C8

0956748 ORDER NO: AAD87-13295

SYNTHESIS AND EVALUATION OF AMMONIUM ANALOGS OF CARBOCATIONIC INTERMEDIATES IN SQUALENE BIOSYNTHESIS

Author: CAPSON, TODD LEO
Degree: PH.D.
Year: 1987
Corporate Source/Institution: THE UNIVERSITY OF UTAH (0240)
Source: Volume 4803B of Dissertations Abstracts International.
PAGE 757 . 157 PAGES
Descriptors: CHEMISTRY, ORGANIC
Descriptor Codes: 0490

The syntheses of primary amine, N- 2- 4,8-dimethyl-3,7-(3E)-nonadienyl -2-methyl-3- 2,6,10-trimethyl-1,5,9-(1E,5E)-undecatrienyl -trans-1,2-trans-1,3-cyclopropylamine (35) and secondary amine, N- 2- 4,8-dimethyl-3,7-(3E)-nonadienyl -2-methyl-3- 2,6,10-trimethyl-1,5,9-(1E,5E)-undecatrienyl -1,2-trans-1,3-cyclopropylamino -N-propyl-3-phosphonophosphate (80) are described. Both amines were converted to their ammonium forms and tested as inhibitors of squalene synthetase.

Both amines were synthesized from presqualene alcohol (38), which was oxidized to aldehyde 62 by a Swern oxidation and then to acid 37 with tertabutylammonium permanganate or sodium chlorite. Acid 37 was converted to the isocyanate, which was treated with 2-trimethylsilylethanol to afford carbamate 63. Treatment of 63 with tetrabutylammonium fluoride afforded primary amine 35. Reaction of 63 with potassium hydride and then with diethyl (3-p-toluenesulfonyloxy)propyl phosphonate afforded N-alkylated phosphonocarbamate 83. Hydrolysis of 83 afforded phosphonic acid 100, which was treated sequentially with carbonyldiimidazole, tetrabutylammonium dihydrogen phosphonate, and tetrabutylammonium fluoride to afford 80.

Radioactive substrates of squalene synthetase were prepared for enzymatic studies. 1-('3)H Farnesyl pyrophosphate (1-('3)H farnesyl)PP, 1-('3)H 9) was synthesized from 1-('3)H farnesyl bromide (1-('3)H 41) and tris-tetrabutylammonium pyrophosphate. 1-('3)H Presqualene pyrophosphate (1-('3)H presqualene PP, 1 -('3)H 13) was synthesized from presqualene alcohol (1-('3)H 38), bis-triethylammonium phosphate, and trichloro-acetonitrile.

Squalene synthesis from farnesyl PP was unaffected in the presence 33 (20 (mu)M) in the absence of inorganic pyrophospate (PP(,i)). In the presence of 2 mM PP(,i), synthesis of squalene from farnesyl PP was inhibited 75%. In the absence of PP(,i), 33 inhibited the conversion of presqualene PP to squalene by 34%. In the presence of 1 mM PP(,i), synthesis of squalene from presqualene PP was inhibited by 73%.

Secondary ammonium compound 79 inhibited squalene synthetase in the absence of PP(,i). In the presence of 18 (mu)M 79, squalene synthesis from farnesyl PP and presqualene PP was inhibited by 80% and 90%, respectively. The proton release assay was used to measure the conversion of farnesyl PP to presqualene PP. When proton release and squalene synthesis were measured simultaneously in the same assay, presqualene PP and squalene production from farnesyl PP was inhibited to virtually the same degree (56%). Inhibition of squalene synthetase by 33 and 79 was interpreted as evidence for the existence for carbocationic intermediates that exist as ion pairs with PP(,i) during the exzymatic rearrangement of presqualene PP to squalene.