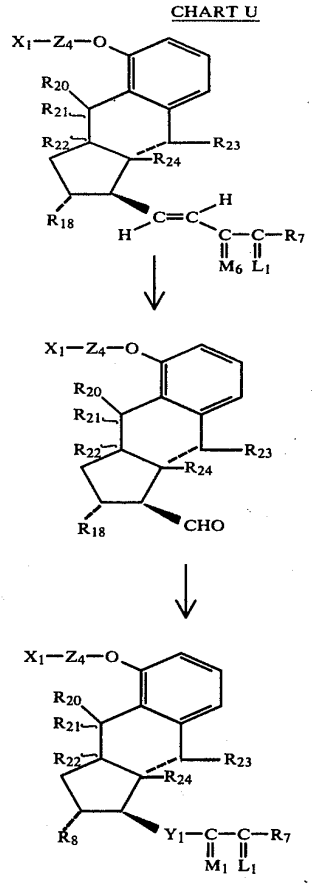


CCXII



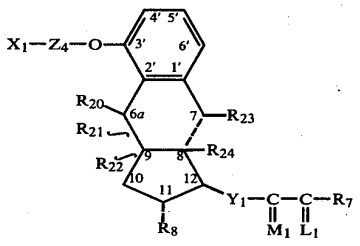
CCXIII

CHART U



I claim:

1. A carbacyclin analog of formula XI:



wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro;

wherein M_1 is α-OH:β-R_5 or α-R_5:β-OH, wherein R_5 is hydrogen or methyl;

wherein R_7 is

(1) $-C_mH_{2m}-CH_3$, wherein m is an integer from one to 5, inclusive,

(2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different,

(3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl,

(4) cis—CH=CH—CH_2—CH_3,

(5) —$(CH_2)_2$—CH(OH)—CH_3, or

(6) —$(CH_2)_3$—CH=$C(CH_3)_2$;

wherein —$C(L_1)$—R_7 taken together is

(1) (C_4-C_7)cycloalkyl optionally substituted by one to 3 (C_1-C_5) alkyl;

(2) 2-(2-furyl)ethyl,

(3) 2-(3-thienyl)ethoxy, or

(4) 3-thienyloxymethyl;

wherein R_8 is hydroxy, hydroxymethyl, or hydrogen;

wherein

(1) R_{20}, R_{21}, R_{22}, R_{23}, and R_{24} are all hydrogen with R_{22} being either α-hydrogen or β-hydrogen,

(2) R_{20} is hydrogen, R_{21} and R_{22} taken together form a second valence bond between C-9 and C-6a, and R_{23} and R_{24} taken together form a second valence bond between C-8 and C-9 or are both hydrogen, or

(3) R_{22}, R_{23}, and R_{24} are all hydrogen, with R_{22} being either α-hydrogen or β-hydrogen, and

(a) R_{20} and R_{21} taken together are oxo, or

(b) R_{20} is hydrogen and R_{21} is hydroxy, being α-hydroxy or β-hydroxy;

wherein X_1 is

(1) —$COOR_1$, wherein R_1 is

(a) hydrogen,

(b) (C_1-C_{12})alkyl,

(c) (C_3-C_{10})cycloalkyl,

(d) (C_6-C_{12})aralkyl,

(e) phenyl, optionally substituted with one, 2 or 3 chloro or (C_1-C_3)alkyl,

(f) phenyl substituted in the para position by

(i) —NH—CO—R_{25},

(ii) —CO—R_{26},

(iii) —O—CO—R_{54}, or

(iv) —CH=N—NH—CO—NH_2 wherein R_{25} is methyl, phenyl, acetamidophenyl, benzamidophenyl, or —NH_2; R_{26} is methyl, phenyl, —NH_2, or methoxy; and R_{54} is phenyl or acetamidophenyl; inclusive, or

(g) a pharmacologically acceptable cation;

(2) —CH_2OH,

(3) —COL_4, wherein L_4 is

(a) amino of the formula —$NR_{51}R_{52}$, wherein R_{51} and R_{52} are

(i) hydrogen,

(ii) (C_1-C_{12})alkyl,

(iii) (C_3-C_{10})cycloalkyl,

(iv) (C_7-C_{12})aralkyl,

(v) phenyl, optionally substituted with one, 2 or 3 chloro, (C_1-C_3)alkyl, hydroxy, carboxy, (C_2-C_5)alkoxycarbonyl, or nitro,

(vi) (C_2-C_5)carboxyalkyl,

(vii) (C_2-C_5)carbamoylalkyl,

(viii) (C_2-C_5)cyanoalkyl,

(ix) (C_3–C_6)acetylalkyl,
(x) (C_7–C_{11})benzoalkyl, optionally substituted by one, 2 or 3 chloro, (C_1–C_3)alkyl, hydroxy, (C_1–C_3)alkoxy, carboxy, (C_2–C_5)alkoxycarbonyl, or nitro,
(xi) pyridyl, optionally substituted by one, 2 or 3chloro, (C_1–C_3)alkyl, or (C_1–C_3)alkoxy,
(xii) (C_6–C_9)pyridylalkyl optionally substituted by one, 2 or 3 chloro, (C_1–C_3)alkyl, hydroxy, or (C_1–C_3)alkyl,
(xiii) (C_1–C_4)hydroxyalkyl,
(xiv) (C_1–C_4)dihydroxyalkyl,
(xv) (C_1–C_4)trihydroxyalkyl,

with the further proviso that not more than one of R_{51} and R_{52} is other than hydrogen or alkyl,

(b) cycloamino selected from the group consisting of pyrolidino, piperidino, morpholino, piperazino, hexamethyleneimino, pyrrolino, or 3,4-didehydropiperidinyl optionally substituted by one or 2 (C_1–C_{12})alkyl of one to 12 carbon atoms, inclusive,
(c) carbonylamino of the formula $—NR_{53}COR_{51}$, wherein $R5_3$ is hydrogen or (C_1–C_4)alkyl and R_{51} is other than hydrogen, but otherwise as defined above,
(d) sulfonylamino of the formula $—NR_{53}SO_2R_{51}$, wherein R_{51} and R_{53} are as defined in (c),
(4) $—CH_2NL_2L_3$, wherein L_2 and L_3 are hydrogen or (C_1–C_4)alkyl, being the same or different, or the pharmacologically acceptable acid addition salts thereof when X_1 is $—CH_2NL_2L_3$,

wherein Y_1 is trans—CH=CH—, cis—CH=CH—, $—CH_2CH_2—$, or —C≡C—; and
wherein Z_4 is $—CH_2—$ or $—(CH_2)_f—CF_2$, wherein f is zero, one, 2, or 3.

2. 9-Deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1, methyl ester, a compound according to claim **1**.

3. 9-Deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1, a compound according to claim **1**.

4. 9-Deoxy-16,16-difluoro-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 or its methyl ester, a compound according to claim **1**.

5. 9-Deoxy-13,14-dihydro-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 or its methyl ester, a compound according to claim **1**.

6. 9-Deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1, amide, a compound according to claim **1**.

7. (15(R)-9-Deoxy-2′,9α-methano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 or its methyl ester, a compound according to claim **1**.

8. 9-Deoxo-2′,9-metheno-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGE_1 or its methyl ester, a compound according to claim **1**.

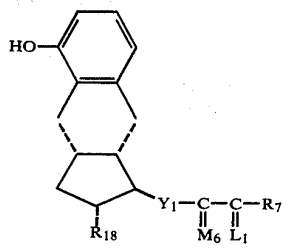
9. 9-Deoxo-7,8-dihydro-2′,9-metheno-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGE_1 or its methyl ester, a compound according to claim **1**.

10. 9-Deoxy-2′,9-hydroxymethano-3-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 or its methyl ester, a compound according to claim **1**.

11. 9-Deoxy-2′,9α-carbonyl-oxa-4,5,6-trinor-3,7-(1′,3′-inter-phenylene)-PGF_1 or its methyl ester, a compound according to claim **1**.

12. A compound according to formula IX:

IX



wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro;
wherein M_6 is α-OR_{10}:β-R_5 or α-R_5:β-OR_{10}, wherein R_5 is hydrogen or methyl and R_{10} is an acid hydrolyzable protective group;
wherein R_{27} is

(1) $—C_mH_{2m}—CH_3$, wherein m is an integer from one to 5, inclusive,
(2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1–C_3)alkyl, or (C_1–C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_{27} is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different,
(3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1–C_3)alkyl, or (C_1–C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl,
(4) cis—CH=CH—CH_2—CH_3,
(5) $—(CH_2)_2—CH(OR_{10})—CH_3$, wherein R_{10} is as defined above, or
(6) $—(CH_2)_3—CH=C(CH_3)_2$;

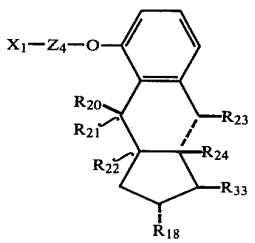
wherein $—C(L_1)—R_{27}$ taken together is

(1) (C_4–C_7)cycloalkyl optionally substituted by one to 3 (C_1–C_5) alkyl;
(2) 2-(2-furyl)ethyl,
(3) 2-(3-thienyl)ethoxy, or
(4) 3-thienyloxymethyl;

wherein R_{18} is hydrogen, hydroxy, hydroxymethyl, $—OR_{10}$ or $—CH_2OR_{10}$, wherein R_{10} is an acid-hydrolyzable protective group; and wherein Y_1 is trans—CH=CH—, cis—CH=CH—, $—CH_2CH_2—$, or —C≡C—.

13. A compound according to formula VIII:

VIII



wherein R_{18} is hydrogen, hydroxy, hydroxymethyl, $-OR_{10}$ or $-CH_2OR_{10}$, wherein R_{10} is an acid-hydrolyzable protective group;

wherein

(1) R_{20}, R_{21}, R_{22}, R_{23}, and R_{24} are all hydrogen with R_{22} being either α-hydrogen or β-hydrogen,

(2) R_{20} is hydrogen, R_{21} and R_{22} taken together form a second valence bond between C-9 and C-6a, and R_{23} and R_{24} taken together form a second valence bond between C-8 and C-9 or are both hydrogen, or

(3) R_{22}, R_{23}, and R_{24} are all hydrogen, with R_{22} being either α-hydrogen or β-hydrogen, and

(a) R_{20} and R_{21} taken together are oxo, or

(b) R_{20} is hydrogen and R_{21} is hydroxy, being α-hydroxy or β-hydroxy;

wherein R_{33} is $-CHO$ or $-CH_2OR_{32}$, wherein R_{32} is hydrogen or a hydroxyl hydrogen replacing group;

wherein X_1 is

(1) $-COOR_1$, wherein R_1 is

(a) hydrogen,

(b) (C_1-C_{12})alkyl,

(c) (C_3-C_{10})cycloalkyl,

(d) (C_7-C_{12})aralkyl,

(e) phenyl, optionally substituted with one, 2 or 3 chloro or (C_1-C_3)alkyl,

(f) phenyl substituted in the para position by

(i) $-NH-CO-R_{25}$,

(ii) $-CO-R_{26}$,

(iii) $-O-CO-R_{54}$, or

(iv) $-CH=N-NH-CO-NH_2$ wherein R_{25} is methyl, phenyl, acetamidophenyl, benzamidophenyl, or $-NH_2$; R_{26} is methyl, phenyl, $-NH_2$, or methoxy; and R_{54} is phenyl or acetamidophenyl; inclusive, or

(g) a pharmacologically acceptable cation;

(2) $-CH_2OH$,

(3) $-COL_4$, wherein L_4 is

(a) amino of the formula $-NR_{51}R_{52}$, wherein R_{51} and R_{52} are

(i) hydrogen,

(ii) (C_1-C_{12})alkyl,

(iii) (C_3-C_{10})cycloalkyl,

(iv) (C_7-C_{12})aralkyl,

(v) phenyl, optionally substituted with one, 2 or 3 chloro, (C_1-C_3)alkyl, hydroxy, carboxy, (C_2-C_5)alkoxycarbonyl, or nitro,

(vi) (C_2-C_5)carboxyalkyl,

(vii) (C_2-C_5)carbamoylalkyl,

(viii) (C_2-C_5)cyanoalkyl,

(ix) (C_3-C_6)acetylalkyl,

(x) (C_7-C_{11})benzoalkyl, optionally substituted by one, 2 or 3 chloro, (C_1-C_3)alkyl, hydroxy, (C_1-C_3)alkoxy, carboxy, (C_2-C_5)alkoxycarbonyl, or nitro,

(xi) pyridyl, optionally substituted by one, 2 or 3 chloro, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy,

(xii) (C_6-C_9)pyridylalkyl optionally substituted by one, 2 or 3 chloro, (C_1-C_3)alkyl, hydroxy, or (C_1-C_3)alkyl,

(xiii) (C_1-C_4)hydroxyalkyl,

(xiv) (C_1-C_4)dihydroxyalkyl,

(xv) (C_1-C_4)trihydroalkyl,

with the further proviso that not more than one of R_{51} and R_{52} is other than hydrogen or alkyl,

(b) cycloamino selected from the group consisting of pyrolidino, piperidino, morpholino, piperazino, hexamethyleneimino, pyrrolino, or 3,4-didehydropiperidinyl optionally substituted by one or 2 (C_1-C_{12})alkyl of one to 12 carbon atoms, inclusive,

(c) carbonylamino of the formula $-NR_{53}COR_{51}$, wherein R_{53} is hydrogen or (C_1-C_4)alkyl and R_{51} is other than hydrogen, but otherwise as defined above,

(d) sulfonylamino of the formula $-NR_{53}SO_2R_{51}$, wherein R_{51} and R_{53} are as defined in (c),

(4) $-CH_2NL_2L_3$, wherein L_2 and L_3 are hydrogen or (C_1-C_4)alkyl, being the same or different, or the pharmacologically acceptable acid addition salts thereof when X_1 is $-CH_2NL_2L_3$; and

wherein Z_4 is $-CH_2-$ or $-(CH_2)_f-CF_2$, wherein f is zero, one, 2, or 3.

* * * * *

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 4,306,075 Dated December 15, 1981

Inventor(s) Paul A. Aristoff

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 3, line 63, "R_{47}" should read -- R_{17} --.

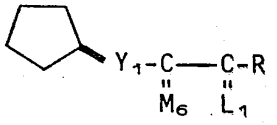
Column 33, line 11, "acetone:acetone" should read -- acetate: acetone --.

Column 33, line 27, "-dimethylphosphonom ethyl-5-keto-PFE_1" should read -- -dimethylphosphonomethyl-5-keto-PGE_1 --.

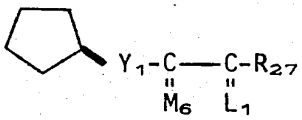
Column 36, line 55, "$-CH_3OH$" should read -- $-CH_2OH$ --.

Column 49, line 67, "$-(CH_2)_{32}$-"should read -- $-(CH_2)_3-$ --.

Column 78, lines 30-37, that portion of Formula LV reading



should read

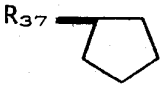


Column 81, Chart G, the fourth structural formula now labeled "LXXIV" should read -- LXXXIV --.

Column 81, Chart G, following the fourth structural formula, the instructions "To LXXXVIII and LXXXIV From LXXXIV" should read -- To LXXXVIII and LXXXIX From LXXXIV --.

Column 83, Chart G, that portion of Formula LXXXVII reading

should read



Column 83, Chart G, the fourth structural formula now labeled "LXXIX" should read -- LXXXIX --.

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 4,306,075 Dated December 15, 1981

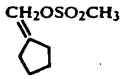
Inventor(s) Paul A. Aristoff

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

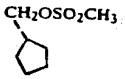
Column 90, Chart P, that portion of Formula CLXXIII reading

should read

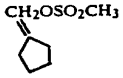
Column 90, Chart P, that portion of Formula CLXXIV reading



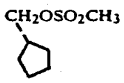
should read



Column 90, Chart P, that portion of Formula CLXXV reading

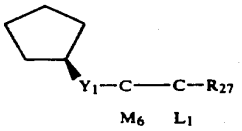


should read

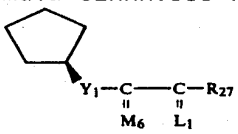


Column 90, Chart P, the fifth structural formula now labeled "CLXXVI" should read -- CLXXVII --.

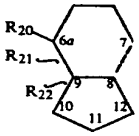
Column 91, Chart Q, that portion of Formula CLXXXVIII reading



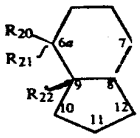
should read



Column 95, Claim 1, lines 47-57, that portion of Formula XI now reading



should read



UNITED STATES PATENT OFFICE

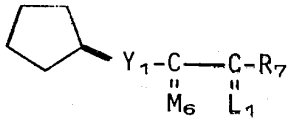
CERTIFICATE OF CORRECTION

Patent No. 4,306,075 Dated December 15, 1981

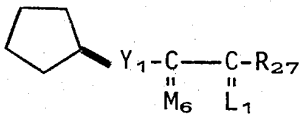
Inventor(s) Paul A. Aristoff

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 96, line 42, "(C_6-C_{12})" should read -- (C_7-C_{12}) --.
Column 97, line 59, "-dihydro-" should read -- -didehydro- --.
Column 98, lines 1-13, that portion of Formula IX reading



should read



Column 100, line 21,"(C_1-C_4)trihydroalkyl" should read -- (C_1-C_4)trihydroxyalkyl --.

Signed and Sealed this

Seventeenth Day of May 1983

[SEAL]

Attest:

GERALD J. MOSSINGHOFF

Attesting Officer

Commissioner of Patents and Trademarks

US005153222A

United States Patent [19]

Tadepalli et al.

[11] Patent Number: 5,153,222

[45] Date of Patent: Oct. 6, 1992

[54] METHOD OF TREATING PULMONARY HYPERTENSION WITH BENZIDINE PROSTAGLANDINS

[75] Inventors: **Anjaneyulu S. Tadepalli**, Durham; **Walker A. Long**, Chapel Hill; **James W. Crow**, Raleigh; **Kenneth B. Klein**, Chapel Hill, all of N.C.

[73] Assignee: **Burroughs Wellcome Co.**, Research Triangle Park, N.C.

[21] Appl. No.: **715,439**

[22] Filed: **Jun. 14, 1991**

Related U.S. Application Data

[62] Division of Ser. No. 367,090, Jun. 16, 1989, abandoned.

[30] **Foreign Application Priority Data**

Jun. 17, 1988 [GB] United Kingdom 8814438

[51] **Int. Cl.**[5] **A61K 31/19**

[52] **U.S. Cl.** **514/571**

[58] **Field of Search** 524/454, 569; 514/571

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,028,628	7/1991	Tadepalli	514/573
4,306,075	12/1981	Aristoff	560/56
4,499,085	2/1985	Masuda	514/58

FOREIGN PATENT DOCUMENTS

0005768A1	12/1979	European Pat. Off. .
0217419A2	4/1987	European Pat. Off. .
0229844A1	7/1987	European Pat. Off. .
0347243A1	12/1989	European Pat. Off. .

OTHER PUBLICATIONS

Aristoff, et al., J. Amer. Chem. Soc., vol. 107, No. 26, 1985, p. 7968, Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction.

Abstract Supplement—R. J. Lambert, et al., Chest. 89, p. 459S, Jun. (1986).

Abstract—New England Journal of Medicine, vol. 312, No. 14, pp. 932-936 (1985).

Praveen Tyle, Review, Pharmaceutical Research, vol. 3, No. 6, 1986, Iontophoretic Devices for Drug Delivery, pp. 318-326.

Whittle, et al., Progress in Medicinal Chemistry, vol. 21, pp. 235-279, 1984, 6 Antithrombotic Assessment and Clinical Protential of Protential of Prostacyclin Analogues.

Rubin, et al., The American Heart Association, Circulation, vol. 66, No. 2, Aug., 1982, Prostacyclin-induced Acute Pulmonary Vasodilation in Primary Pulmonary Hypertension, pp. 334-338.

Whittle, et. al., The American Heart Association, Circulation, vol. 72, No. 6, Dec., 1985, Platelet Actions of Stable Carbocyclic Analogues of Prostacyclin, pp. 1219-1225.

Long, et al., Am. Rev. Respir. Cns, 1987, 136, pp. 773-776, Prostacyclin and Pge, Treatment of Pulmonary Hypertension.

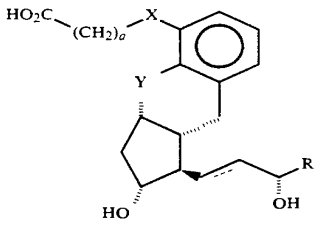
Grossman, et al., Pulmonary Hypertension, 24, The Normal Pulmonary Circulation, pp. 835-851, 1981.

Aristoff et al., Advances in Prostaglandin, Thranboxane, and Leukotriene Research, vol. 15, pp. 275-277, 1985.

Primary Examiner—Frederick E. Waddell
Assistant Examiner—Kimberly R. Jordan
Attorney, Agent, or Firm—Donald Brown; Lawrence A. Nielsen

[57] **ABSTRACT**

The present invention is concerned with methods for the prophylaxis, treatment and diagnosis of pulmonary hypertension which comprise hte administrative of an effective amount of a compound of formula (I)



(I)

wherein a is an integer of from 1 to 3;
X and Y, which may be the same or different, are selected from —O— and —CH_2—;
R is —$(CH_2)_5R^1$ wherein R^1 is hydrogen or methyl, or R is cyclohexyl, or
R is —$CH(CH_3)CH_2C{\equiv}CCH_3$; and
the dotted line represents an optional double bond;
or of a physiologically acceptable salt or acid derivative thereof.

Medicaments and diagnostic aids for use in the methods of the invention are also within the scope of the invention.

2 Claims, No Drawings

METHOD OF TREATING PULMONARY HYPERTENSION WITH BENZIDINE PROSTAGLANDINS

This is a divisional of copending application(s) Ser. No. 07/367,090 filed on Jun. 16, 1989, now abandoned.

The present invention is concerned with prostaglandins, specifically benzindene prostaglandins, for use in the treatment, or diagnosis of pulmonary hypertension. Their use in the manufacture of medicaments for the treatment of pulmonary hypertension and in the manufacture of diagnostic aids for identifying PPH patients having active pulmonary vasoconstriction and the medicaments and diagnostic aids obtained thereby are within the scope of the invention.

All blood is driven through the lungs via the pulmonary circulation in order, among other things, to replenish the oxygen which it dispenses in its passage around the rest of the body via the systemic circulation. The flow through both circulations is in normal circumstances equal, but the resistance offered to it in the pulmonary circulation is generally much less than that of the systemic circulation. When the resistance to pulmonary blood flow increases, the pressure in the circulation is greater for any particular flow. This is referred to as pulmonary hypertension. Generally, pulmonary hypertension is defined through observations of pressures above the normal range pertaining in the majority of people residing at the same altitude and engaged in similar activities.

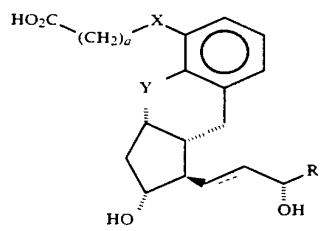
Most often pulmonary hypertension is a manifestation of an obvious or explicable increase in resistance, such as obstruction to blood flow by pulmonary emboli, malfunction of the heart's valves or muscle in handling blood after its passage through the lungs, diminution in pulmonary vessel calibre as a reflex response to hypoventilation and low oxygenation, or a mismatch of vascular capacity and essential blood flow, such as shunting of blood in congenital abnormalities or surgical removal of lung tissue. Such pulmonary hypertension is referred to as secondary hypertension.

There remain some cases of pulmonary hypertension where the cause of the increased resistance is as yet inexplicable. They are described as primary pulmonary hypertension (PPH) and are diagnosed by and after exclusion of the causes of secondary pulmonary hypertension. Despite the possibility of a varied aetiology, cases of primary pulmonary hyptertension tend to comprise a recognisable entity. Approximately 65% are female and yound adults are most commonly afflicted, though it has occurred in children and patients over 50. Life expectancy from the time of diagnosis is short, about 3 to 5 years, though occasional reports of spontaneous remission and longer survival are to be expected given the nature of the diagnostic process. Generally, however, progress is inexorable via syncope and right heart failure and death is quite often sudden. Until now, no successful treatment was known.

U.S. Pat. No. 4,306,075 describes novel benzindene prostaglandins which produce various pharmacological responses, such as inhibition of platelet aggregation, reduction of gastric secretion, and bronchodilation. It is indicated that the compounds have useful application as anti-thrombotic agents, anti-ulcer agents, and anti-asthma agents. There is no indication that these compounds may be used in the treatment of any form of hypertension.

We have now discovered that within the class of benzindene prostaglandins described in the U.S. Patent, there is a sub-class of compounds of formula (I) as defined hereinbefore which are suitable for use in the treatment of pulmonary hypertension. The term "pulmonary hypertension" is used herein to include both primary and secondary pulmonary hypertension as ordinarily understood by clinicians (vide supra). The compounds of the invention may also be used in the treatment of Raynaud's disease. PPH patients having active pulmonary vasoconstriction are considered suitable candidates for long-term oral vasodilator therapy (R J Lambert et al, Chest 89, 459S (1986)). The ability of the compounds of the invention to reduce pulmonary vascular resistance in such patients provides a useful diagnostic aid for identifying suitable candidates for long-term vasodilator therapy.

According to the present invention, therefore, there is provided a compound of formula (I)



(I)

for use in the treatment, or diagnosis of pulmonary hypertension

wherein a is an integer of from 1 to 3;

X and Y, which may be the same or different, are selected from —O— and —CH_2—;

R is —$(CH_2)_5R^1$ wherein R^1 is hydrogen or methyl, or R is cyclohexyl, or

R is —$CH(CH_3)CH_2C{\equiv}CCH_3$; and

the dotted line represents an optional double bond;

and pharmaceutically acceptable salts and acid derivatives thereof.

The term "acid derivative" is used herein to describe C_{1-4} alkyl esters and amides, including amides wherein the nitrogen is optionally substituted by one or two C_{1-4} alkyl groups.

The invention also includes bioprecursors or "prodrugs" of the above-defined compounds, that is, compounds which are converted in vivo to compounds of formula (I) or pharmaceutically active derivatives thereof.

Further aspects of the present invention are concerned with the use of a compound of formula (I), or a pharmaceutically acceptable salt or acid derivative thereof, in the manufacture of a medicament for the treatment of pulmonary hypertension or in the manufacture of a diagnostic aid for identifying PPH patients having active pulmonary vasoconstriction and with medicaments and diagnostic aids obtained thereby which may be administered when primary or secondary pulmonary hypertension is indicated.

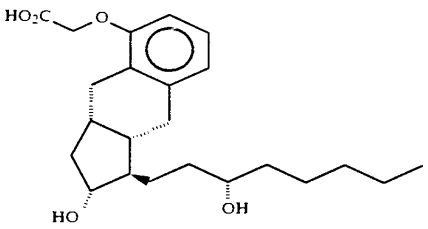
Preferred compounds of formula (I) having particularly desirable pulmonary anti-hypertensive properties include those wherein

X is —O—;

Y is —CH_2—; and

R is —$(CH_2)_4CH_3$.

A particularly preferred compound of formula (I) having exceptional pulmonary anti-hypertensive properties is 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-13,14-dihydro-prostaglandin F_1, which has the following structure:



and pharmaceutically acceptable salts and acid derivatives thereof.

Other compounds of the invention which show pulmonary anti-hypertensive activity include:

9-Deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-prostaglandin F_1

9-Deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-15-cyclohexylprostaglandin F_1

9-Deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-20-methylprostaglandin F_1

(15S,16RS)-9-Deoxy-2',9α-methano-16-methyl-3-oxa-18,18,19,19-tetradehydro-4,5,6-trinor-3,7-(1',3'-interphenylene)prostaglandin F_1

The present invention extends to non-physiologically acceptable salts of the compounds of formula (I) which may be used in the preparation of the pharmacologically active compounds of the invention. The physiologically acceptable salts of compounds of formula (I) include salts derived from bases. Base salts include ammonium salts, alkali metal salts such as those of sodium and potassium, alkaline earth metal salts such as those of calcium and magnesium, salts with organic bases such as dicyclohexylamine and N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine.

Quaternary ammonium salts can be formed, for example, by reaction with lower alkyl halides, such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides, with dialkyl sulphates, with long chain halides, such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides, and with aralkyl halides, such as benzyl and phenethyl bromides.

The amount of a compound of formula (I), or a physiologically acceptable salt or acid derivative thereof, which is required in a medication or diagnostic aid according to the invention to achieve the desired effect will depend on a number of factors, in particular the specific application, the nature of the particular compound used, the mode of administration, and the condition of the patient. In general, a daily dose per patient for the treatment of pulmonary hypertension is in the range 25 μg to 250 mg; typically from 0.5 μg to 2.5 mg, preferably from 7 μg to 285 μg, per day per kilogram bodyweight. For example, an intravenous dose in the range 0.5 μg to 1.5 mg per kilogram bodyweight per day may conveniently be administered as an infusion of from 0.5 ng to 1.0 μg per kilogram bodyweight per minute. Infusion fluids suitable for this purpose contain, for example, from 10 ng to 10 μg per milliliter. Ampoules for injection contain, for example, from 0.1 μg to 1.0 mg and orally administrable unit dose formulations, such as tablets or capsules, contain, for example, from 0.1 to 100 mg, typically from 1 to 50 mg. For diagnostic purposes, a single unit dose formulation may be administered. In the case of physiologically acceptable salts, the weights indicated above refer to the weight of the active compound ion, that is, the ion derived from the compound of formula (I).

In the manufacture of a medicament or diagnostic aid according to the invention, hereinafter referred to as a "formulation", the compounds of formula (I) and their physiologically acceptable salts and acid derivatives are typically admixed with, inter alia, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a tablet, which may contain from 0.05% to 95% by weight of the active compound. One or more compounds of formula (I) and/or their physiologically acceptable salts or acid derivatives may be incorporated in the formulations of the invention, which may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components.

In addition to compounds of formula (I), other pharmacologically active substances may be present in the formulations of the present invention. For example, the compounds of the invention may be present in combination with tissue plasminogen activator, a substance known to dissolve the fibrin network of blood clots which has found utility in the treatment of thrombotic disorders (see, for example, The New England Journal of Medicine, 312(14), 932, (1985)).

The formulations of the invention include those suitable for oral, rectal, topical, buccal (e.g. sub-lingual), parenteral (e.g. subcutaneous, intramuscular, intradermal, or intravenous) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular compound of formula (I), or the physiologically acceptable salt or acid derivative thereof, which is being used.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of a compound of formula (I) or a physiologically acceptable salt or acid derivative thereof; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Moulded tablets may be

made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sub-ligual) administration include lozenges comprising a compound of formula (I), or a physiologically acceptable salt or acid derivative thereof, in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of a compound of formula (I), or a physiologically acceptable salt or acid derivative thereof, which preparations are preferably isotonic with the blood of the intended recipient. These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood. Injectable formulations according to the invention generally contain from 0.1 to 5% w/v of active compound and are administered at a rate of 0.1 ml/min/kg.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing a compound of formula (I), or a physiologically acceptable salt or acid derivative thereof, with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include vaseline, lanoline, polyethylene glycols, alcohols, and combinations of two or more thereof. The active compound is generally present at a concentration of from 0.1 to 15% w/w, for example, from 0.5 to 2% w/w. Formulations for transdermal administration may be delivered by iontophoresis (see, for example, Pharmaceutical Research 3(6), 318, (1986)) and typically take the form of an optionally buffered aqueous solution of a compound of formula (I) or of a salt or acid derivative thereof. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

The compounds of the present invention are conveniently prepared by methods the same as or analogous to those described in U.S. Pat. No. 4,306,075.

For a better understanding of the invention, the following Examples are given by way of illustration.

EXAMPLES

The effects of 9-deoxy-2', 9α-methano-3-oxa-4,5-6-trinor-3,7-(1',3'-interphenylene)-13,14-dihydro-prostaglandin F_1 monitored in experimental pulmonary hypertension models. In Example 1, the model used was an open chest preparation of an anaesthesised cat (anaesthetic: chloralose and urethane). In Example 2, the model was a conscious spontaneously hypertensive rat.

EXAMPLE 1

A series of glycine buffer solutions of the test compound were successively administered to each animal by i.v. infusion at doses equivalent to 100 ng, 300 ng, 1 μg, and 3 μg/kg/min. Each solution was infused over a period of 20 minutes, hypoxia being induced in the animal during the last 5 minutes of infusion by ventilating with 10% oxygen in nitrogen. A 15-minute 'recovery' period was observed between successive infusions. Following surgery, the animal was allowed to stabilize for 30 minutes, after which two 5-minute hypoxic challenges were given 15 minutes apart which were averaged to obtain the control hypoxic responses. 15 minutes after the second control hypoxic challenge, the animal started to receive the test compound. The averaged control hypoxic responses were compared with those obtained during infusion of the test compound.

The following parameters were monitored during the course of each experiment: systemic arterial pressure (MAP), pulmonary arterial (PAP) and venous (PVP) pressure, and cardiac output (CO, aortic blood flow). From the values obtained, the systemic vascular resistance (MAP/CI where CI=CO/body weight in kg) and the pulmonary vascular resistance (PAP/CI) were calculated.

The test compound was found to reduce hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance in a dose-related manner without appreciably affecting cardiac output or heart rate. At higher doses, the test compound reduced systemic arterial pressure and systemic vascular resistance. Thus hypoxia-induced pulmonary vasoconstriction could be reduced without disturbing the systemic haemodynamics by suitably adjusting the dose. The hypoxia-induced vasoconstriction did not return to its control value within 15 minutes of terminating the final infusion indicating a relatively long duration of action for the compound.

EXAMPLE 2

The test compound was administered to a series of animal at doses of 0.1, 0.3, 1.0 and 3.0 mg/kg P.O. and the systolic and diastolic pressures and heart rate of each animal were monitored for 24 hours after administration of the compound. At doses of 0.3 mg/kg P.O. and above, a dose-dependent fall in systolic and diastolic pressures were observed for a period of up to 8 hours after administration indicating that the compound had good oral bioavailability.

What is claimed:

1. A method of treating pulmonary hypertension in a patient, which comprises administering to said patient an effective pulmonary hypertension treatment amount of the compound 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-13,14-dihydro-prostaglandin F_1.

2. A method of treating pulmonary hypertension in a patient, which comprises administering to said patient an effective pulmonary hypertension treatment amount of a pharmaceutically acceptable salt of the compound 9-deoxy-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-13,14-dihydroprostaglandin F_1.

* * * * *

Q7 Implementation Working Group

ICH Q7 Guideline: Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients

Questions and Answers

Current version

dated 10 June 2015

In order to facilitate the implementation of the Q7 Guidelines, the ICH Experts have developed a series of Q&As:

Q7 Q&As
Document History

Code	History	Date
Q7 Q&As	Approval by the ICH Steering Committee under *Step 4*	10 June 2015

References

These documents are published at www.ich.org.

ICH E2E	Pharmacovigilance Planning	November 2004
ICH Q1A(R2)	Stability testing of new drug substance and products February 2003	
ICH Q5A	Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin	September 1999
ICH Q5B	Quality of biotechnological products: Analysis of the construct in cells used for the production of r-DNA derived protein products	November 2005
ICH Q5D	Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products	July 1997
ICH Q6B	Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products	March 1999
ICH Q7	Good Manufacturing Practice of APIs	November 2000
ICH Q8(R2)	Pharmaceutical Development	August 2009
	Part I: 'Pharmaceutical Development'	November 2006
	Part II: 'Annex to Pharmaceutical Development',	November 2008
ICH Q9	Quality Risk Management and the ICH Q9 Briefing pack	November 2005
ICH Q10	Pharmaceutical Quality Systems	June 2008
ICH Q-IWG	Training Programme for ICH Q8/Q9/Q10	November 2010
ICH Q11	Development and Manufacturing of Active Pharmaceutical Ingredients	May 2012

Table of Contents

PREFACE

Since the ICH Q7 Guidance was finalised, experience with implementing the guidance worldwide has given rise to requests for clarification of uncertainties due to the interpretation of certain sections. This Question and Answer (Q&A) document is intended to respond to those requests.

The ICH Q7 document should be read in its entirety regardless of the nature of the manufacturing activities being conducted to fully understand the linkages between certain sections and successfully implement appropriate Good Manufacturing practices (GMPs) at all stages of the Active Pharmaceutical Ingredients (API) supply chain, including distribution. A table is provided as an Annex of this document showing the link between each Q&A and the relevant Sections of ICH Q7 and other ICH Quality guidance.

ICH would like to acknowledge the work undertaken by the Pharmaceutical Inspection Co-operation Scheme (PIC/S). PIC/S contributed to this document by selecting and reviewing relevant Q&As that had been collected from training sessions since the implementation of Q7 and transferred the output of these reviews to the ICH Q7 IWG for consideration and consolidation, as appropriate. Additional questions were developed based on responses from an ICH survey. PIC/S further contributed to the development of the document as an ICH Interested Party.

Please note that ICH Q7 should be applied in combination with the principles laid down for development and manufacturing in ICH Q11 (see definition of API starting material; see also ICH Q8(R2) Part II), Quality Risk Management (ICH Q9), and Pharmaceutical Quality Systems (ICH Q10). GMP principles as described in ICH Q7 should be applied regardless which approach is taken in pharmaceutical development and manufacturing.

ICH Q7 also describes principles of GMPs to be applied in the manufacture of APIs for use in clinical trials (Section 19) and for APIs manufactured by cell culture/fermentation (Section 18).

Q7 Questions and Answers

1. INTRODUCTION - SCOPE

#	Date of Approval	Questions	Answers
1.1	**June 2015**	Should GMP according to ICH Q7 be applied for manufacturing Steps before the defined 'API starting material' i.e., Steps not identified in grey in Table 1?	ICH Q7 does not apply to Steps prior to the introduction of the API starting material. However, there is an expectation that an appropriate level of controls suitable for the production of the API starting material should be applied [ICH Q7, Section 1.3]. Normally, the 'API-starting material' is defined in the regulatory filing by the applicant and approved in the regulatory reviewing process. Additional guidance is provided to define and justify 'API starting material' derived from various sources [ICH Q11, Section 5]; for master cell banks, see [ICH Q5B; ICH Q5D].
1.2	**June 2015**	Does ICH Q7 apply to manufacturing Steps for the addition of substance(s) to an API (e.g., to stabilise the API)?	When a mixture is classified in the regulatory filing as an API in a region or country in which it is used in a drug product, ICH Q7 should be applied to the manufacturing of these mixtures [ICH Q7, Section 1.2, 20 – see Glossary for definition of 'API'].

2. QUALITY MANAGEMENT

#	Date of Approval	Questions	Answers
2.1	**June 2015**	What is meant by 'quality unit(s) independent from production'?	The intent of the term 'independent' is to prevent any conflict of interest and ensure unbiased decision-making regarding quality-related decisions in the organisation structure. The person in the quality unit who is responsible for final decision-making (e.g., batch release decision) should not have responsibilities for production activities [ICH Q7, Section 2.13].
2.2	**June 2015**	Does ICH Q7 expect that the quality unit performs API release testing?	While the quality unit has responsibility for the release of the API, which includes oversight of the testing and results, ICH Q7 does not prescribe specifically who performs testing. 'quality control' in the ICH Q7 Glossary [ICH Q7, Section 20] refers to the activities, not the organisational structure. For examples of quality responsibility related to testing and release, refer to [ICH Q7, Sections 2.13, 2.22, and 11.12]. Appropriate laboratory controls should be followed [ICH Q7, Sections 11.10, 16.10] regardless of who performs the testing.
2.3	**June 2015**	Can other departments outside of the quality unit be held responsible for	Yes. The quality unit is responsible for establishing a system to release or reject raw materials, intermediates, packaging, and labelling materials. This responsibility cannot be delegated [ICH Q7,

		releasing raw materials and intermediates?	Section 2.22(2)]. The system established by the quality unit may allow 'other departments' to release raw materials and intermediates (except intermediates that are for use outside the control of the manufacturer [ICH Q7, Section 2.22(1)] as long as oversight and the overall responsibility of this system remains with the quality unit.
2.4	**June 2015**	Does ICH Q7 expect that sampling be performed by the quality unit?	No. ICH Q7 does not prescribe specifically who should perform the sampling [ICH Q7, Section 2.22]. However, the quality unit has responsibility for reviewing and approving sampling plans [ICH Q7, Section 11.12] and procedures. Sampling should be performed by adequately trained personnel [ICH Q7, Section 3.10] and be appropriately documented as per [ICH Q7, Section 6.52].
2.5	**June 2015**	What should be the frequency of a product quality review?	A product quality review is generally expected annually. Review timeframes can be appropriately adjusted based upon manufacturing and campaign duration with adequate justification. Even if no manufacturing has occurred in the review period, the quality review should be conducted as per section [ICH Q7, Section 2.50] and include stability, returns, complaints, and recalls. For example, a product quality review may encompass more or less than 12 months depending upon product campaign duration [ICH Q7, Section 2.50; ICH Q10, Section 2.6].
2.6	**June 2015**	Should the product quality review of results include trend analysis?	Trend analysis is usually an important element in verifying the consistency of the process as part of the product quality review [ICH Q7, Sections 2.50, 2.51]. Potential tools to use are described in [ICH Q9, Annex I.9].

3. PERSONNEL

#	Date of Approval	Questions	Answers
3.1	**June 2015**	What is the intent of the statement in [ICH Q7, Section 3.12] 'training should be periodically assessed'?	In [ICH Q7, Section 3.12], the statement 'training should be periodically assessed' refers to a system to evaluate if personnel remain proficient and competent in their job tasks and responsibilities, and whether more frequent, additional, or new training is needed and recurring training is up to date.
3.2	**June 2015**	Does ICH Q7 expect the use of a consultant and can a company delegate tasks and/or responsibility to a consultant?	ICH Q7 does not expect the use of a consultant. Consultants may perform delegated tasks and/or provide advice. However, the ultimate responsibility for API quality must not be delegated [ICH Q10, Section 2.7, ICH Q7, Sections 2.2, 3.3].

4. BUILDINGS AND FACILITIES – CONTAINMENT

#	Date of Approval	Questions	Answers
4.1	June 2015	When are dedicated production areas expected?	ICH Q7 expects dedicated production areas for highly sensitising materials such as penicillins and cephalosporins because of the patient risk (e.g., anaphylactic shock to penicillin-allergic patients) from trace amounts of these compounds in other medicines [ICH Q7, Section 4.40]. For materials of an infectious nature or high pharmacological activity or toxicity, a risk-based approach should be used to determine appropriate containment measures, which may include validated inactivation, cleaning and/or dedicated production areas [ICH Q7, Section 4.41]. While ICH Q7 does not define high pharmacological activity or toxicity, these are generally determined by evaluating relevant animal and human data collected during research and development. Important considerations in this evaluation of pharmacological activity or toxicity may include Occupational Exposure Limit (OEL), Permitted Daily Exposure (PDE), Acceptable Daily Exposure (ADE), Threshold for Toxicological Concerns (TTC), No Observed Adverse Effect Level (NOAEL) [ICH S Guidelines, ICH E2E, Section 2.1.1], and the consequences of cross-contamination [ICH Q9, Section 4.3].
4.2	June 2015	To what extent can quality risk management be used in establishing appropriate containment measures to prevent cross-contamination?	The principles of quality risk management [ICH Q9, Annex II.4] should be applied to the design of buildings, facilities and controls for the purpose of containment, taking into consideration the pharmacological/toxicological/chemical/biological properties of the raw material, intermediate and/or API to be handled or manufactured. Appropriate containment measures and controls [ICH Q7, Section 4.42] include but are not limited to the following: • Technical controls (e.g., dedicated production areas, closed/dedicated Heating Ventilation and Air Conditioning (HVAC) system, closed manufacturing systems, use of disposable technologies, design of facility and equipment for containment and ease of cleaning); and • Procedural (organisational) controls (e.g., cleaning, personnel flow, environmental monitoring and training). Monitoring systems are important to check the effectiveness of the containment controls.

5. PROCESS EQUIPMENT – CLEANING

#	Date of Approval	Questions	Answers
5.1	**June 2015**	For dedicated equipment, is 'visually clean' acceptable for verification of cleaning effectiveness, (i.e., no expectation for specific analytical determination)?	'Visually clean' may be acceptable for dedicated equipment based on the ability to visually inspect and sufficient supporting data from cleaning studies (e.g., analytical determination to demonstrate cleaning effectiveness) [ICH Q7, Section 12.76]. Equipment should be cleaned at appropriate intervals (e.g., time or number of batches) to prevent build-up and carryover of contaminants (e.g., degradants or objectionable levels of microorganisms) so that they do not adversely alter the quality of the API [ICH Q7, Sections 5.23, 12.7].
5.2	**June 2015**	Should acceptance criteria for residues be defined for dedicated equipment?	Yes. Regardless of whether equipment is dedicated or not, it is expected that acceptance criteria for residues be defined and that the equipment be cleaned at appropriate intervals to prevent build-up and carry-over of contaminants. Intervals can be based on number of batches, product change-over, time, etc. [ICH Q7, Sections 5.22, 5.23, 5.24, 5.25, 8.50]. Cleaning intervals and acceptance criteria should be established based on an understanding of the process/reactions/degradation, taking into account solubility, potency, toxicity, etc. Establishment of acceptance criteria does not necessarily imply sampling and testing after every cleaning. Visual inspection of equipment for cleanliness is an expectation of [ICH Q7, Section 5.21]. Where validation data has confirmed effective cleaning, cleaning procedures should be monitored at appropriate intervals [ICH Q7, Section 12.76].
5.3	**June 2015**	Is it expected that equipment cleaning time limits be confirmed in cleaning validation?	Yes. Equipment cleaning is addressed in two sections in ICH Q7. While the cleaning validation [ICH Q7, Section 12.7] does not specifically address time limits for cleaning, [ICH Q7, Section 5.21] indicates that the maximum time between completion of processing and equipment cleaning (dirty hold time) should be established by the company. This maximum established dirty hold time is the time period for which evidence is available to demonstrate that the equipment can still be reliably cleaned. This maximum established dirty hold time is confirmed during the initial cleaning validation and can be extended with appropriate supporting data. While ICH Q7 does not specify the need for time limits between equipment cleaning and use in the next process (clean hold time), [ICH Q7, Section 5.21] does state that written procedures should include instructions for the protection of clean equipment from contamination prior to use and inspection of equipment for cleanliness immediately before use, if practicable.
5.4	**June 2015**	Is it expected that campaign manufacturing be addressed in cleaning validation?	Yes. The cleaning validation section [ICH Q7, Section 12.7] does not specifically address campaign manufacture. However, sections [ICH Q7, Sections 5.23, 8.50] set forth the expectations that equipment be cleaned at appropriate intervals (e.g., time or number of batches) to prevent build-up and carryover

			of contaminants so that they do not adversely alter the quality of the API. The appropriate interval is confirmed during cleaning validation.
5.5	**June 2015**	At product changeover, are both visual examination and analytical testing necessary to verify that equipment is clean?	Appropriate cleaning validation verifies that the cleaning process is effective. During cleaning validation, both visual examination and analytical testing should be used to verify cleaning effectiveness [ICH Q7, Sections 12.72 to 75]. Once the cleaning process is validated, routine monitoring of cleanliness of equipment at product changeover should include visual inspection [ICH Q7, Section 12.76]. Frequency of analytical testing to verify ongoing effectiveness of the validated cleaning process is determined by the API manufacturer using a risk-based approach. In situations where the cleaning process is not yet validated, both visual examination and analytical testing are expected.

6. DOCUMENTATION AND RECORDS

#	Date of Approval	Questions	Answers
6.1	**June 2015**	What is meant by 'completely distributed' in [ICH Q7, Section 6.13], which states that 'records should be retained for at least 3 years after the batch is completely distributed'?	For APIs with a retest date, [ICH Q7, Section 6.13] states that records related to production, control and distribution should be retained for at least 3 years after the API batch is 'completely distributed', which is understood as the complete distribution of the entire batch of the API by the API manufacturer to the next party in the supply chain. In the case of APIs handled by agents, brokers, traders, distributors, repackers, and relabellers [ICH Q7, Section 17], 'completely distributed' refers to distribution of the received quantity of the batch of API. The intent of ICH Q7 is to retain records for the period of time that the API could be on the market in order to investigate any problems and/or product complaints. Based on accepted industry practice at the time ICH Q7 was written, it was not anticipated that a manufacturer would set a retest date longer than 3 years. However, the use of 'at least three years' in this section of ICH Q7 covers longer record retention periods, which is in alignment with the basic GMP principle and/or regional requirements that records be retained for the entire period the material is available on the market. It is good industry practice to consider retaining records for the period of time the drug product(s) in which the API was used may be available on the market.
6.2	**June 2015**	Does a batch numbering system need to be sequential?	No, [ICH Q7, Section 6.51] says only that batch production records should have a unique batch or ID number.
6.3	**June 2015**	Who is responsible for the issuance of batch production records?	[ICH Q7, Section 2.3] does not specify who is responsible for the issuance of batch production records [ICH Q7, Section 6.5] as long as the issuance process is described in writing and approved by the quality unit [ICH Q7, Section 2.21].

7. MATERIALS MANAGEMENT

#	Date of Approval	Questions	Answers
7.1	**June 2015**	Does the phrase 'grouping of containers' have the same meaning in [ICH Q7, Sections 7.20 and 7.24]?	The phrase 'grouping of containers' should be read in the context of each sentence. A grouping of containers refers to multiple containers physically secured by the supplier (e.g., shrink-wrapped pallet, etc.) usually intended for ease of shipment and reconciliation. [ICH Q7, Section 7.20] is referring to incoming visual examination of materials before acceptance into the facility under quarantine. The phrase in [ICH Q7, Section 7.24], 'grouping of containers (batches)' contains an additional word 'batches' because this section is addressing the need to establish batch traceability for the incoming material.
7.2	**June 2015**	What is expected in terms of evaluation of suppliers of materials?	Different phrases are used to describe the expectation for evaluation of suppliers of materials [ICH Q7, Sections 7.11, 7.12, 7.31], including traders, if any. [ICH Q7, Section 7.12] states that all materials are purchased against a specification and from suppliers approved by the quality unit [ICH Q7, Section 7.31]. Prior to approval of any supplier, an evaluation should be conducted using a risk-based approach [ICH Q9, Appendix II.5; ICH Q7, Section 7.31]. More extensive evaluation is needed for suppliers of those materials classified as 'critical' [ICH Q7, Section 7.11].
7.3	**June 2015**	What is meant by 'full analysis' [ICH Q7, Section 7.31] on batches of raw materials to qualify a supplier?	A 'full analysis' should include all tests specified by the user of the raw material in the regulatory filing. In cases where no filing is required, the full analysis should include tests in other formal written specifications issued by the user of the raw material [ICH Q7, 7.31]. A raw material supplier's Certificate of Analysis (CoA) may not necessarily align with the user's specifications.
7.4	**June 2015**	Are on-site audits required in the evaluation of suppliers?	No. An on-site audit is not required; however, an on-site audit could be a useful tool in the evaluation of a supplier. A risk assessment of the material or the service provided can be used to develop an audit strategy and manage the ongoing evaluation of suppliers [ICH Q7, Sections 7.11, 7.31].
7.5	**June 2015**	Which tests are considered to be identity tests?	For incoming production materials, identity tests and related methods should be used as described in the relevant sections of a Pharmacopoeia monograph, in an approved regulatory filing or in an in-house specification (including method/analytical procedure) [ICH Q7, Section 7.30]. When available, a discriminating test should be considered for identification testing. The visual examination of a label or the material is not considered sufficient except in the cases described in [ICH Q7, Section 7.32].
7.6	**June 2015**	Is it possible to extend the expiry date or retest date of a raw material and what is the acceptable practice to	Manufacturing and labelling of raw materials for use by API manufacturers is outside the scope of ICH Q7. As such, retest and expiry dates, as defined in ICH Q7, do not strictly apply to raw materials and

		determine how long it may be extended for?	may be used in a different manner by the raw material supplier. Expiry date, as defined in the glossary of [ICH Q7, Section 20], applies specifically to the API. API manufacturers may re-evaluate [ICH Q7, Section 7.5] and then use a raw material after the 'expiry date' or 'retest date', based on an appropriate scientific and risk-based justification (e.g., understanding of material attributes, testing, and stability). Similar justifications may be used to extend the date by which the material should be re-evaluated. It is the responsibility of the API manufacturer to ensure the raw materials are appropriate for the intended use at the time of use.

8. PRODUCTION AND IN-PROCESS CONTROLS

#	Date of Approval	Questions	Answers
8.1	**June 2015**	Can yield ranges defined for the first batch differ from latter batches within a campaign?	Yes. Differing yield ranges [ICH Q7, Section 8.14] may be described and justified in the manufacturing procedure/master batch record explaining the ranges [ICH Q7, Section 6.41]. For example, the first batch in the series of production of batches of the same material (campaign) may leave residual material in the equipment, resulting in a low yield in the first batch and contributing to an increased yield in a subsequent batch of the campaign.
8.2	**June 2015**	What is meant by 'appropriate specifications (of each batch) prior to blending' [ICH Q7, Section 8.41]?	As a principle, no batches with Out of Specification (OOS) results should be blended [ICH Q7, Section 8.41]. Blending is defined in [ICH Q7, Section 8.40]. Individual intermediate and/or API batches should demonstrate conformance with the filed specifications prior to blending. In regions or circumstances where there are intermediates and/or APIs that do not require filing, conformance with the release specification should be demonstrated.

9. PACKAGING AND IDENTIFICATION LABELLING OF APIS AND INTERMEDIATES

No Q&A.

10. STORAGE AND DISTRIBUTION

#	Date of Approval	Questions	Answers
10.1	**June 2015**	What is meant by 'APIs and intermediates can be transferred under quarantine to another unit under the company's control when...'	[ICH Q7, Section 10.20] states 'APIs and intermediates should only be released for distribution to third parties after they have been released by the quality unit(s). APIs and intermediates can be transferred under quarantine to another unit under the company's control when authorised by the quality unit(s) and if appropriate controls and documentation are in place'.

		and is this applicable to contract manufacturers?	The second sentence in [ICH Q7, Section 10.20] describes transport situations that are not considered distribution. It provides for physical movement (transfer but not release) of quarantined material to another unit. This unit can be on the same site, different site (within the same company), or a contract manufacturer (see final paragraph below). The goal of transfer under quarantine is to allow transportation and testing in parallel. Material that is transferred under quarantine is not to be used for further processing until all testing and quality review is complete and the material is released by the quality unit as defined in [ICH Q7, Section 2.22]. This provision for transfer under quarantine is included in ICH Q7 for situations where a company is shipping APIs or intermediates from one unit to another and has both the need to expedite the shipping and the material management system in place to prevent use of the material before full release. Examples of circumstances where transfer under quarantine may be needed include extraordinary supply chain requirement(s) (e.g., short shelf-life), and materials with a lengthy timeframe for required test(s) (e.g., some microbiological tests, etc.). With appropriate oversight as described in [ICH Q10, Section 2.7], including a written agreement as described in [ICH Q7, Section 16.12], and appropriate ongoing controls, a contract manufacturer may be considered a 'unit under the company's control'. There is a joint responsibility by both parties to clearly justify and document the need to transfer the unreleased intermediate or API, and to ensure appropriate control is maintained to prevent use before full release.

11. LABORATORY CONTROLS

#	Date of Approval	Questions	Answers
11.1	June 2015	What is expected in terms of impurities for APIs extracted from herbal or animal tissue origin [ICH Q7, Section 11.2]?	In cases where the API itself is the extract from an herbal or animal tissue preparation, all constituents of this extract (concomitant constituents) might be considered to be part of the API. Therefore, a production process-related impurity profile (except, for example, solvents used in the process), would generally not be expected. However, for all APIs derived from herbal or animal sources, tests and limits for possible contaminants originating from these sources (e.g., pesticides, mycotoxins, viruses, herbicides, elemental impurities and wrong species) should be established, based on a risk assessment. In cases where herbal or animal sources provide material that is further processed to yield a chemically-defined API, all constituents other than the API are considered impurities. In this situation, the API manufacturer would be expected to establish an impurity profile as well as an API release specification that would include impurity limits.

			In any case, it is the API manufacturer's responsibility to establish batch release specifications for APIs to ensure that they are safe and of high quality, consistent with appropriate regulatory requirements, applicable compendial specifications and regional expectations [ICH Q7, Section 11.21; ICH Q9; ICH Q11].
11.2	**June 2015**	In cases where an API test method is changed, which method should be used for stability studies already in progress?	The company should decide and justify the decision of which method to use. All test methods for stability studies [ICH Q1A] should be validated and demonstrated to be stability indicating prior to use [ICH Q7, Section 11.51]. Any changes to stability test methods should be documented. Applicability of the changes to the existing stability studies should be assessed and may require filing in accordance with regional requirements for post-approval changes [ICH Q7, Section 13.11].
11.3	**June 2015**	When is it acceptable for an API manufacturer to extend an API retest date [ICH Q7, Section 11.6]?	The purpose of a retest date is to ensure that the API is still suitable for use. The API manufacturer can extend the retest date of a specific batch based on good science and long-term stability results for that API and testing of the specific batch that has been stored according to the label conditions. In some regions, regulatory authority approval of the retest date extension for the batch may be required. If an API manufacturer wants to change (i.e., extend) the retest date for future batches of an API, then it should conduct stability testing sufficient to support the change, and include the new retest date and supporting data in a regulatory filing, as determined by regional requirements.
11.4	**June 2015**	What is meant by 'completely distributed' in [ICH Q7, Section 11.71], which indicates reserve/retention samples should be retained for 3 years after the batch is completely distributed by the manufacturer?	'Completely distributed' refers to the distribution of the entire batch of the API by the API manufacturer to the next party in the supply chain. It should be noted that this applies to all parties that physically process or repackage the API [ICH Q7, Section 20 – see Glossary for definition of 'manufacture'). The intent of ICH Q7 is to retain samples for the period of time that the API could be in the market in order to investigate any problems and/or product complaints. Based on accepted industry practice at the time ICH Q7 was written, it was not anticipated that a manufacturer would set a retest date longer than 3 years. It is a basic GMP principle that reserve samples be retained for the entire period the material is available on the market. For example, if a company sets a retest date of 5 years and the API is completely distributed immediately after manufacturing, it was never intended that the reserve sample be destroyed before the 5 year retest date was reached.
11.5	**June 2015**	Why does ICH Q7 permit the use of a packaging system for reserve/retention samples that is 'more protective than the marketed packaging system' [ICH Q7, Section 11.72]?	Unlike stability samples, the purpose of the reserve/retention sample is not to represent the quality of the batch in the market place but to allow future evaluation of the quality of the original API batch (e.g., in evaluation of potential counterfeits, etc.). Therefore, reserve/retention samples may be stored in packaging (and conditions) that better preserve the original state of the API.

12. VALIDATION

#	Date of Approval	Questions	Answers
12.1	**June 2015**	Is the lifecycle approach to process validation acceptable for APIs under ICH Q7?	Yes, ICH Q7 does not preclude the lifecycle approach [ICH Q7, Section 12.10, ICH Q10, ICH Q11].
12.2	**June 2015**	Can the range of a process parameter be expanded based only on a process deviation(s)?	No. However, information from the investigation into a process deviation(s) can be used to support expanding the range of a process parameter. Additional work and studies are normally needed to adequately demonstrate that the expanded range for the process parameter consistently produces API of the necessary quality [ICH Q7, Sections 2.16, 12.11, 13.13].
12.3	**June 2015**	Would additional process validation studies be needed to support a change in the source of an API starting material?	Any change in the API starting material should be assessed for impact on the API manufacturing process and the resulting API quality [ICH Q7, Section 7.14]. Additional validation studies of the API process may be warranted if the change in the API starting material is deemed significant. In most cases, validation would be expected for a different source of the starting material unless otherwise justified [ICH Q7, Sections 12.1, 13.13].
12.4	**June 2015**	Is a retrospective approach to validation still acceptable?	Prospective validation is normally expected for processes introduced since the publication of ICH Q7. The concept of retrospective validation remains acceptable as an exception for existing, well established products prior to the implementation of ICH Q7 [ICH Q7, Section 12.44]. If regulatory discussions redefine a step as critical, which had previously been considered non-critical, a protocol describing retrospective analysis of data together with the commitment for concurrent or prospective validation may be an option. Regardless of the type of validation, the quality system should confirm the ongoing robustness of the process (e.g., product quality review).

13. CHANGE CONTROL

#	Date of Approval	Questions	Answers
13.1	**June 2015**	Who is responsible for notifying the drug product manufacturer about relevant changes in API manufacturing?	Each party in the supply chain is responsible for transferring information related to quality or regulatory changes to the next customer in the supply chain. The intention is that the information is transferred

			along the supply chain to the drug product manufacturer in a timely manner [ICH Q7, Sections 13.17, 17.60].

14. REJECTION AND REUSE OF MATERIALS

#	Date of Approval	Questions	Answers
14.1	**June 2015**	Should rejected materials be stored under physical and secure segregation?	ICH Q7 does not specify a need for physical and secure segregation. Both [ICH Q7, Sections 4.14 and 10.11] include the provision for the use of alternative control systems for storage of rejected material. Whatever control system is used, the purpose should be to prevent the unintentional or unauthorised use of the rejected material [ICH Q7, Sections 7.44, 10.11, 14.1].
14.2	**June 2015**	Does the definition of expiry date in ICH Q7 preclude the rework or reprocess of an expired API?	According to the definition, material should not be used after the expiry date. The original intent of this definition in ICH Q7 was that expired API should not be used in drug product formulation. It may be acceptable to reprocess [ICH Q7, Section 14.2] or rework [ICH Q7, Section 14.3] the expired API where the API manufacturer has all related historical GMP documentation and additional stability data on the reworked or reprocessed API. There may be registration/filing considerations that are beyond the scope of ICH Q7 in addition to the GMP considerations.
14.3	**June 2015**	Is validation expected for the recovery of material from mother liquor?	It depends. Recovery of material(s) from mother liquor is a process and the need for validation should be assessed as for any other process step [ICH Q7, Section 14.40]. Recovery of material from mother liquor in any process step that must be controlled within predetermined criteria to ensure the API meets its specification is, by definition, a critical process step and should be validated. For example, recovery of API from mother liquor would be considered a critical process step and should be validated [ICH Q7, Sections 12.11, 12.12, 14.41, 14.43, 20 – see Glossary for definitions of 'critical', 'materials', 'mother liquor', and 'validation'].

15. COMPLAINTS AND RECALLS

#	Date of Approval	Questions	Answers
15.1	**June 2015**	Can quality defects of released APIs that are identified by another entity belonging to the same company be handled outside of the API	Yes. After the release of an API for further use, any identified quality defect should be investigated and addressed according to the API manufacturer's complaint system or equivalent (i.e., non-conformance, deviations, etc.) [ICH Q7, Sections 15.10 to 15.12]. Where equivalent systems are used, such defects should be categorised in a manner that provides clear visibility that the defect was discovered after being released by the API site.

		manufacturer's complaint procedure?	
15.2	**June 2015**	Must a quality related return, at the request of the API manufacturing site, from another site within the same company be recorded as a 'recall'?	No, provided that no portion of the batch left direct control of the company for sale or use. It must be clearly visible in the API site's Quality System as a return triggered by the API manufacturing site so this is clear in quality system trend reporting and in the Product Quality Review [ICH Q7, Sections 2.50, 15.13; and 15.14].

16. CONTRACT MANUFACTURERS (INCLUDING LABORATORIES)

#	Date of Approval	Questions	Answers
16.1	**June 2015**	Does ICH Q7 preclude a contract manufacturer's independent quality unit from performing the main responsibilities as described in [ICH Q7, Section 2.22]?	No. The original intent of Section 2.2 was to distinguish the main responsibilities (e.g., batch record review, review of non-conformances and investigations, sampling, testing, release or rejection of intermediate or API, etc.) of the independent quality unit from other departments within a company. Contract manufacturers are expected to have an independent quality unit that meet the responsibilities defined in [ICH Q7, Section 2.2] for all activities performed. Given the potential complexity of outsourcing contract manufacturing arrangements, GMP responsibilities should be clearly defined between both parties in detail in a written agreement [ICH Q7, Section 16.12]. However, the overall responsibility for API quality must not be delegated.
16.2	**June 2015**	Which outsourced activities are covered by ICH Q7?	In the context of ICH Q7, contract manufacturing is the outsourced activity. The term 'outsourced activities', as defined and described in [ICH Q10, Section 2.7, Glossary], aligns with the description of 'contract manufacturer' in [ICH Q7, Section 16]. ICH Q7 defines 'manufacture' as '*all operations of receipt of materials, production, packaging, repackaging, labelling, relabeling, quality control, release, storage, and distribution of APIs and related controls*.' 'Related controls' include any activities or services necessary to support production (e.g., maintenance, calibration, etc.). ICH Q7 applies to any activities performed by the original manufacturer or the company that is performing the activity on behalf of the original manufacturer.

16.3	**June 2015**	What is meant by 'where subcontracting is allowed' [ICH Q7, Section 16.14]?	Subcontracting as used in [ICH Q7, Section 16.14] refers to the contract acceptor further contracting out a specific activity to another party (third party). This should only be done when the written and approved contract, as described in [ICH Q7, Section 16.12], specifically allows for such subcontracting. Even when subcontracting is allowed, the original contract giver should approve specific subcontracting before it occurs as stated in [ICH Q7, Section 16.14].

17. AGENTS, BROKERS, TRADERS, DISTRIBUTORS, REPACKERS, AND RELABELLERS

#	Date of Approval	Questions	Answers
17.1	**June 2015**	What does ICH Q7 mean by 'Agents, brokers, traders, distributors, repackers, or relabellers'?	Regardless of what they are referred to in different regions, ICH Q7 applies to all parties in the supply chain after the original API/intermediate manufacturer to the drug product manufacturer, in order to maintain the integrity, traceability, and transparency of the supply chain [ICH Q7, Section 17.1].
17.2	**June 2015**	Could a distributor of an API engage a contract manufacturer for production Steps?	No. If a distributor [ICH Q7, Section 17.1] of an API contracts out production Steps (e.g., drying, micronisation, milling, or sieving), then the distributor becomes a manufacturer and is subject to the entirety of ICH Q7. This includes, but is not limited to, appropriate written agreements as stated in [ICH Q7, Section 16.12] defining responsibilities of each party. In addition, these contracted production steps must be described in registration documents, applications, or equivalent as per regional requirements.
17.3	**June 2015**	Is it acceptable to replace the original label, which contains the information of the original manufacturer?	Any relabeling operations are considered manufacturing by definition [ICH Q7, Section 20] and should be performed under appropriate GMP controls [ICH Q7, Section 17.40]. With appropriate justification, manufacturers including repackagers and relabellers may replace the original label. The new label should contain information as per [ICH Q7, Sections 9.42, 9.43]. However, distributors should not remove an original label, but only add additional labels. Information about the original manufacturer must be provided to the customers [ICH Q7, Section 17.61]. Overall, the traceability of the supply chain needs to be maintained [ICH Q7, Section 17.2].
17.4	**June 2015**	Who is considered to be the original manufacturer of the API for purposes of the Certificate of Analysis (CoA)?	The CoA should document the original manufacturer to support traceability throughout the supply chain [ICH Q7, Sections 11.4, 17.6]. The original manufacturer would be the facility where the final purified API/intermediate is produced. Further physical processing (e.g., drying, micronisation, milling, sieving) of an API would not make the manufacturer performing such operations the original manufacturer. All authentic CoAs including those of the original manufacturer should be available [ICH Q7, Section 17.20].

18. SPECIFIC GUIDANCE FOR APIS MANUFACTURED BY CELL CULTURE/FERMENTATION

#	Date of Approval	Questions	Answers
18.1	**June 2015**	Does ICH Q7 expect validation for viral removal/viral inactivation steps for biological/biotechnological products?	Yes. According to [ICH Q7, Section 18.51], viral inactivation/removal steps are considered critical for some processes (e.g., cell lines of human and animal origin [ICH Q5A, Section 1]. Parameters for validation should be established in accordance with [ICH Q5A, Q5D and Q6B]. Due to the potential for contamination [ICH Q5A, Section 2.B], viral inactivation studies should be performed in a separate and typically smaller laboratory facility [ICH Q11, Section 7.2] and not in a clinical or commercial manufacturing facility.
18.2	**June 2015**	Do [ICH Q7, Sections 18.14, 18.2] apply to classical fermentation and biotechnology?	For 'classical fermentation', the text from [ICH Q7, Section 18.14] '*…this guide covers cell culture/fermentation from the point at which a vial of the cell bank is retrieved for use in manufacturing*' refers to 'classical fermentation' and not to the 'biotechnology fermentation/cell culture'. Although the entire ICH Q7 Guideline does not apply prior to the introduction of cells into the classical fermentation process, as shown in Table 1 of [ICH Q7, Section 1.3], an appropriate level of GMP controls suitable for cell banks should be established. For 'biotechnology fermentation/cell culture' [ICH Q7, Section 18.2] on '*Cell Bank Maintenance and Record Keeping*' applies specifically to biotechnology fermentation/cell culture because ICH Q7 starts with the maintenance of the working cell bank [ICH Q7, Section 1.3, Table 1]. Although for biotech products the entire ICH Q7 Guideline does not apply prior to the maintenance of the working cell bank, an appropriate level of GMP controls suitable for cell banks should be established. See also [ICH Q5B, ICH Q5D].

19. APIS FOR USE IN CLINICAL TRIALS

#	Date of Approval	Questions	Answers
19.1	**June 2015**	Is it permitted to use the same equipment to manufacture materials to be used in pre-clinical and clinical trials?	Yes. As long as operations are conducted under GMP conditions according to ICH Q7, including the establishment of effective cleaning methods, safe residue limits and appropriate containment measures [ICH Q7, Section 19.3].

20. GLOSSARY

#	Date of Approval	Questions	Answers
20.1	**June 2015**	Are the terms 'deviation' and 'non-conformance' synonyms?	No. However, they are related. The term 'deviation', as used in ICH Q7, refers to a '*departure from an approved instruction or established standard'* that may or may not have an impact on the quality of the material. '*Non-conformance*' refers to a status as a result of a failure of the material to meet specifications or appropriately established standards that impacts the quality of the material [ICH Q7, Sections 2.50, 14.30, 20].

21. ANNEX: Q&As linked to the respective Sections of ICH Q7

Sections of ICH Q7	1: Introduction	2: Quality Management	3: Personnel	4: Buildings and Facilities	5: Process Equipment	6: Documentation and Records	7: Materials Management	8: Production and In-Process Controls	9: Packaging and Identification Labelling of APIs and Intermediates	10: Storage and Distribution	11: Laboratory Controls	12: Validation	13: Change Control	14: Rejection and Re-use of Materials	15: Complaints and Recalls	16: Contract Manufacturers (including Laboratories)	17: Agents, Brokers, Traders, Distributors, Repackers, and Relabellers	18: Specific Guidance for APIs manufactured by Cell Culture/Fermentation	19: APIs for Use in Clinical Trials	20: Glossary	Other ICH Guidelines
1. Introduction – Scope																					
1	1.3																				Q11 Q5B Q5D
2	1.2																			20	
2. Quality Management																					
1		2.13																			
2		2.13 2.22									11.12 11.10					16.10				20	
3		2.22																			
4		2.22	3.10			6.52					11.12										
5		2.50																			Q10
6		2.50 2.51																			Q9
3. Personnel																					
1			3.12																		
2		2.2	3.3																		Q10
4. Buildings and Facilities – Containment																					
1				4.40 4.41																	E2E Q9
2				4.42																	Q9
5. Process Equipment – Cleaning																					
1					5.23							12.76 12.7									
2					5.21 to 5.25			8.50				12.76									
3					5.21							12.7									
4					5.23			8.50				12.7									
5												12.72to 12.76									
6. Documents and Records																					
1						6.13											17				
2						6.51															
3		2.21 2.3				6.5															
7. Materials Management																					
1							7.20 7.24														
2							7.11 7.12 7.31														Q9
3							7.31														
4							7.11 7.31														
5							7.30 7.32														
6							7.5													20	
8. Production and In-Process Control																					
1						6.41		8.14													
2								8.40 8.41													
9. Packaging and Identification Labelling of APIs and Intermediates																					
10. Storage and Distribution																					