Unexpectedly Facile Hydrolysis of the 2-Benzoate Group of Taxol and Syntheses of Analogs with Increased Activities

Ashok G. Chaudhary, Milind M. Gharpure, John M. Rimoldi, Mahendra D. Chordia, A. A. Leslie Gunatilaka, and David G. I. Kingston*

Department of Chemistry Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

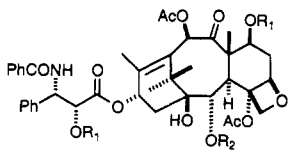
Surinder Grover, Chii M. Lin, and Ernest Hamel*

Laboratory of Molecular Pharmacology Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute National Institutes of Health Bethesda, Maryland 20892

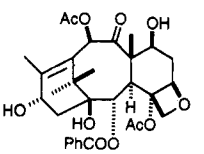
Received November 11, 1993

The diterpenoid taxol (**1**), first isolated from *Taxus brevifolia*,[1] has excellent clinical activity against ovarian and breast cancers and shows promise in the treatment of other cancers.[2] There have been extensive studies on the chemistry and structure–activity relationships of taxol,[3] but the preparation of 2-acyl analogs has not been reported because of the inability to debenzolyate selectively at the C-2 position. Previously, only hydrolysis of the side chain and/or the 10-acetate,[1,4] with no detectable hydrolysis of the 2-benzoate, has occurred. Hydrolysis of baccatin III (**2**) also proceeds in the order 10-acetate > 4-acetate > 2-benzoate,[5] although low-yield debenzoylations of 7,13-bis(triethylsilyl)-baccatin III[6] and of 7-(triethylsilyl)-10-deacetylbaccatin III[7] have been reported. Reacylation of a 2-debenzoylbaccatin III or taxol has not been described, in part because of the ready attack of the 2-hydroxyl group on the oxetane ring.[5-7] We now report that *a surprisingly selective hydrolysis of the C-2 benzoate ester can be achieved* and also that *reacylation with certain meta-substituted benzoic acids yields derivatives more cytotoxic than taxol and with greater activity in promoting tubulin assembly.*

Since most previous hydrolytic studies were carried out in methanolic solution and since taxol would be expected to adopt a different conformation in a non-hydroxylic environment, we investigated the use of phase-transfer catalysis to effect selective hydrolysis and obtained dramatic results. Treatment of 2',7-bis(triethylsilyl)taxol (**3**) with dilute NaOH under phase-transfer conditions[8] gave the 2-debenzoyl product **4** in 69% yield. Reacylation of **4** (RCOOH, DCC, PP) gave the 2-acyl-2-debenzoyl product **5**, avoiding the undesired intramolecular opening of the oxetane ring.[5-7] Deprotection (HCl/MeOH) gave the final product **6**. This procedure allows the synthesis of a 2-acyltaxol analog from taxol in four steps with an unoptimized overall yield of 57%. Support for the hypothesis that selective hydrolysis is favored by a solvent-dependent conformational change comes from recent NMR and molecular modeling studies,[9] which conclude that taxol adopts a globular conformation in aqueous media but a nonglobular conformation in chloroform. A nonglobular conformation opens the C-2 benzoate to nucleophilic attack, whereas such attack would be inhibited by the globular conformation.

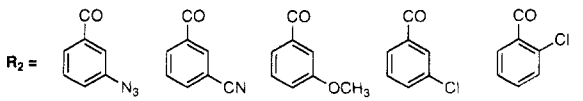


1 R_1 = H, R_2 = PhCO
3 R_1 = $SiEt_3$, R_2 = PhCO
4 R_1 = $SiEt_3$, R_2 = H
5 R_1 = $SiEt_3$, R_2 = ArCO
6 R_1 = H, R_2 = ArCO

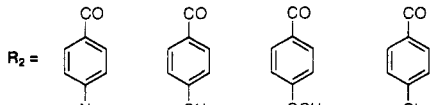


2

We prepared several meta- and para-substituted benzoyl analogs of taxol. Unexpectedly, *these two classes show strikingly different bioactivities.* In the P-388 murine leukemia cell line, the para derivatives **6b**, **6d**, and **6h** were much less cytotoxic than taxol, while the meta derivatives **6a**, **6c**, **6e**, and **6g** were all significantly more active than taxol, with cytotoxicities 150, 5, 800, and 700 times that of taxol.[10] A similar large difference between the meta and para derivatives was observed in the HL-60 human leukemia cell line, although here the increase in activity of the meta derivatives was less marked.[11] Analogs **6a** and **6e** were 3 times as cytotoxic as taxol, and **6c** was equipotent with taxol; **6b** and **6d** were orders of magnitude less cytotoxic than taxol. Of particular importance, **6a** was at least 10-fold more cytotoxic than taxol in five cell lines of the NCI tumor panel screen.



6a **6c** **6e** **6g** **6i**



6b **6d** **6f** **6h**

The morphology of drug-treated cells demonstrated that **6a** and taxol caused mitotic arrest in HL-60 cells, and a quantitative comparison of the interactions of the taxol and of **6a** and **6b** with tubulin was thus carried out.[12] The para derivative **6b** was 14-fold less active than taxol, and the meta derivative **6a** was slightly more active than taxol. However, *compound **6a** at 10 μM caused tubulin polymerization at 0 °C*, an effect not observed with taxol.

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.

(2) For reviews: (a) Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Sem. Oncol.* **1992**, *19*, 646–662. (b) Slichenmyer, W. J.; Von Hoff, D. D. *Anti-Cancer Drugs* **1991**, *2*, 519–530.

(3) Recent reviews on the chemistry and structure–activity relationships of taxol: (a) Kingston, D. G. I. *Pharmacol. Ther.*, **1991**, *52*, 1–35. (b) 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, Ch., Eds.; Springer-Verlag: Vienna, 1993; Vol. 61, pp 1–206. (c) Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160–167.

(4) (a) Miller, R. W.; Powell, R. G.; Smith, C. R., Jr.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1981**, *46*, 1469–1474. (b) Ringel, I.; Horwitz, S. B. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 692–698.

(5) (a) Farina, V.; Huang, S. *Tetrahedron Lett.* **1993**, *33*, 3979–3982. (b) Samaranayake, G.; Neidigh, K. A.; Kingston, D. G. I. *J. Nat. Prod.* **1993**, *56*, 884–898.

(6) Chen, S.-H.; Wei, J.-M.; Farina, V. *Tetrahedron Lett.* **1993**, *34*, 3205–3206.

(7) Wahl, A.; Guéritte-Voegelein, F.; Guénard, D.; Le Goff, M.-T.; Potier, P. *Tetrahedron* **1992**, *48*, 6965–6974.

(8) Experimental details of the conversions of **3** to **4** and **4** to **6a** are provided as supplemental data to this paper.

(9) (a) Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. *Tetrahedron* **1993**, *49*, 6545–6560. (b) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 11650–11651.

(10) Cytotoxicities against P-388 murine leukemia cells were determined by Dr. W. Lichter of the University of Miami; we gratefully acknowledge his assistance.

(11) HL-60 cells were inoculated into RPMI-1640 medium (supplemented with 0.03% glutamine, 10 mM Hepes buffer, and 9% fetal calf serum) at 2 × 10^5 cells/mL and counted after 24 h of growth at 37 °C.

(12) Interactions were quantitated by examining the effects of low concentrations on the cold-stability of polymer formed from electrophoretically homogeneous bovine brain tubulin in 1 M monosodium glutamate–0.1 mM GTP,[14] based on the method described by Lataste et al.[15] Polymerization also occurs under this reaction condition in the absence of drug.

0002-7863/94/1516-4097$04.50/0

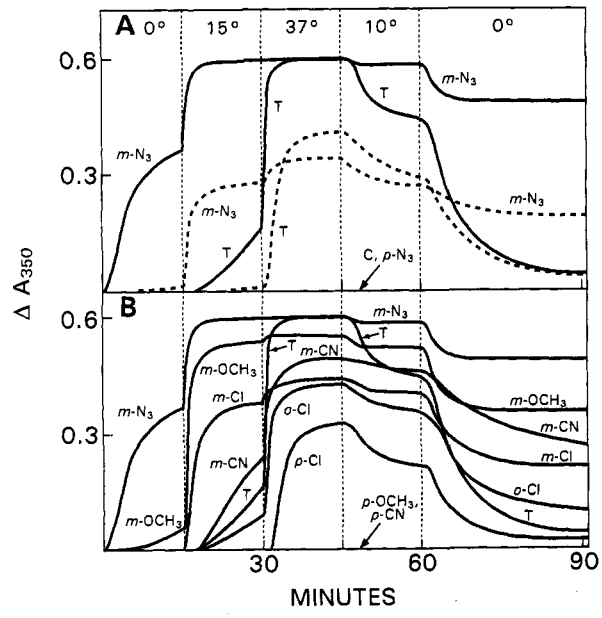


Figure 1. Effects of taxol (T) and compounds **6a–i** (indicated by their substituents) on tubulin polymerization in 0.1 M Mes. Each 0.25-mL reaction mixture contained 1.0 mg/mL (10 μM) tubulin, 0.1 M Mes (pH 6.9 with NaOH), 0.5 mM $MgCl_2$, 100 μM GTP, 4% (v/v) dimethyl sulfoxide, and drugs as indicated; the dashed curves are for 10 μM drug and the solid curves for 40 μM drug. All components except drug were added to cuvettes held at 0 °C by an electronic temperature controller, and initial base lines were established. Drugs were added individually to reaction mixtures, the samples were rapidly mixed, and turbidity changes were followed for 15 min at 0 °C. The arrows pointing to the abscissas, labeled "C, *p*-N_3" in panel A and "*p*-OCH_3, *p*-CN" in panel B, indicate that no change in turbidity occurred without drug or in the presence of 10 or 40 μM **6b**, **6d**, or **6f**.

This striking difference between taxol and **6a** on the assembly of tubulin at 0 °C led us to examine the tubulin–**6a** interaction in detail. Major differences between agents were observed in a system in which tubulin will not polymerize without drug.[13] In this system (Figure 1, panel A), 10 or 40 μM **6b** failed to induce polymerization. With 10 μM taxol, significant assembly occurred only at 37 °C, yielding polymer stable at 10 °C but not at 0 °C. With 10 μM **6a**, brisk polymerization occurred at 15 °C, and the polymer was over 50% stable at 0 °C. When drug concentration was increased to 40 μM, there was a slow polymerization reaction with taxol at 15 °C, followed by a rapid reaction at 37 °C, but the polymer formed was still cold-labile at 0 °C. With 40 μM **6a**, the initial reaction was shifted to 0 °C, and *this reaction was both more rapid and more extensive than the taxol reaction at 15 °C.* Polymerization was complete at 15 °C, and the polymer formed, unlike that with taxol, was largely stable in the cold. These results show that *the analog* **6a** *promotes tubulin assembly under conditions where taxol is inactive.*

Examination of additional analogs **6c–6i** provided additional information on the tubulin-assembly properties of these compounds (Figure 1, panel B). Although none was as potent as **6a** in inducing tubulin polymerization, all the meta-substituted derivatives were more active than taxol (N_3 > OCH_3 > Cl > CN). The para substituted derivatives were all much less active than taxol, with only the chloro derivative able to induce formation of a cold-labile polymer. Thus far a single ortho-substituted compound (the chloro derivative **6i**) has been prepared, and it was slightly less active than taxol. In the chloro series, the order of activity is meta > ortho > para.

Table 1. Comparison of Taxol and C-2 Derivatives on Assembly Rates at 15 °C and Extent of Assembly at 37 °C

	10 μM drug		40 μM drug	
compound	maximum rate at 15 °C[a] (munits ΔA_{350}/min)	extent[b] (ΔA_{350})	maximum rate at 15 °C[a] (munits ΔA_{350}/min)	extent[b] (ΔA_{350})
taxol (**1**)	1.2 (1)	0.38 (1)	23 (1)	0.60 (1)
6a (*m*-N_3)	119 (99)	0.34 (0.9)	NM[c]	0.60 (1)
6e (*m*-OCH_3)	51 (43)	0.37 (1)	271 (12)	0.54 (0.9)
6g (*m*-Cl)	28 (23)	0.38 (1)	128 (6)	0.44 (0.7)
6c (*m*-CN)	1.3 (1.1)	0.40 (1.1)	33 (1.4)	0.51 (0.9)
6i (*o*-Cl)	0.7 (0.6)	0.25 (0.7)	9.3 (0.4)	0.43 (0.7)
6h (*p*-Cl)	0 (0)	0.15 (0.4)	0 (0)	0.32 (0.5)
6f (*p*-OCH_3)	0 (0)	0 (0)	0 (0)	0 (0)
6d (*p*-CN)	0 (0)	0 (0)	0 (0)	0 (0)
6b (*p*-N_3)	0 (0)	0 (0)	0 (0)	0 (0)

[a] The maximum assembly rate at 15 °C was determined from the experiments shown in Figure 1, together with additional experiments performed with analogs at 10 μM that are not presented here. The number in partheses is relative to taxol. Individual experiments were performed with four samples in the spectrophotometers, with a dwell time at each position of 5 s. About 0.42 min elapsed between successive readings at each position. The maximum interval increase in reading was used to calculate the maximum rate. [b] Maximum extent of assembly was determined from the same experiments, subtracting the initial turbidity reading at 0 °C from the final reading at 37 °C. [c] NM, not meaningful, since the reaction at 0 °C was so extensive (see Figure 1).

The data of Figure 1, including additional experiments performed with the taxol analogs at 10 μM, are presented in a quantitative comparison in Table 1. The maximum assembly rates at 15 °C and the final turbidity values are compared. The most active agents differed little from taxol in the extent of tubulin assembly, but striking increases in the assembly rates at 15 °C were observed for **6a**, **6e**, and **6g**.

The enhanced activity of **6a**, **6c**, **6e**, and **6g** and the reduced activity of **6b**, **6d**, **6f**, and **6h** relative to taxol is consistent with the recent observation that the C-2 substituent is critical for taxol's activity.[6] Additional insight is provided by studies on the conformation of taxol and taxotere in aqueous solution by NMR spectroscopy and molecular modeling.[9,15] These showed that hydrophobic interactions between the C-2 benzoate residue and the phenyl group of the C-13 side chain are important in the overall conformation of active compounds. Our results suggest that para groups disrupt these side-chain interactions while meta groups enhance them. Such findings raise the interesting question of whether it is the sidechain complex itself and subtle constraints that such hydrophobic sidechain interactions impose on the conformation of the taxane skeleton that are the key molecular features recognized by tubulin. We should also note that although both **6a** and **6b** are potentially photoreactive, we have no evidence that photoactivation plays a major role in the properties described here.

In summary, we have described a method to deacylate taxol at position C-2 and place alternate aroyl groups at this position. Two classes of analogs that we have prepared, the meta-substituted benzoyl derivatives **6a**, **6c**, **6e**, and **6g** and the para-substituted benzoyl derivatives **6b**, **6d**, **6f**, and **6h**, differ dramatically from each other and from taxol in their biological properties. Our results indicate that modification of taxol at C-2 is a promising approach to the preparation of taxol analogs with improved anticancer activity.[16]

Supplementary Material Available: Experimental details of the conversion of **3** to **4** and **4** to **6a** (1 page). This material is contained in many 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.

(13) Rimoldi, J. M.; Kingston, D. G. I.; Chaudhary, A. G.; Samaranayake, G.; Grover, S.; Hamel, E. *J. Nat. Prod.* **1993**, *56*, 1313–1330.
(14) Lataste, H.; Senilh, V.; Wright, M.; Guénard, D.; Potier, P. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4090–4094.
(15) Dubois, J.; Guénard, D.; Guéritte-Voegelein, F.; Guedira, N.; Potier, P.; Gillet, B.; Beloeil, J.-C. *Tetrahedron* **1993**, *49*, 6533–6544.

(16) Support of this work by the National Cancer Institute, Grants No. CA 55131 and CA 48974, is gratefully acknowledged.