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ABSTRACT: Paclitaxel and other taxanes have complex structures that include the presence of numerous hydrolytically sensitive ester groups. The present study attempts to make sense of the kinetics of the base catalyzed hydrolysis of various ester groups found in paclitaxel by also studying the hydrolysis of 7-epi-taxol, 10-deacetyltaxol, 7-epi-10-deacetyltaxol, baccatin III, 10-deacetylbaccatin III and *N*-benzoyl-3-phenylisoserine ethyl ester. Kinetics were studied as function of pH, buffer concentration and temperature, and analyzed using a stability indicating HPLC assay and LC/MS to identify degradation products. The kinetics were complicated by the epimerization reaction occurring at the 7-position but isolation of the hydrolytic components of the kinetics was possible. All ester hydrolysis reactions observed above pH 6–7 were, as expected, base catalyzed. After epimerization at the C7, paclitaxel hydrolysis occurs mainly due to cleavage of the side chain with further hydrolysis of the ester bonds at C10, C2 and C4 with the C10 acetate hydrolysis being the relatively next most facile. By studying the hydrolysis of 10-deacetyltaxol, 7-epi-10-deacetyltaxol, baccatin III, 10-deacetylbaccatin III and *N*-benzoyl-3-phenylisoserine ethyl ester, good insight into the hydrolysis of the larger more complex taxanes was possible. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:3100–3108, 2008

Keywords: paclitaxel; taxol; 7-epi-taxol; baccatin III; epimerization; hydrolysis; degradation; pH; stability

INTRODUCTION

This study was undertaken to investigate the degradation kinetics of paclitaxel and related compounds in aqueous solutions in the neutral to basic pH range where both epimerization at the C7 site and hydrolysis were observed. An earlier article described the role of pH on the C7 epimerization and identified the likely mechanism for this base catalyzed reaction.[1] An additional goal was to provide insight into the stability of other taxanes and paclitaxel analogues in aqueous solution.

The chemistry and therapeutic benefits of paclitaxel and related taxanes has been studied extensively since the 1980s.[2–7] Limited quantitative information is available on the chemical stability of paclitaxel in aqueous solution because of its poor aqueous solubility, its complex structure and its nonadherence to first-order kinetics under neutral to basic pH conditions. However, some useful studies have been published.[8–10] Tian and Stella[1] quantified the C7 epimerization of paclitaxel and several related taxanes in aqueous

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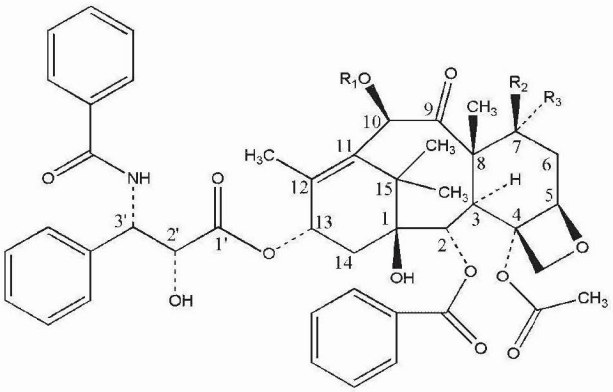
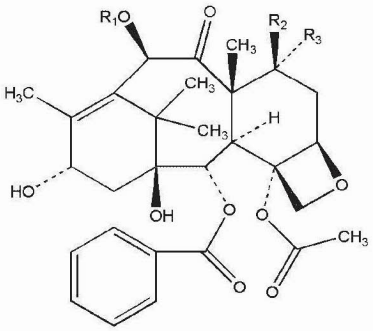
solution and the rate constants for the interconversion of the 7*R*- and 7*S*-epimers. The focus of the present study was to investigate the kinetics of the further degradation of these epimers under neutral and basic pH conditions and to study the influence of the ring system and substituents.

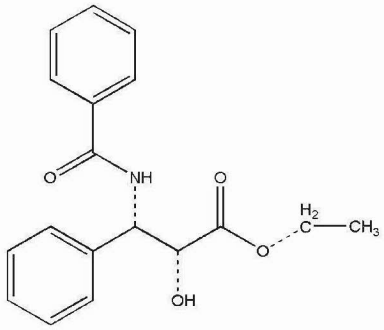
The structures of paclitaxel and its related compounds used in this study are illustrated in Figure 1. Baccatin III and 10-deacetyl baccatin III represent the diterpene ring structure of paclitaxel and 10-deacetyltaxol, and *N*-benzoyl-3-phenylisoserine ethyl ester was selected as a mimic of the side chain of paclitaxel.



(1) $R_1 = CH_3CO$ $R_2 = OH$ $R_3 = H$
(2) $R_1 = CH_3CO$ $R_2 = H$ $R_3 = OH$
(3) $R_1 = H$ $R_2 = OH$ $R_3 = H$
(4) $R_1 = H$ $R_2 = H$ $R_3 = OH$



(5) $R_1 = CH_3CO$ $R_2 = OH$ $R_3 = H$
(6) $R_1 = H$ $R_2 = OH$ $R_3 = H$



(7)

Figure 1. The structures of paclitaxel and related compounds: paclitaxel (**1**) 7-epi-taxol (**2**), 10-deacetyltaxol (**3**), 7-epi-10-deacetyltaxol (**4**), baccatin III (**5**), 10-deacetylbaccatin III (**6**) and *N*-benzoyl-3-phenylisoserine ethyl ester (**7**).

EXPERIMENTAL

Materials

All of the chemicals, solvents and buffer solutions used in this study were identical to those described in greater detail in an earlier study.[1]

pH

The pH of the solutions was controlled throughout the reaction by using appropriate buffer solutions and dilute sodium hydroxide solutions. The preparation of buffer solutions was described previously.[1] The kinetic measurements at pH 11 and 12 were performed in dilute sodium hydroxide solutions of appropriate concentration. Buffer concentration, when not varied, was 1.0 mM except for the measurement at pH 12. No significant change of pH was observed throughout the reaction.

Analytical HPLC Assays and Mass Spectrometry

The starting compounds, their epimers, and other degradation products were simultaneously measured using an isocratic HPLC-UV assay. The stability indicating assay and the HPLC system employed in this study were described earlier[1] as was the identification and profiling of epimerization and degradation products using a Waters Alliance 2690 HPLC system connected to a Micromass Quattro Micro Tandem Quadruple mass spectrometer. The system was operated at an electrospray source block temperature of 120°C, a desolvation temperature of 50°C, a cone voltage of 18 kV. For HPLC-UV–MS mode, the flow rate was 0.833 mL/min versus 0.167 mL/min for UV and MS respectively. The molecules undergo electron spray ionization in the positive ion mode.

Kinetic Procedure

The reaction kinetics for further degradation of both *R*- and *S*-epimers was investigated in aqueous solutions at pH 6–12. For the kinetic experiments at 25°C, 24.2 mL appropriate buffer solutions were equilibrated in a water bath at 25.0 ± 0.1°C, and the epimerization was initiated by adding 0.8 mL stock solution (125 μg/mL in acetonitrile) into the reaction buffer. This resulted in an initial reaction concentration of 2.0 μg/mL. At various time intervals, aliquots (0.8 mL) of the reaction solutions were withdrawn, and assayed by HPLC. The reactions at basic pH were quenched by adding dilute hydrochloric acid solution to adjust the pH close to 5 before HPLC analysis.

For the stability studies at elevated temperatures 37, 50, and 70°C respectively, vials containing sample solutions were placed in thermostatically controlled ovens. The reaction solutions were maintained at the desired temperature throughout the stability study. Portions were removed from the reaction solution at appropriate intervals. The samples were quickly cooled in ice water to quench the reaction followed by immediate HPLC analysis. The time interval between sampling and HPLC injection was less than three minutes so that the experimental error was minimized.

RESULTS AND DISCUSSION

Total Degradation of 10-Deacetylbaccatin III

Figure 2 shows HPLC-UV–MS chromatograms of the degradation of 10-deacetyl baccatin III. The starting compound, the *S*-epimer, and the epimerization and degradation products were adequately separated from one another. After the initial epimerization of the C7 hydroxyl, both 7*S*- and 7*R*-epimers further degraded into more fragmented products. For example, some benzoic acid was confirmed by its strong UV

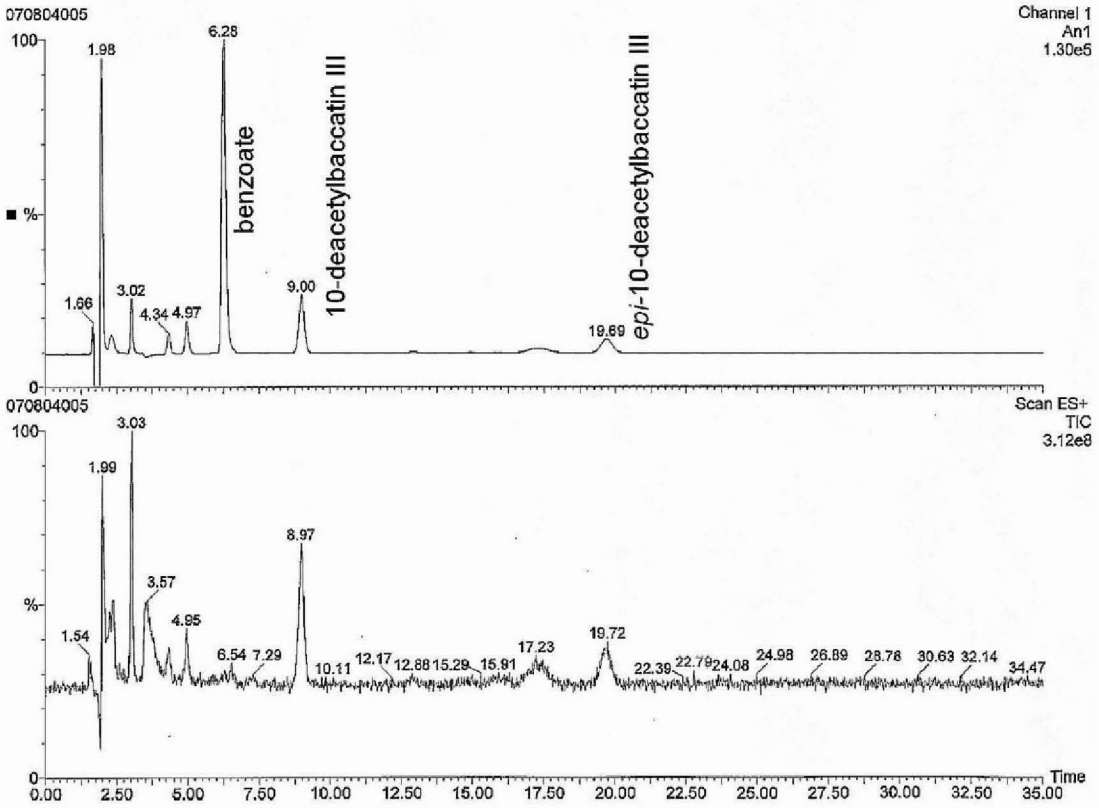


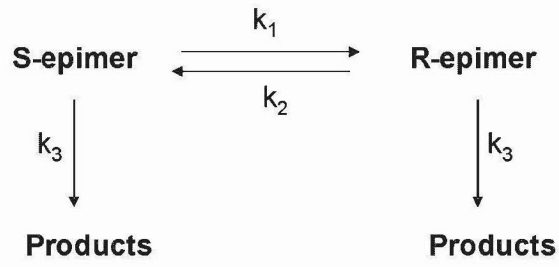
Figure 2. HPLC chromatogram and MS data for the hydrolytic degradation of 10-deacetyl baccatin III and its epimer in aqueous solution at pH 10.8, 50°C, reaction time = 3 h. After the initial epimerization of the C7 hydroxyl, both 10-deacetylbaccatin III and its epimer degrade to further products such as benzoic acid.

absorbance and by added standards while it was hardly detected by MS due to its low molecular weight. This result indicated some hydrolytic cleavage of the benzoate ester bond at C13 position occurred, however, this was a relatively minor overall pathway.

Tian and Stella[1] discussed the C7 epimerization reaction of 10-deacetylbaccatin III proceeds according to Scheme 1: k_1 is the epimerization rate constant from the 7*S*-epimer to the 7*R*-epimer, and k_2 is the reverse. The observed base-catalyzed epimerization in near neutral to higher pH range suggests a possible rapid deprotonation/protonation of the C7 –OH, followed by a structural rearrangement through a retroaldol/aldol mechanism to form the 7-epimer. The rate-limiting step of structure rearrangement most likely occurs with the formation of an enolate intermediate. k_3 is the sum of all the degradation rate constants for primary hydrolytic deacylation of 10-deacetylbaccatin III (both 7*R*- and 7*S*-epimers). Due to the similarity of the chemical structures of the *R*- and *S*-epimers, it is assumed that they have identical rate constants for hydrolytic degradation. Despite neither epimer following simple first-order kinetics due to the relatively fast preequilibrium, the overall degradation loss of the sum of the two epimers did follow pseudo-first-order kinetics. Thus:

$$[\mathrm{R}] + [\mathrm{S}] = \{[\mathrm{R}]_0 + [\mathrm{S}]_0\} \exp(-k_3 t) \qquad (1)$$

where $[\mathrm{R}]_0$ and $[\mathrm{S}]_0$ are the initial concentration of the 7*R*- and 7*S*-epimers, and k_3 is the overall apparent degradation rate constant. Therefore, the values of k_3 were readily calculated from the slope of the linear semilogarithmic plot of the percent residual total 10-deacetylbaccatin III, sum of both epimers, versus time. Similarly, the total loss of 10-deacetylbaccatin III was measured at various pH values (6–12). The experimental results were plotted in Figures 3 and 4. The overall degradation follows pseudo-first-order kinetics at any constant pH and temperature. The pseudo-first-order rate constants, k_3, are obtained from best linear regression using SigmaPlot (v 7.101).



Scheme 1. Proposed reaction scheme for the C7 epimerization and subsequent hydrolysis of paclitaxel and related analogues.

Total Degradation of Baccatin III

Under basic pH condition, baccatin III quickly forms its 7*R*-epimer, followed by further hydrolysis of various ester bonds, leading to multiple products, such as 10-deacetylbaccatin III, 7-epi-10-deacetylbaccatin III and other minor hydrolytic products. The degradation time course was described earlier.[1] The ester groups at C2, C4, and C10 positions all undergo hydrolysis simultaneously, and, therefore, the value of the overall degradation rate constant k_3 is the sum of all hydrolytic rate constants contributing to hydrolysis. Among the hydrolytic products, 10-deacetylbaccatin III and 7-epi-10-deacetylbaccatin III were observed as the primary products, indicating that the 10-acetyl ester hydrolyzed faster than the other two, ester groups. Therefore, the proposed initial degradation pathways of

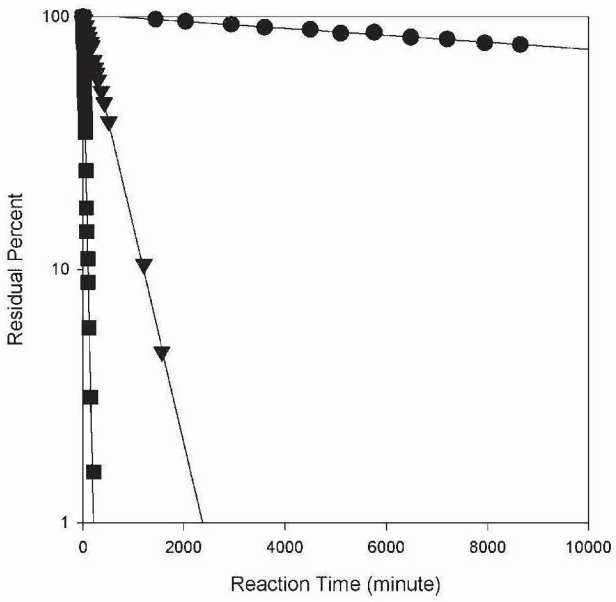


Figure 3. Semi-log plot of the total loss of 10-deacetyl baccatin III (sum of 7*R*- and 7*S*-epimers) at pH 9.04 (●), 10.78 (▼), 11.82 (■), and 25°C. The total loss of 10-deacetylbaccatin III, including both epimers, follows pseudo-first-order kinetics in the high pH range.

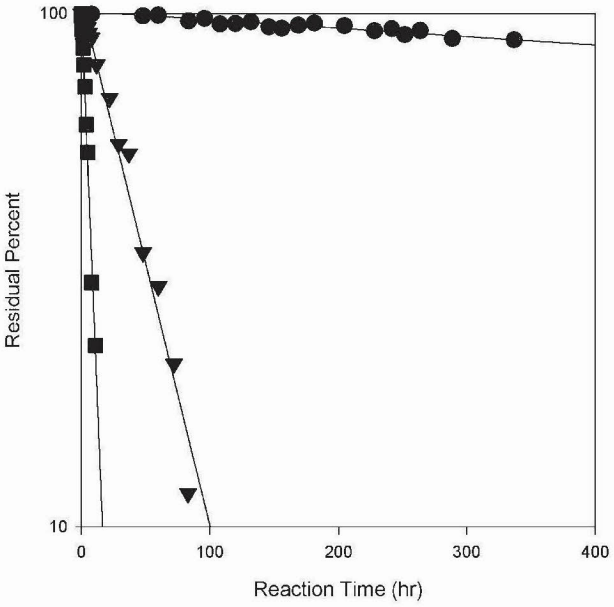


Figure 4. Semi-log plot of the total loss of 10-deacetyl baccatin III (sum of 7*R*- and 7*S*-epimers) at pH 7.58 at 25°C (●), 50°C (▼), and 70°C (■).

baccatin III in the basic pH range is shown in Figure 5.

pH-Rate Profiles for Total Degradation of 10-Deaceylbaccatin III and Baccatin III

The first-order rate constants for total degradation, k_3, for 10-deaceylbaccatin III and baccatin III were determined in aqueous solution at various pH conditions. The pH dependencies of the pseudo first-order degradation rate constants of 10-deacetylbaccatin III and baccatin III at 25°C, are shown in Figure 6. The observed rates of the degradation increased rapidly and uniformly with increasing pH. Since the slopes of these straight-line portions of $\log k$ versus pH profiles are close to unity, it is likely that the overall degradation is base-catalyzed with little evidence of a water term in this pH range. The solid lines in Figure 6 were drawn from fits with the slope fixed at unity to show the relative stability of the derivatives. In addition, no significant buffer catalysis was

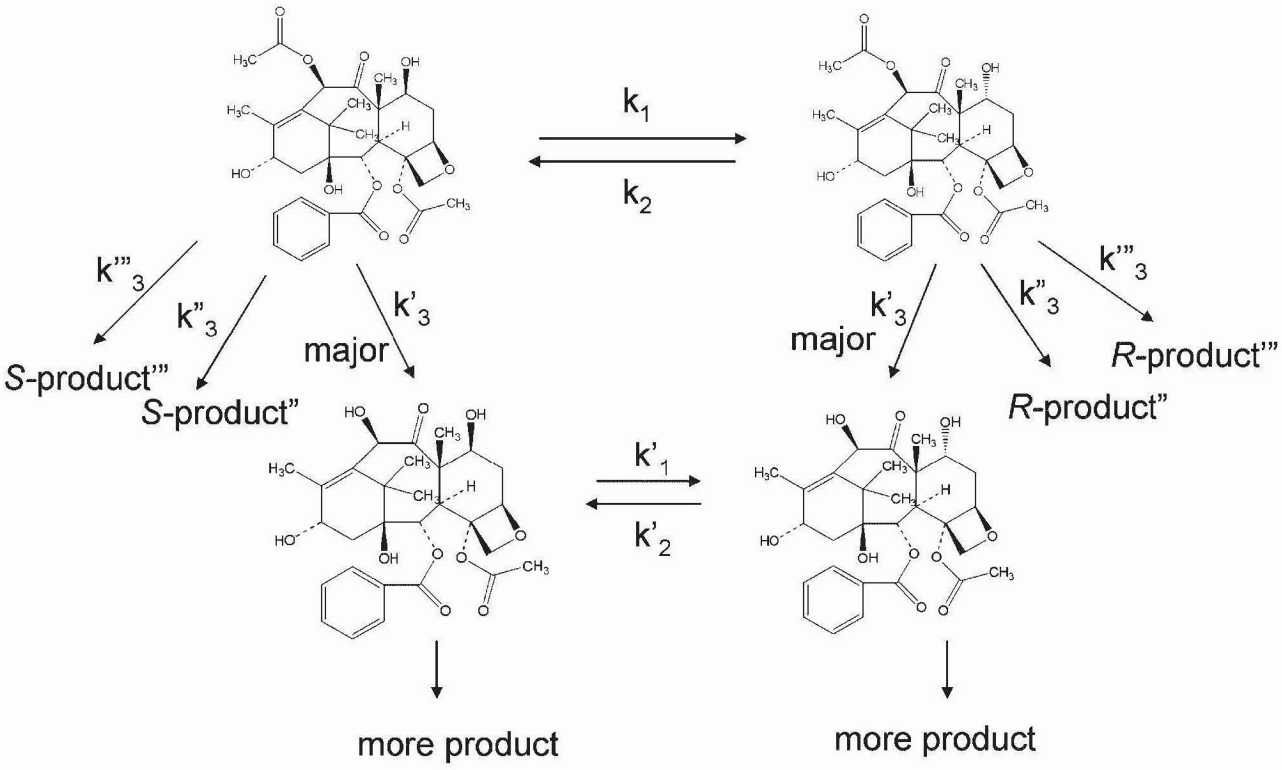


Figure 5. The proposed degradation pathways of baccatin III in the basic pH range. A fast epimerization forms its 7*R*-epimer, followed by further hydrolysis of ester bonds. The value of the overall degradation rate constant, k_3, is the sum of all hydrolytic rate constants contributing to hydrolysis. Thus, $k_3 = k'_3 + k''_3 + k'''_3$.

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