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MODIFIED TAXOLS, 6.[1] PREPARATION OF WATER-SOLUBLE PRODRUGS OF TAXOL

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ABSTRACT.—Three H_2O-soluble prodrugs of taxol have been prepared using a sulfonate group as the H_2O-solubilizing group. The first two contain taurine or 3-amino-1-sulfopropionic acid linked to the 2′-position of taxol through a succinate group, and a novel method of preparing these derivatives is described. The third derivative is prepared by the Michael addition of bisulfite to 2′-acryloyltaxol. A 2′-(γ-aminobutyryl)taxol salt was too unstable to be isolable.

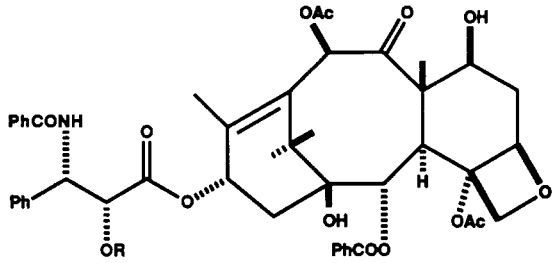
The diterpenoid natural product taxol [**1**], first isolated by Wani *et al.* (1), is of great current interest because of its clinical anticancer activity. In clinical trials it shows excellent activity against ovarian cancer (2), even in patients resistant to *cis*-platin (3), and clinical trials in other cancers are in progress. These is thus little doubt that taxol will make a major contribution to cancer treatment.

One of the major difficulties in the use of taxol as an anticancer agent is its almost complete insolubility in H_2O, which has necessitated a complex emulsion formulation in the current clinical trials (2,3). In attempts to address this problem, both we (4) and Deutsch *et al.* (5) have prepared H_2O-soluble prodrugs of taxol. The compounds prepared to date have been based on ammonio or carboxylate groups as solubilizing groups. In this study the use of sulfonate groups as solubilizing groups has been investigated; this group was selected because sulfonate salts are neutral, and sulfonate derivatives might thus prove to be more stable than simple carboxylate salts. The good activity seen for simple carboxylate salts (5) also raised the hope that a sulfonate salt might prove even more active. Two independent methods of preparing sulfonate derivatives of taxol have been developed, and in addition, two other 2′-acyltaxol derivatives have been prepared.

The preferred position for the preparation of prodrugs of taxol is the 2′-position, since many 2′-acyl taxols hydrolyze fairly rapidly back to taxol (4,6). The preparation of 2′-succinyltaxol [**2**] has previously been described (4,5), and the first approach thus consisted in the preparation of amide derivatives of **2** with taurine or its homologue 3-amino-1-sulfopropionic acid.

A major practical problem in the use of taurine or its homologue is the fact that both compounds are essentially insoluble in organic solvents. Previous work on the use of these compounds to prepare prodrugs circumvented this problem by the use of aqueous DMF as a solvent for the coupling reaction (7), but this solution is undesirable in the case of taxol derivatives because of their lability in basic aqueous conditions. It was thus necessary to develop an alternate procedure for the coupling reaction; this was achieved by conversion of taurine or its homologue to their organic-soluble tetrabutylammonium salts. Coupling of 2′-succinyltaxol with the tetrabutylammonium salt of either taurine or its homologue was achieved in THF solution by the mixed anhydride

[1]For Part 5, see S.G.S. Samaranayake and D.G.I. Kingston, *J. Org. Chem.*, **56**, 5114 (1991).



1 R=H
2 R=$COCH_2CH_2COOH$
3 R=$COCH_2CH_2CONHCH_2CH_2SO_3^-N^+Bu_4$
4 R=$COCH_2CH_2CONHCH_2CH_2SO_3^-N^+Bu_4$
5 R=$COCH_2CH_2CONHCH_2CH_2SO_3^-Na^+$
6 R=$COCH_2CH_2CONHCH_2CH_2CH_2SO_3^-Na^+$
7 R=$COCH=CH_2$
8 R=$COCH_2CH_2SO_3^-Na^+$
9 R=$COCH_2CH_2CH_2NHCOOCH_2C_6H_5$
10 R=$COCH_2CH_2CH_2NH_3^+HCOO^-$

method. The resulting tetrabutylammonium salts **3** and **4** were converted to the corresponding sodium salts **5** and **6** by ion exchange.

The second approach used the nucleophilicity of sodium bisulfite in a Michael reaction as the key step. 2′-Acryloyltaxol [7] was prepared by the mixed anhydride method and was reacted with sodium metabisulfite in aqueous iPrOH. Michael addition to the αβ-unsaturated ester occurred readily to yield the sodium sulfonate **8**.

The relative H_2O solubilities of the three sulfonates **5**, **6**, and **8** were determined by octanol/H_2O partition (8). The sulfonates had partition coefficients ($C_{water}/C_{octanol}$) that were, respectively, 191, 118, and 210 times greater than that of taxol.

In addition to the sulfonate derivatives **5**, **6**, and **8**, two additional 2′-acyltaxols were prepared. In earlier work (4) the preparation of 2′-(β-alanyl) taxol was described; this compound had useful solubility characteristics but reverted rather rapidly to taxol. The preparation of 2′-glycyltaxol was also attempted (9), but it proved to be even less stable than the β-alanyl derivative. The preparation of 2′-(γ-aminobutyryl) taxol was attempted to determine whether a lengthening of the carbon chain might prove beneficial. Taxol was coupled with *N*-carbobenzyloxy-γ-aminobutyric acid to yield 2′-(*N*-Cbz-γ-aminobutyryl) taxol, which was deprotected with HCO_2H over palladium. The resulting 2′-(γ-aminobutyryl) taxol could be detected in the crude product by ^{1}H-nmr spectroscopy, but it decomposed to taxol on standing in an organic solvent for a few hours and is thus even less stable than the 2′-(β-alanyl) derivative described earlier.

The relative stability of the 2′-(ω-aminoacyl) taxols thus appears to be greatest for 2′-(β-alanyl) taxol, with both 2′-glycyl taxol and 2′-(γ-aminobutyryl) taxol being less stable. The lack of stability of the γ-aminobutyryl derivative may be explained by its ability to undergo intramolecular displacement on the 2′-acyl group through a 5-membered ring intermediate, while the instability of the 2′-glycyl derivative may be due to a simple inductive effect of the protonated amino group assisting attack of external nucleophiles on the 2′-acyl group. The 2′-β-alanyl group minimizes both of these effects and is thus relatively more stable.

Recently a patent describing the preparation of a range of 2′- and 7′-aminoacyl taxol derivatives has appeared (10). These investigators prepared 2′-(*N*,*N*-dimethylglycyl) taxol and 2′-(*N*,*N*-diethyl-β-alanyl) taxol, among other compounds. These dialkylamino derivatives (as their methane sulfonate salts) appear to be more stable than the formate salts described above, but they show the same stability trend, with

TABLE 1. In vitro Cytotoxicity of Compounds **5**, **6**, and **8**.[a]

Compound	Cell line				
	A549	A549/VP	B16-PRIM	HCT-116	HCT/VP35
Taxol [**1**]	4.79	<0.02	<0.02	<0.02	<0.02
5	0.09	<0.02	<0.02	<0.02	0.03
6	0.19	0.11	<0.02	<0.02	0.04
8	0.40	0.19	0.02	<0.02	0.05

[a]Table entries are IC_{50} (μg/ml).

the β-alanyl derivative being more stable than the glycyl derivative in pH 4.5 acetate buffer.

The biological activity of compounds **5**, **6**, and **8** was investigated using four different cell lines in vitro and P-388 lymphocytic leukemia in vivo. The results, shown in Tables 1 and 2, indicate that these compounds have somewhat reduced activity as compared to taxol.

TABLE 2. In vivo P-388 Activity of Compounds **5**, **6**, and **8**.[a]

Compound	T/C (dose, mg/kg)				
Taxol [**1**]	155 (16)	132 (8)	150 (4)		
5	132 (64)	136 (32)	127 (16)	114 (8)	109 (4)
6	127 (48)	123 (24)	118 (12)	114 (6)	109 (3)
8	136 (40)	136 (20)	127 (10)	123 (5)	109 (2.5)

[a]ip injection, schedule Q01D × 5; 1.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The general procedures used were as previously described (4). The ^{1}H-nmr spectra of the taxol ring systems of compounds **3–8** were essentially identical to those previously reported for other 2′-acyl taxols (4), and thus are not listed in the experimental sections below.

TETRABUTYLAMMONIUM SALTS OF TAURINE AND 3-AMINO-1-SULFOPROPIONIC ACID.—Taurine (250 mg, 2 mmole) was dissolved in a minimum volume of distilled H_2O and treated with tetrabutylammonium hydroxide (1.0 ml of 40 wt % aqueous solution, 1.56 mmol). The solution was stirred for 1 h at room temperature and evaporated to dryness. The residue was dissolved in dry THF, filtered to remove unreacted taurine, and evaporated to dryness for quantitation. A similar procedure was used with 3-amino-1-sulfopropionic acid.

2′-({ 4-[(2-SULFOETHYL)AMINO]-1,4-DIOXOBUTYL } OXY) TAXOL TETRABUTYLAMMONIUM SALT [**3**].—2′-Succinyl taxol [**2**] (4) (122 mg) was dissolved in dry THF (4 ml), Et_3N (50 μl) was added, and the solution was cooled to 0°. Isobutyl chloroformate (50 μl) was added, and the mixture was warmed to room temperature over 15 min. Taurine tetrabutylammonium salt (91 mg in 0.5 ml THF) was added, and the reaction mixture was stirred at room temperature for 5 h. After this time reaction was complete, as indicated by tlc [SiO_2, EtOAc-MeOH (2:1)], and the solution was filtered and evaporated to dryness. The product was purified by flash chromatography on Si gel [300 × 15 mm, CH_2Cl_2-MeOH (7:1)] to yield the tetrabutylammonium salt **3** (168 mg, 100%): mp 168–170°; $[\alpha]^{20}D$ −24.06° (c = 0.0086, MeOH); ir (KBr) 3460, 3000, 1760, 1740, 1670, 1560, 1400, 1260, 1180, 1060 cm^{-1}; ^{1}H nmr 2.82 (2H, t, 8, $OCOCH_2CH_2CONH$) 2.74 (2H, t, 8, $OCOCH_2CH_2CONH$), 3.77 (1H, t, 7, NH), 3.6 (2H, m, $-NHCH_2CH_2SO_3^-$), 2.94 (2H, m, $-NHCH_2CH_2SO_3^-$), 3.28 [8H, t, 9, $N^+(CH_2CH_2CH_2CH_3)_4$, 1.68 [8H, m, $N^+(CH_2CH_2CH_2CH_3)_4$], 1.45 [8H, m, $N^+(CH_2CH_2CH_2CH_3)_4$], 1.05 [12H, t, $N^+(CH_2CH_2CH_2CH_3)_4$].

2′-({ 4-[(2-SULFOETHYL)AMINO]-1,4-DIOXOBUTYL } OXY)TAXOL SODIUM SALT [**5**].—The tet-

rabutylammonium salt **3** (160 mg) was placed in a beaker with Dowex 50 ion exchange resin in the Na^+ form (3 ml of resin in 3 ml deionized H_2O). The mixture was stirred at room temperature for 1.5 h, then passed through a small resin column containing 2 ml resin in the Na^+ form, with deionized H_2O as solvent. The solution was azeotroped to dryness with MeCN to yield the sodium salt **5** (122 mg, 92%): mp 174–175°; $[\alpha]^{20}D$ −29.8° (c = 0.0055, MeOH); ir (KBr) 3450, 3000, 1760, 1730, 1660, 1560, 1400, 1260, 1190, 1050 cm^{-1}; fabms *m/z* $[MNa]^+$ 1105, $[MH]^+$ 1083; ^1H nmr δ 2.72 (2H, m, -OCO*CH*$_2$CH$_2$CONH-), 2.52 (2H, m, -OCOCH$_2$*CH*$_2$CONH-), 3.58 (1H, t, 7, CO*NH*CH$_2$), 3.58 (2H, m, -NH*CH*$_2$CH$_2$SO$_3^-$), 2.96 (2H, m, -NHCH$_2$*CH*$_2$SO$_3^-$).

2′-({ 4-[(3-SULFOPROPYL)AMINO]-1,4-DIOXOBUTYL } OXY) TAXOL TETRABUTYLAMMONIUM SALT [**4**].—The same procedure described above for compound **3** was employed with 2-succinyltaxol and 3-amino-1-sulfopropionic acid tetrabutylammonium salt. The homogeneous salt **4** was obtained in 71% yield after flash chromatography: mp 165–166°; $[\alpha]^{20}D$ −17.9° (c = 0.0033, MeOH); ir (KBr) 3460, 3000, 1760, 1740, 1670, 1560, 1400, 1260, 1180, 1060 cm^{-1}; ^1H nmr δ 2.79 (2H, t, -OCO*CH*$_2$CH$_2$CONH), 2.55 (2H, t, 7, -OCOCH$_2$*CH*$_2$CONH-), 3.28 (2H, m, -NH*CH*$_2$CH$_2$CH$_2$SO$_3^-$), 1.98 (2H, m, -NHCH$_2$*CH*$_2$CH$_2$SO$_3^-$), 2.87 (2H, t, 7, -NHCH$_2$CH$_2$*CH*$_2$SO$_3^-$), 3.25 [8H, t, 9, N^+(*CH*$_2$CH$_2$CH$_2$CH$_3$)$_4$], 1.69 [8H, m, N^+(CH$_2$*CH*$_2$CH$_2$CH$_3$)$_4$, 1.43 [8H, m, N^+(CH$_2$CH$_2$*CH*$_2$CH$_3$)$_4$], 1.09 [12H, t, 9, N^+(CH$_2$CH$_2$CH$_2$*CH*$_3$)$_4$].

2′-({ 4-[(3-SULFOPROPYL)AMINO]-1,4-DIOXOBUTYL } OXY) TAXOL SODIUM SALT [**6**].—Treatment of compound **4** with Dowex 50 resin in the Na^+ form as described for compound **3** yielded the sodium salt **6** in 79% yield: mp 168–169°; $[\alpha]^{20}D$ −29° (c = 0.001, MeOH); ir (KBr) 3480, 3000, 1760, 1740, 1660, 1550, 1400, 1260, 1050 cm^{-1}; fabms *m/z* $[MNa]^+$ 1119, $[MH]^+$ 1097; ^1H nmr δ 2.75 (2H, t, 7, -OCO*CH*$_2$CH$_2$CONH$^-$), 2.54 (2H, t, 7, -OCOCH$_2$*CH*$_2$CONH$^-$), 3.25 (2H, m, -NH*CH*$_2$CH$_2$CH$_2$SO$_3^-$), 1.98 (2H, m, -NHCH$_2$*CH*$_2$CH$_2$SO$_3^-$), 2.85 (2H, t, 7, -NHCH$_2$CH$_2$*CH*$_2$SO$_3^-$).

2′-ACRYLOYLTAXOL [**7**].—A solution of triethylamine (50 μl) and acrylic acid (30 μl) in dry THF (5 ml) under argon was cooled to 0° and treated with isobutylchloroformate (50 μl). The solution was warmed to room temperature and treated with taxol (100 mg), and the resulting solution was stirred at 60° for 15 h. The precipitated triethylamine hydrochloride was filtered off and the filtrate evaporated; the residue was purified by flash chromatography [SiO_2, CH_2Cl_2-EtOAc (1:1)] to yield 100 mg of compound **7** (94%): mp 160–161°; $[\alpha]^{20}D$ −32° (c = 0.002, MeOH); ir (KBr) 3500, 2970, 2370, 1740, 1660, 1385, 1260, 1190, 990 cm^{-1}; fabms *m/z* $[MNa]^+$ 930, $[MH]^+$ 908; ^1H nmr δ 6.20 (1H, dd, 12, 10, OCO*CH*=CH$_2$), 5.95 (1H, dd, 3, 10, OCCH=*CH*$_2$), 6.45 (1H, dd, 3, 12, OCOCH=*CH*$_2$).

2′-[(3-SULFO-1-OXOPROPYL)OXY] TAXOL SODIUM SALT [**8**].—2-Acryloyltaxol [**7**] (85 mg) was dissolved in 3 ml distilled iPrOH and treated with sodium metabisulfite (85 mg in 1 ml H_2O). The reaction mixture was stirred at 60° for 15 h, until reaction was complete as judged by tlc [SiO_2, CH_2Cl_2-MeOH (10:1)]. The solvents were removed in vacuo, and the product was purified by flash chromatography [SiO_2, CH_2Cl_2-iPrOH (2:1)] to yield the sodium salt **8** (84 mg, 85%): mp 175–176°; $[\alpha]^{20}D$ −30° (c = 0.0012, MeOH); ir (KBr) 3500, 2950, 1760, 1730, 1660, 1380, 1250, 1190, 1100, 800 cm^{-1}; fabms *m/z* $[MNa]^+$ 1034, $[MH]^+$ 1012; ^1H nmr δ 2.93 (2H, t, 8, -OCO*CH*$_2$CH$_2$SO$_3^-$), 3.14 (2H, t, 8, -OCOCH$_2$*CH*$_2$SO$_3^-$).

2′-*N*-(CARBOBENZYLOXY)-γ-AMINOBUTYRYL TAXOL [**9**].—Taxol (20 mg) was treated in dry MeCN (4 ml) with dicyclohexylcarbodiimide (40 mg) and *N*-Cbz-γ-aminobutyric acid (20 mg). The mixture was stirred at room temperature for 30 h, the precipitated dicyclohexyl urea was filtered off, and the solvent was removed in vacuo. The product was purified by preparative tlc [SiO_2, hexane-EtOAc (45:55)] to yield **9** (19.1 mg, 76%): mp 168–170°; $[\alpha]^{20}D$ −22.5 (c = 0.002, MeOH); ir (KBr) 3450, 2950, 1740, 1720, 1660, 1530, 1375, 1240, 1070, 1020 cm^{-1}; ^1H nmr 3.14 (2H, m, -OCO*CH*$_2$CH$_2$CH$_2$NH−), 2.42 (2H, m, -OCOCH$_2$*CH*$_2$CH$_2$NH−), 3.4 (2H, m, -OCOCH$_2$CH$_2$*CH*$_2$NH−), 4.87 (1H, t, 7, -OCOCH$_2$CH$_2$CH$_2$*NH*CO), 4.95 (2H, s, O*CH*$_2$C$_6$H$_5$), 7.4–8.1 (5H, m, OCH$_2$*C*$_6$*H*$_5$).

2′-(γ-AMINOBUTYRYL) TAXOL FORMATE [**10**].—The Cbz-aminotaxol **9** was dissolved in MeOH (15 ml), and HCO_2H (1 ml) and 5% Pd/C (5 mg) were added. The solution was stirred at room temperature for 26 h and filtered to remove Pd/C, and the solvents were removed in vacuo. The crude product showed ^1H nmr δ 3.14 (2H, m, -OCO*CH*$_2$CH$_2$CH$_2$N$^+$H$_3$), 2.42 (2H, m, -OCOCH$_2$*CH*$_2$CH$_2$N$^+$H$_3$), 3.40 (2H, m, -OCOCH$_2$CH$_2$*CH*$_2$N$^+$H$_3$) and gave no signals for the benzyloxy group. However, on standing in solution at room temperature for 15 h, the aminotaxol **10** reverted back to taxol, as indicated by tlc and ^1H-nmr evidence.

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