A Novel Benzoyl Group Migration: Synthesis and Biological Evaluation of 1-Benzoyl-2-des(benzoyloxy)paclitaxel

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The structurally complex diterpenoid paclitaxel (Taxol), first isolated by Wall and co-workers[1] in 1971, has been found to be an effective anticancer agent and was approved for the treatment of drug-refractory ovarian cancer by the FDA in 1992. Because of this clinical activity, paclitaxel has become an important target for studies of structure–activity relationships, and several conclusions can be drawn from published work.[2] The northern hemisphere of paclitaxel, comprising the region from C-7 to C-12, can be varied within certain limits without making large changes in activity.[3] The southern hemisphere, comprised of C-14 and C-1 to C-5, plays an important role in expressing the bioactivity, and changes at these positions can have dramatic effects on activity.[4] The oxetane ring and the phenylisoserine side chain at the C-13 position are likewise essential components for bioactivity.[2,5]

Various reports[4a,b] of the effect of modifying the C-2 benzoyl group have appeared, and from these reports it can be concluded that this group is necessary for bioactivity. However, no report had appeared on modification of the C-1 OH group until a recent paper by Chen et al.[6] in which the preparation of a 1-benzoyl-2-des(benzoyloxy)baccatin III was reported. In our ongoing studies of the chemistry of paclitaxel, we had prepared a similar product from paclitaxel, but our interpretation of the reaction mechanism differed from that reported by Chen. We now present our findings, together with evidence that indicates that acyl migration occurs during xanthate formation and not during the deoxygenation reaction as proposed by Chen et al.[6]

In an attempt to deoxygenate the C-1 hydroxyl group, paclitaxel was first converted to its 2′,7-bis(triethylsilyl) derivative **2** (Scheme 1). Treatment of **2** with NaH and carbon disulfide in THF at room temperature followed by quenching with methyl iodide led to formation of the novel benzoyl group migration product **3**. The ^{1}H NMR spectrum of compound **3** showed the appearance of the methyl signal of the xanthyl group as a singlet at 2.51 ppm, together with a downfield shift to 3.72 ppm of one of the C-14 protons as a doublet of doublets and a downfield shift of the C-2 proton from 5.70 to 6.83 ppm. These shifts suggested that both the C-1 and C-2 oxygens were acylated, but did not distinguish clearly between the desired structure **7** and the isomeric structure **3**. A distinction between these structures was made on the basis of ^{13}C NMR and HMBC data. The HMBC spectrum of **3** showed a three-bond correlation between the C-1 benzoyl carbonyl group at 164.95 ppm and the *ortho* benzoyl protons at 7.80 ppm and between the xanthate thiocarbonyl carbon at 215.25 ppm and the xanthate methyl protons at 2.51 ppm. The HMBC spectrum thus established unambiguously the assignment of each carbon signal. To our surprise, only the thiocarbonyl carbon at 215.25 ppm showed a three-bond correlation with H-2 at 6.83 ppm, establishing that **3** has the rearranged structure rather than the structure **7** expected for a direct acylation reaction.

Although 1,2-acyl migrations are common reactions under basic conditions, such a migration involving the 2-benzoyl group of paclitaxel has not previously been observed. The reaction is unusual in that it involves migration from a secondary to a tertiary position and would not appear to be thermodynamically favored. We thus propose that the reason for benzoate migration during xanthylation of **2** relates to the relative ease of attack of the appropriate alkoxide ions on carbon disulfide. If the initial alkoxide **8** is in equilibrium with a very small amount of the isomeric anion **9** (Scheme 2), and if attack of **9** on CS_2 occurs very much faster than attack of **8**, then the reaction would lead to eventual formation of **3**.

Deprotection of **3** under standard conditions (5% methanolic HCl at room temperature) gave compound **4**, which is the first C-1-acylated paclitaxel analog reported. Compound **3** when subjected to radical deoxygenation conditions[7] (tributyltin hydride/AIBN in toluene at 90 °C) gave 1-benzoyl-2-des(benzoyloxy)-2′,7-bis(triethylsilyl)-paclitaxel (**5**). The ^{1}H NMR spectrum of compound **5** showed the disappearance of the methyl singlet of the xanthyl group (2.51 ppm) and the downfield doublet for the C-2 proton (6.83 pm), together with the appearance of signals at 2.06 and 2.54 ppm for the C-2 methylene protons. The ^{13}C NMR spectrum also revealed the deoxygenation of the C-2 carbon by the upfield shift of its signal from 83.52 to 33.74 ppm. Deprotection of the silyl groups of **5** with freshly prepared 5% methanolic HCl at room temperature gave 1-benzoyl-2-des(benzoyloxy)-paclitaxel (**6**). The novel benzoyl group migration in the above reaction sequence once again indicated the pro-

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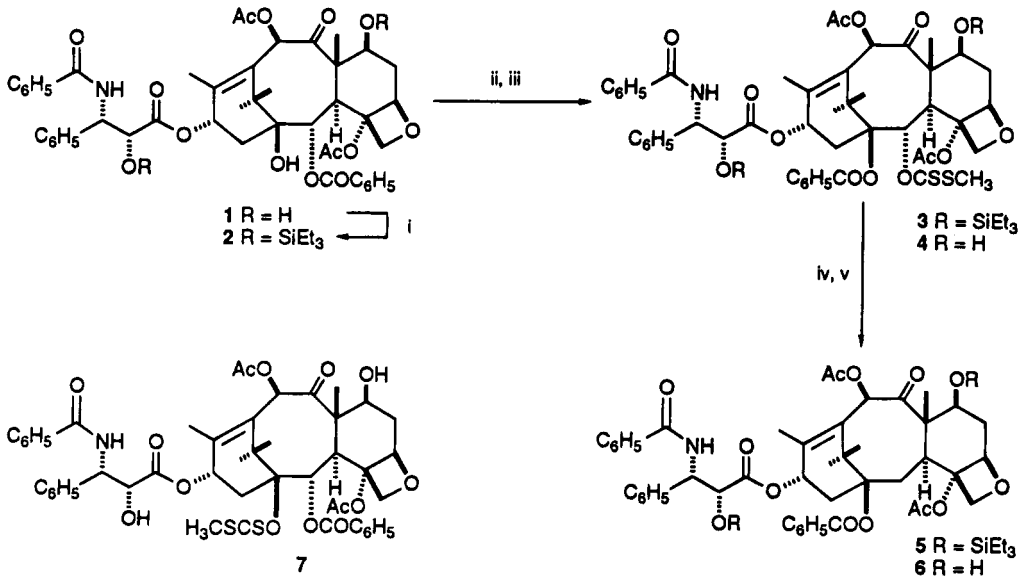
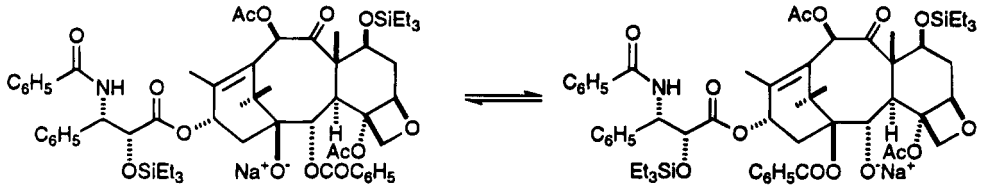
0022-3263/95/1960-3260$09.00/0

Scheme 1



[a] (i) TESCl, imidazole, DMF, rt; (ii) NaH, CS_2, MeI, THF, rt; (iii) 5% HCl, methanol; (iv) TBTH/AIBN, toluene, 90 °C; (v) 5% HCl, methanol.

Scheme 2



pensity of paclitaxel to undergo unusual chemistry with different reagents under a variety of reaction conditions.[8]

Biological evaluation of the two derivatives of paclitaxel obtained above was carried out in the P-388 leukemia cytotoxicity system.[9] Both 1-benzoyl-2-[(*S*-methyldithio)-carbonyl]paclitaxel (**4**) and 1-benzoyl-2-des(benzoyloxy)-paclitaxel (**6**) were found to be less active than paclitaxel in this assay. Compounds **4** and **6** exhibited ED_{50} 0.28 and 1.70 μg/mL, respectively, as compared with paclitaxel, which has an ED_{50} of 0.03 μg/mL in this system. These data thus confirm the essential nature of the C-2 benzoyl group for the bioactivity of paclitaxel.

Experimental Section

General Methods. All chemicals were obtained from Aldrich Chemical Co. and were used without further purification. All anhydrous reactions were performed under argon. THF was dried over sodium/benzophenone. All reactions were monitored by TLC (silica gel, GF) and analyzed with UV light and developed with vanillin spray. ^{1}H NMR spectra were obtained in $CDCl_3$ at 270 and 400 MHz and were assigned by comparison of chemical shifts and coupling constants with those of related compounds and by appropriate 2D NMR techniques; coupling constants are reported in hertz. ^{13}C NMR spectra were assigned with the aid of HETCOR and DEPT spectra. ^{1}H NMR spectra showed the presence of traces of ethyl acetate; paclitaxel and its derivative retain ethyl acetate very tightly, and it cannot be removed completely even on prolonged treatment in vacuo at 38 °C. Exact mass measurements were performed at the Midwest Center for Mass Spectrometry, an NSF Regional Instrumentation Facility. IUPAC nomenclature for paclitaxel derivatives is used for title compounds.

1-Benzoyl-2-[(*S*-methyldithio)carbonyl]-2′,7-bis(triethylsilyl)paclitaxel (3). 2′,7-Bis(triethylsilyl)paclitaxel[4a] (54.75 mg, 0.05 mmol) was dissolved in freshly distilled dry THF (0.5 mL), and sodium hydride (3.5 mg, 0.15 mmol) followed by carbon disulfide (0.1 mL) was added at room temperature. After stirring for 5 min methyl iodide (50 μL, 0.8 mmol) was added to the reaction mixture and stirred for an additional 30 min. The reaction mixture was then diluted with EtOAc (10 mL) and washed with water followed by brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product thus obtained was purified by PTLC (SiO_2, 500 μm, ethyl acetate:hexane, 1:4) to yield compound **3** as a pale yellow solid (53.5 mg, 91%): ^{1}H NMR ($CDCl_3$) δ 0.45 (q, J = 7.8, 6H), 0.59 (q, J = 7.8, 6H), 0.82 (t, J = 7.8, 9H), 0.93 (t, J = 7.8, 9H), 1.24 (s, 3H), 1.37 (s, 3H), 1.75 (s, 3H), 2.04 (bs, 3H), 2.17 (s, 3H), 2.32 (m, 1H), 2.45 (s, 3H), 2.51 (s, 3H), 2.50 (m, 1H), 3.72 (dd, J = 8.2, 16.2, 1H), 4.00 (d, J = 6.6, 1H), 4.49 (m, 1H), 4.50 (d, J = 8.7, 1H), 4.60 (d, J = 8.7, 1H), 4.72 (d, J = 2.2, 1H), 4.99 (bd, J = 7.8, 1H), 5.73 (dd, J = 8.8, 2.2, 1H), 6.47 (bt and s, 2H), 6.83 (d, J = 6.6, 1H), 7.09 (d, J = 8.8, 1H), 7.30–7.55 (m, 11H), 7.80 (m, 4H); ^{13}C NMR ($CDCl_3$) 4.35, 5.29, 6.51, 6.74, 10.64, 14.34, 18.43, 20.84, 22.93, 23.01, 29.26, 30.45, 37.15, 45.13, 46.55, 55.66, 58.61, 71.15, 72.11, 74.68, 74.99, 76.84, 80.89, 83.52, 84.38, 89.07, 126.47, 127,22, 127.84, 128.05, 128.59, 128.60, 129.63, 130.65, 131.56, 131.69, 132.83, 134.25, 138.43, 141.55, 164.95, 166.99, 169.20, 170.35, 171.37, 201.36, 215.25; HRMS calcd for $C_{61}H_{81}O_{14}NS_2Si_2$ (MH^+) m/z 1172.4726, found 1172.4732.

1-Benzoyl-2-debenzoyl-2-[(*S*-methyldithio)carbonyl]-paclitaxel (4). 2′,7-Bis(triethylsilyl)-1-benzoyl-2-[(*S*-methyldithio)carbonyl]paclitaxel (**3**) (11.7 mg, 0.01 mmol) was dissolved in freshly prepared methanolic HCl (5% v/v, 0.2 mL) and

(8) A similar benzoyl group migration was proposed by us earlier in our studies on the preparation of 7-deoxypaclitaxel,[3a] but the structure of the intermediate was not confirmed at that time.

(9) The cytotoxicity data against P-388 murine leukemia cells for these compounds were determined by Dr. W. Lichter of the University of Miami; we gratefully acknowledge his assistance.

the solution stirred at room temperature for 30 min. The solution was then diluted with EtOAc (10 mL) and washed with water, dilute $NaHCO_3$, and finally with brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product thus obtained was purified further by PTLC (SiO_2, 500 μm, EtOAc:hexane, 1:1) to yield compound **4** as a white solid (8.5 mg, 90%): ^1H NMR ($CDCl_3$) δ 1.27 (s, 3H), 1.29 (s, 3H), 1.73 (s, 3H), 1.78 (s, 3H), 2.23 (s, 3H), 2.32 (m, 1H), 2.34 (s, 3H), 2.42 (s, 3H), 2.50 (m, 2H), 3.82 (dd, J = 8.2, 16.2, 2H), 3.93 (d, J = 6.4, 1H), 4.38 (m, 1H), 4.49 (d, J = 8.6, 1H), 4.56 (d, J = 8.6, 1H), 4.79 (dd, J = 2.9, 5.0, 1H), 4.98 (bd, J = 7.4, 1H), 5.80 (dd, J = 2.9, 8.8, 1H), 6.28 (s, 1H), 6.42 (t, 1H), 6.79 (d, J = 6.4, 1H), 7.18 (m, 1H), 7.30–7.55 (m, 11H), 7.80 (m, 4H); ^{13}C NMR ($CDCl_3$) 10.18, 14.94, 18.59, 20.81, 22.54, 23.18, 29.31, 30.74, 35.65, 44.86, 45.58, 55.10, 58.80, 71.79, 71.97, 73.59, 75.29, 76.68, 80.92, 83.42, 84.48, 89.11, 126.99, 127,15, 128.14, 128.23, 128.62, 128.91, 129.62, 130.52, 131.11, 131.83, 133.02, 133.71, 137.83, 143.34, 165.17, 167.04, 170.75, 171.07, 172.24, 203.22, 216.10; HRMS calcd for $C_{49}H_{53}O_{14}NS_2$ (MNa^+) m/z 966.2805, found 966.2818.

1-Benzoyl-2-des(benzoyloxy)-2′,7-bis(triethylsilyl)paclitaxel (5). Compound **3** (29.3 mg, 0.025 mmol) was dissolved in dry toluene (0.2 mL) and heated on an oil bath at 90 °C. AIBN (2.0 mg, catalytic) and tributyltin hydride (25.0 μL, 0.12 mmol) were added to the solution, and heating and stirring were continued for 1 h. The reaction mixture was then cooled to room temperature, diluted with CH_3CN (5 mL), and extracted with hexane (5 mL × 2). The hexane layer was discarded and the CH_3CN layer evaporated under reduced pressure to yield a thick syrup. Purification of this syrup over PTLC (SiO_2, 500 μm, ethyl acetate:hexane, 1:4) yielded compound **5** (21.0 mg, 79%) as a glassy solid: ^1H NMR ($CDCl_3$) δ 0.47 (q, J = 7.8, 6H), 0.59 (q, J = 7.8, 6H), 0.82 (t, J = 7.8, 9H), 0.93 (t, J = 7.8, 9H), 1.31 (s, 3H), 1.32 (s, 3H), 1.58 (s, 3H), 1.87 (m, 1H), 1.96 (bs, 3H), 2.06 (dd, J = 7.6, 15.1, 1H), 2.19 (s, 3H), 2.44 (s, 3H), 2.54 (m, 3H), 2.88 (dd, J = 9.3, 15.8, 1H), 3.27 (d, J = 7.6, 1H), 4.30 (d, J = 7.9, 1H), 4.41 (dd, J = 6.7, 10.2, 1H), 4.52 (d, J = 7.9, 1H), 4.60 (d, J = 1.9, 1H), 4.96 (bd, J = 9.0, 1H), 5.56 (dd, J = 1.9, 8.7, 1H), 6.09 (t, J = 9.1, 1H), 6.44 (s, 1H), 7.13 (d, J = 8.7, 1H), 7.30–7.60 (m, 11H), 7.75 (dd, J = 7.0, 1.4, 2H), 7.94 (dd, 2H, J = 1.3, 7.0); ^{13}C NMR ($CDCl_3$) 4.32, 5.21, 6.45, 6.71, 10.15, 14.09, 20.80, 22.74, 22.82, 25.53, 33.74, 37.60, 37.64, 40.44, 44.74, 55.83, 58.34, 71.41, 72.25, 74.58, 74.81, 74.92, 82.12, 82.99, 85.64, 126.52, 127.03, 127.90, 128.37, 128.51, 128.56, 129.43, 131.13, 131.51, 132.94, 133.33, 134.18, 138.59, 140.28, 165.37, 166.73, 169.16, 169.95, 171.63, 202.22; HRMS calcd for $C_{59}H_{79}O_{13}NSi_2$ (MNa^+) m/z 1088.4987, found 1088.5002.

1-Benzoyl-2-des(benzoyloxy)paclitaxel (6). Compound **5** (21.0 mg) was dissolved in freshly prepared methanolic HCl (5% v/v, 0.2 mL) and the solution stirred at room temperature for 30 min. The solution was then diluted with EtOAc (10 mL) and washed with water, dilute $NaHCO_3$, and finally with brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product thus obtained was purified further by PTLC (SiO_2, 500 μm, ethyl acetate:hexane, 1:1) to yield compound **6** as a white solid (14.3 mg, 87%): ^1H NMR ($CDCl_3$) δ 1.22 (s, 3H), 1.35 (s, 3H), 1.55 (s, 3H), 1.71 (s, 3H), 1.84 (dd, J = 6.6, 7.2, 1H), 2.01 (dd, J = 8.3, 15.1, 1H), 2.22 (s, 3H), 2.26 (s, 3H), 2.32 (d, J = 4.1, 1H), 2.42 (dd, J = 8.0, 16.1, 1H), 2.54 (m, 1H), 2.57 (d, J = 15.4, 1H), 3.01 (dd, J = 9.1, 16.1, 1H), 3.21 (d, J = 7.6, 1H), 3.86 (d, J = 5.9, 1H), 4.27 (d, J = 8.0, 1H), 4.32 (m, 1H), 4.48 (d, J = 8.0, 1H), 4.69 (dd, J = 3.0, 5.9, 1H), 4.94 (d, J = 8.3, 1H), 5.68 (dd, J = 3.0, 8.7, 1H), 6.08 (t, 1H), 6.24 (s, 1H), 7.09 (d, J = 8.7, 1H), 7.30–7.65 (m, 11H), 7.73 (dd, J = 1.3, 7.1, 2H), 7.95 (dd, J = 1.2, 7.1, 2H); ^{13}C NMR ($CDCl_3$) 9.53, 14.72, 20.82, 22.26, 23.25, 25.82, 33.75, 35.84, 38.09, 39.42, 44.44, 55.41, 58.50, 71.80, 72.15, 73.32, 74.51, 75.48, 81.95, 83.12, 85.76, 127.05, 127.09, 128.32, 128.48, 128.57, 128.91, 129.42, 131.01, 131.80, 132.71, 133.12, 133.62, 137.95, 142.15, 165.50, 167.03, 170.38, 170.89, 172.47, 204.38; HRMS calcd for $C_{47}H_{51}O_{13}N$ (MH^+) m/z 838.3439, found 838.3456.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **3–6** and ^1H NMR peak assignments of **3–6** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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