Selective Deesterification Studies on Taxanes: Simple and Efficient Hydrazinolysis of C-10 and C-13 Ester Functionalities

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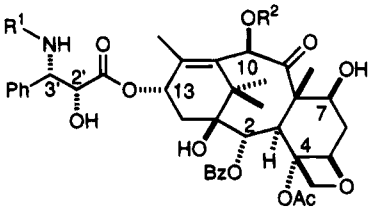
The novel antitumor agent paclitaxel (**1a**, Figure 1) has proven to be a most exciting discovery in the field of cancer chemotherapy.[1] Intense efforts are being directed to further modify this unique drug to design and synthesize more active, less toxic, and more water-soluble analogues.[2,3] The structure–activity profile of paclitaxel thus continues to be the focus of considerable attention to elucidate the paclitaxel pharmacophore.[2–7] In this context, the four ester groups of paclitaxel have been recognized to be potential sites, where conversion of these esters to the parent hydroxy compounds followed by further transformation will lead to the syntheses of a new generation of paclitaxel analogues.

Surprisingly, till to date, only a few methods have been reported for the selective hydrolysis of these ester groups. Magri *et al.* have reported on the selective reductive cleavage of the C-13 side chain of paclitaxel, using tetrabutylammonium borohydride.[8] The selective hydrolysis of the benzoate group at C-2 has only been achieved recently by three research groups.[9–11] In one method reaction of a 7,13-diprotected baccatin III with Red-Al afforded the corresponding 2-debenzoylated derivative in 78% yield.[9] In the second method, hydrolysis of 2′,7-diprotected paclitaxel with NaOH under phase transfer conditions formed the corresponding 2-debenzoylpaclitaxel derivative in moderate yield.[10] Also, our efforts to perform selective hydrolysis of the C-2 and C-4 ester groups of baccatin III resulted in the development of a protocol where we could utilize the conformation of the baccatin moiety and the free hydroxy groups at C-13 or C-1 as internal nucleophiles to selectively remove the C-4 acetate or C-2 benzoate groups respectively.[11]



1a, R^1 = Benzoyl, R^2 = Ac (Paclitaxel)
1b, R^1 = Tigloyl, R^2 = Ac (Cephalomannine)
1c, R^1 = *tert*-BuOCO, R^2 = H (Docetaxel)

Figure 1. Structures of taxanes.

In continuation of our hydrolysis studies[11] we decided to further explore and develop alternative methodologies for the selective removal of the esters at the C-10 and C-13 positions, and the results of these studies are reported herein.

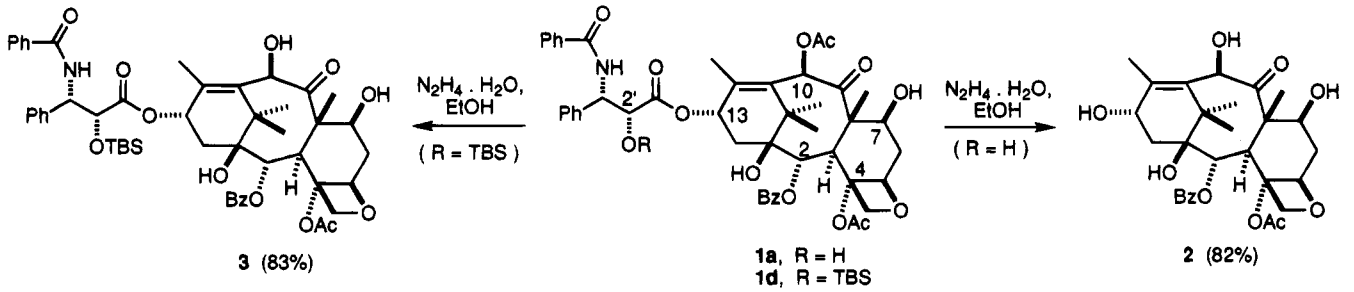
Our attention was drawn to the fact that both ammonia and hydrazine are used for the removal of ester groups under mild conditions and that acetates are cleaved preferentially in the presence of benzoate groups. Thus, we decided to start our intended deesterification studies using hydrazine as the deacylating reagent. When paclitaxel (**1a**) was treated with hydrazine monohydrate in ethanol the only product obtained was found to be 10-deacetylbaccatin III (**2**) (Scheme 1), formed by cleavage of the ester linkages at C-10 and C-13. Gratifyingly, the sterically hindered acetate at C-4 was not effected by the above reaction conditions. Encouraged by this success we decided to extend this reaction to a side cut from paclitaxel isolation, obtained from NCI, containing mainly paclitaxel (**1a**), and cephalomannine (**1b**, Figure 1) along with a small amount of other unidentified compounds, separation of which are often laborious and time consuming. Subjecting the NCI mixture to the above reaction cleanly afforded the expected 10-deacetylbaccatin III (**2**), a common semisynthetic precursor for both paclitaxel and docetaxel.[2,4–6] This method compares well with Kingston's method of cleaving the C-13 side chain,[8] both in terms of yield and reaction time, with the added advantage of requiring considerably cheaper reagent and solvent, an attractive economical proposition for large scale production.

We were able to further modify this reaction, where placement of a bulky *tert*-butyldimethylsilyl group at the 2′-oxygen (**1d**) presumably blocked the approach of the nucleophile toward the ester carbonyl at C-13 and selectively produced the 10-deacetylpaclitaxel analogue **3** (Scheme 1), under the above reaction conditions. This hydrazinolysis reaction at C-10 and/or C-13 was found to be general with both baccatin III (**4**) and 13-acetyl-7-(triethylsilyl)baccatin III (**5**). When reacted with hydrazine monohydrate, baccatin III (**4**) was quantitatively converted to 10-deacetylbaccatin III (**2**) (Scheme 2). *A noteworihy feature of this reaction is the absence of any detectable epimerization at C-7, a known side reaction in the other hydrolytic methods, thereby eliminating the necessity of protection and subsequent deprotection of the C-7 hydroxy group.*[12] The baccatin analogue **5**,[13] when subjected to the above reaction afforded 10-deacetyl-7-

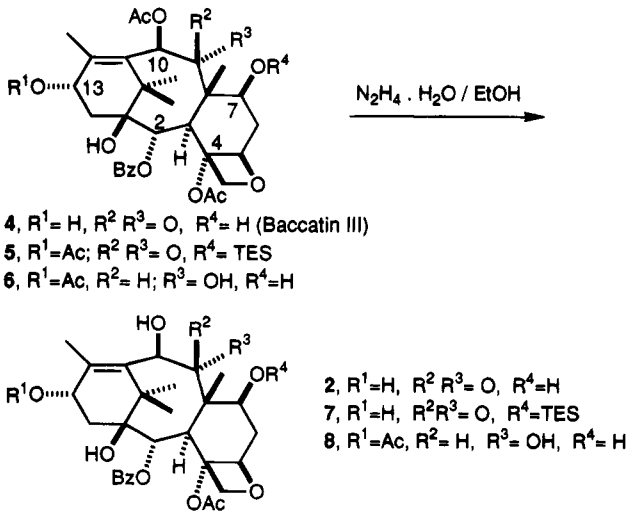
(1) For several reviews on paclitaxel see: *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. C., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995.
(2) For review: Suffness, M. *Annu. Rep. Med. Chem.* **1993**, *28*, 305–314.
(3) For review: Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995 (in press).
(4) For review: Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160–167.
(5) For review: Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Ed.; Springer: New York, 1993; Vol. 61; pp 1–206.
(6) For review: Georg, G. I.; Ali, S. M.; Zygmunt, J.; Jayasinghe, L. R. *Exp. Opin. Ther. Pat.* **1994**, *4*, 109–120.
(7) For review: Hepperle, M.; Georg, G. I. *Drugs Future* **1994**, *19*, 573–584.
(8) Magri, N. F.; Kingston, D. G. I.; Jitrangsri, C.; Piccariello, T. *J. Org. Chem.* **1986**, *51*, 3239–3242.
(9) Chen, S.-H.; Farina, V.; Wei, J.-M.; Long, B.; Fairchild, C.; Mamber, S. W.; Kadow, J. F.; Vyas, D.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 479–482.
(10) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097–4098.
(11) Datta, A.; Jayasinghe, L. R.; Georg, G. I. *J. Org. Chem.* **1994**, *59*, 4689–4690.
(12) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469–1474. For reviews on the facile epimerization at C-7 see ref 3 and 5.
(13) Samaranayake, G.; Neidigh, K. A.; Kingston, D. G. I. *J. Nat. Prod.* **1993**, *56*, 884–898.

0022-3263/95/1960-0761$09.00/0

Scheme 1



Scheme 2



(triethylsilyl)baccatin III (**7**)[14] (Scheme 2). However, 13-acetyl-9-dihydrobaccatin III (**6**)[15] under similar reaction conditions afforded only the corresponding C-10 deacylated product **8** (Scheme 2). Even prolonged reaction time and excess reagent failed to cleave the C-13 acetate. This result is in conformity with an earlier report where C-13 deacetylation of **6** could only be effected by using strong nucleophiles such as methyllithium or *n*-butyllithium.[16,17]

Interestingly, replacement of hydrazine hydrate by 1,1-dimethylhydrazine or phenylhydrazine in this method failed to effect any deesterification, and starting materials were recovered unchanged. Similarly, hydroxylamine also failed to perform the above deesterifications in any appreciable quantity even after prolonged reaction time and elevated temperature. These observations indicate the crucial role of hydrazine hydrate, both in terms of size and nucleophilicity of the reagent, for the success of the present reaction.

In conclusion, a simple and efficient pathway for the cleavage of the ester functionalities at C-10 and/or C-13 positions of both paclitaxel and baccatin analogues was developed. This method also provides an attractive alternative route for converting complex mixtures of various paclitaxel analogues, obtained from natural sources, to 10-deacetylbaccatin III, a known precursor of both paclitaxel and especially docetaxel, thereby augmenting the availability of these precious drugs.

Experimental Section[18]

10-Deacetylbaccatin III (2) from the Reaction of Paclitaxel (1a) with Hydrazine Monohydrate. To a solution of **1a** (35 mg, 0.04 mmol) in 95% ethanol (5 mL) was added hydrazine hydrate (0.5 mL), and the mixture was stirred at room temperature for 2 h. The mixture was then diluted with ethyl acetate (50 mL) and poured into saturated NH_4Cl solution. The organic layer was separated, washed with water and brine, dried (Na_2SO_4), and concentrated, and the residue was purified by flash column chromatography (SiO_2, EtOAc–hexane, 3:1) affording 10-deacetylbaccatin III (**2**), identical with an authentic sample (^1H NMR, MS, mp).[19] Yield, 18 mg (82%), mp 229–232 °C.

10-Deacetyl-2′-(*tert*-butyldimethylsilyl)paclitaxel (3). A solution of 2′-(*tert*-butyldimethylsilyl)paclitaxel (**1d**)[20] (50 mg, 0.05 mmol) in 95% ethanol (5 mL) and hydrazine monohydrate (0.5 mL) was stirred at room temperature for 1.5 h. Workup as described above and purification by flash column chromatography (SiO_2, hexane–EtOAc, 1:1 to 2:3) afforded **3** as a white solid. Yield = 39 mg (83%): mp 218–220 °C; ^1H NMR (300 MHz, $CDCl_3$) δ −0.26 (s, 3H), 0.01 (s, 3H), 0.82 (s, 9H), 1.12 (s, 3H), 1.24 (s, 3H), 1.78 (s, 3H), 1.95 (s, 3H), 2.14 and 2.38 (2m, 2H), 2.60 (s, 3H), 2.62 (m, 1H), 3.96 (d, J = 7.3 Hz, 1H), 4.24 (m, 2H), 4.36 (d, J = 8.4 Hz, 1H), 4.68 (d, J = 1.9 Hz, 1H), 5.0 (br d, J = 8.2 Hz, 1H), 5.21 (s, 1H), 5.73 (m, 2H) 6.32 (t, J = 9.3 Hz, 1H), 7.12 (d, J = 8.9 Hz, 1H), 7.33–7.65 (m, 11H), 7.77 (d, J = 7.3 Hz, 2H) 8.16 (d, J = 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.9, 14.3, 18.1, 21.1, 23.0, 25.5, 26.3, 36.0, 36.9, 43.1, 46.3, 55.6, 57.5, 71.3, 71.9, 74.4, 74.9, 75.1, 78.8, 81.0, 84.1, 126.4, 126.9, 127.9, 128.7, 128.8, 129.1, 130.2, 131.7, 133.6, 134.0, 135.7, 138.3, 138.6, 166.9, 169.7, 170.1, 171.2, 211.4; IR (neat) 3415, 1740, 1720 (br), 1655 cm^{-1}; MS (FAB^+) *m/z* calcd for 926.4147 (M + 1), found 926.4125 (M + 1); 926 (M + 1); $[\alpha]_D$ −43.4° (*c* = 0.8, $CHCl_3$). Anal. Calcd for $C_{51}H_{63}NO_{13}Si$: C, 66.14; H, 6.86; N, 1.51. Found: C, 65.78; H, 7.20; N, 1.47.

Conversion of Baccatin III (4) to 10-Deacetylbaccatin III (2). A solution of **4** (35 mg, 0.06 mmol) and hydrazine monohydrate (0.5 mL) in 95% ethanol (5 mL) was stirred at room temperature for 2 h. Workup as described above and purification by flash column chromatography (SiO_2, EtOAc–hexane, 4:1) afforded **2** as a white solid, yield, 28 mg (87%), mp 229–232 °C.

7-(Triethylsilyl)-10-deacetylbaccatin III (7).[14] A room temperature solution of 13-acetyl-7-(triethylsilyl)baccatin III (**5**)[13] (40 mg, 0.054 mmol) and hydrazine monohydrate (0.5 mL) in 95% ethanol (5 mL) was stirred for 8 h, after which usual workup and purification by flash chromatography (SiO_2, EtOAc–hexane, 2:1) yielded the pure product, **7**, as a white solid. Yield, 28 mg (81%), mp 252–255 °C.

13-Acetyl-10-deacetyl-9-dihydrobaccatin III (8). To a stirred solution of 13-acetyl-9-dihydrobaccatin III (**6**)[16] (35 mg, 0.055 mmol) in 95% ethanol (5 mL) at room temperature was added hydrazine monohydrate (0.5 mL), and the solution was stirred for 2 h. The reaction mixture on usual workup and purification by flash column chromatography (SiO_2, EtOAc–hexane, 1:3) afforded **8** as a white solid. Yield = 27 mg (84%):

(14) Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917–5919.
(15) We thank Dr. Larry L. Klein from Abbott Laboratories for kindly providing us a sample of 13-acetyl-9-dihydrobaccatin III.
(16) Klein, L. L. *Tetrahedron Lett.* **1993**, *34*, 2047–2050.
(17) Klein, L. L.; Yeung, C. M.; Li, L.; Plattner, J. J. *Tetrahedron Lett.* **1994**, *35*, 4707–4710.
(18) For general experimental information please see: Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. *J. Med. Chem.* **1992**, *35*, 4230–4237.
(19) 10-Deacetylbaccatin III is commercially available from Dabur India Limited, New Delhi, India.
(20) Magri, N. F.; Kingston, D. G. I. *J. Nat. Prod.* **1988**, *51*, 298–306.

mp 204–207 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 1.27 (s, 6H), 1.76 (s, 3H), 1.82 (s, 3H), 2.18 (s, 3H), 2.28 (s, 3H), 2.59 (m, 1H), 3.07 (d, J = 5.9 Hz, 1H), 4.18 and 4.24 (2d, J = 8.2 Hz, 2H), 4.32 (dd, J = 2.4, 7.4 Hz), 4.36 (d, J = 10.2 Hz, 1H), 4.90 (d, J = 10.4 Hz, 1H), 4.95 (d, J = 8.3 Hz, 1H), 5.76 (d, J = 6.2 Hz, 1H), 6.18 (t, J = 8.4 Hz, 1H), 7.46–7.64 (m, 3H), 8.09 (d, J = 7.1 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 12.9, 14.9, 21.4, 23.1, 23.3, 28.7, 36.5, 38.2, 44.1, 45.1, 47.8, 49.8, 71.2, 71.5, 74.5, 74.8, 77.5, 78.9, 80.7, 83.0, 85.4, 99.0, 129.3, 130.9, 134.2, 137.3, 139.3, 167.4, 170.9, 172.2; IR (neat) 3400 (br), 1730, 1710, 1600 cm^{-1}; MS (FAB$^+$) m/z calcd for 589.2649 (M + 1), found 589.2650 (M + 1); 589 (M + 1); $[\alpha]_D$ +2.3° (c = 0.35, MeOH). Anal. Calcd for $C_{31}H_{40}O_{11}$: C, 63.24; H, 6.85. Found: C, 62.90; H, 7.10.

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