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MODIFIED TAXOLS, 4.[1] SYNTHESIS AND BIOLOGICAL ACTIVITY OF TAXOLS MODIFIED IN THE SIDE CHAIN

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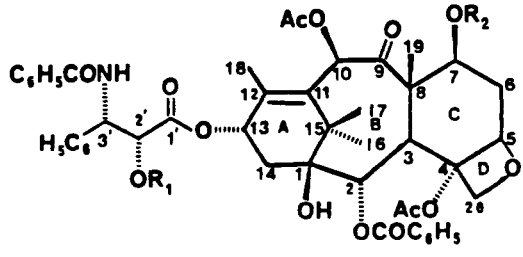
ABSTRACT.—A number of taxol derivatives substituted at the 2′ position of the side chain have been prepared and their biological activities determined in KB cell culture and/or the P-388 in vivo assay. The 2′-(*t*-butyldimethylsilyl)taxol **5** is essentially inactive, indicating the need for a free hydroxyl group at the 2′ position for activity. Epimerization of the 2′ position occurred on treatment of 2′-acetyltaxol derivatives with 1,5-diazabicyclo[5.4.0]undec-7-ene, but treatment of 2′-(2,2,2-trichloroethyloxycarbonyl) taxol derivatives with DBU yielded the novel cyclization products **11** and **12** and, after deprotection at the 7 position, **13**. The derivative **13** is also essentially inactive in the KB test system. Two taxols with increased H_2O solubility were prepared, the 2′-(β-alanyl) derivative **15** and the 2′-succinyl derivative **16**. Although both these derivatives were active in vivo and in vitro, the former was too unstable and the latter not active enough to make suitable H_2O-soluble derivatives of taxol.

The complex diterpenoid taxol [**1**], first reported in 1971 (1), has aroused considerable interest on account of its promising antitumor activity and also because of its unusual mechanism of action (2). Because of the importance of taxol as a potential antitumor drug, we have been studying structure-activity relationships of its derivatives in order to discern those structural features that are essential for activity. In previous papers in this series we have discussed various taxol acetates (3), oxidation products of taxol (4), and baccatin III derivatives (5).

Our previous studies on the synthesis and biological activity of taxol acetates had involved 2′-acetyltaxol [**2**], 7-acetyltaxol [**3**], and 2′,7-diacetyltaxol [**4**] (3). Although these experiments yielded useful data, they did not address the question of the importance of the 2′-hydroxyl group for biological activity, for acyl substituents at the 2′ position are readily hydrolyzed under in vivo conditions, and 2′-acetyltaxol [**2**] and 2′,7-diacetyltaxol [**4**] thus both showed activity in a cell culture bioassay (3). This lability of acyl substituents at the 2′ position suggested that 2′-acyltaxols could serve as prodrug forms of taxol, and we thus investigated the synthesis and biological activity of taxol derivatives substituted at the 2′ position.

RESULTS AND DISCUSSION

Our first approach was to prepare a hydrolytically stable 2′-derivative of taxol, so



1 $R_1=R_2=H$
2 $R_1=Ac$, $R_2=H$
3 $R_1=H$, $R_2=Ac$
4 $R_1=R_2=Ac$

[1]For part 3 in this series, see Magri *et al.* (5).

that the importance of the 2′-hydroxyl group could be accurately assessed. Treatment of taxol with *t*-butyldimethylsilyl chloride in DMF with imidazole as a catalyst yielded 2′-(*t*-butyldimethylsilyl)taxol [**5**] as the major product. Characterization as the 2′-derivative **5** was made by ^{1}H-nmr spectroscopy (Table 1), which showed the expected resonance of the C-2′ proton, and also on the basis of ms, which yielded a strong peak at *m/z* 400 corresponding to the ion $[RCOOH_2]^+$, where RCO is the silylated acyl function of the C-13 position of taxol. Ions of this type are frequently observed in the mass spectra of taxol derivatives (4), and the observation that the ion contains the *t*-butyldimethylsilyl group indicates that, as expected, silylation had occurred at the 2′ position rather than the 7 position.

A second modification of the side chain came about as a result of attempts to deoxygenate taxol at the 7 position. In one of these attempts 2′-acetyltaxol [**2**] was converted to its 7-mesylate **6**, and the latter was then treated with 1,5-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2, because DBU has been shown to be an effective base for bringing about E2 eliminations (6). No elimination of the mesylate was observed, presumably because of the inability of the hindered base to approach the C-6α proton on the underside of the C ring. Instead, a number of other products were formed, three of which were characterized as 2′-*epi*-acetyl-7-mesyltaxol [**7**], 2′-*epi*-7-mesyltaxol [**8**], and 7-mesyltaxol [**9**]. Structural assignments were made on the basis of ^{1}H-nmr spectra and mass spectra; in particular, the coupling constant of the 2′-proton changed from 3 Hz in the normal derivative **6** to 6 Hz in the *epi*-derivative **7**. All other features of the ^{1}H-nmr spectra of **6** and **7** were essentially identical. The taxol derivatives **8** and **9** were similarly characterized.

The formation of a 2′-*epi*-taxol derivative was interesting, but not particularly helpful from a structure-activity standpoint because the presence of the 7-mesyl group pre-

TABLE 1. ^{1}H-nmr Spectra of Modified Taxols.[a]

Proton	Compound					
	5	**6**	**7**	**8**	**9**	**10**
H-2	5.68 (d, 6.5)	5.68 (d, 7)	5.63 (d, 7)	5.63 (d, 7)	5.66 (d, 7)	5.69 (d, 7)
H-3	3.79 (d, 6.5)	3.92 (d, 7)	3.87 (d, 7)	3.89 (d, 7)	3.89 (d, 7)	3.95 (d, 7)
H-5	4.97 (dd, 1, 10)	4.92 (d, 9)	4.91 (d, 8)	4.93 (dd, 1, 9)	4.90 (br d, 9)	4.96 (br d, 10)
H-6	2.2–2.5 (m)	2.9–3.1 (m)	2.9–3.1 (m)	2.9–3.1 (m)	2.9–3.1 (m)	2.4 (m)
H-7	4.41 (dd, 6, 11)	5.34 (dd, 7, 10)	5.30 (dd, 7, 10)	5.31 (dd, 7, 10)	5.30 (dd, 7, 10)	5.56 (dd, 7, 11)
H-10	6.24 (s)	6.50 (s)	6.34 (s)	6.42 (s)	6.46 (s)	6.35 (s)
H-13	6.24 (br t, 8)	6.19 (br t, 9)	6.11 (br t, 8)	6.07 (br t, 9)	6.16 (br t, 9)	6.24 (br t, 9)
H-14	2.2–2.5 (m)	2.1–2.2 (m)	2.2 (m)	2.2–2.4 (m)	2.2–2.4 (m)	2.6 (m)
H-16	1.29 (s)	1.19 (s)	1.23 (s)	1.23 (s)	1.21 (d)	1.20 (s)
H-17	1.13 (s)	1.18 (s)	1.14 (s)	1.13 (s)	1.17 (s)	1.16 (s)
H-18	1.90 (br s)	2.01 (s)	1.78 (s)	1.64 (br s)	1.83 (br s)	1.92 (s)
H-19	1.69 (s)	1.80 (s)	1.56 (s)	1.78 (s)	1.78 (s)	1.82 (s)
H-20	4.17 (d, 8)	4.16 (d, 8)	4.12 (d, 8)	4.10 (d, 8)	4.14 (d, 8)	4.18 (d, 8)
	4.30 (d, 8)	4.31 (d, 8)	4.29 (d, 8)	4.30 (d, 8)	4.29 (d, 8)	4.33 (d, 8)
H-2′	4.62 (d, 2)	5.52 (d, 3)	5.62 (d, 6)	4.89 (d, 4)	4.78 (d, 2)	5.52 (d, 3)
H-3′	5.71 (dd, 2, 9)	5.93 (dd, 3, 9)	5.86 (dd, 6, 8)	5.76 (dd, 4, 8)	5.89 (dd, 2, 9)	6.02 (dd, 3, 9)
NH	7.06 (d, 9)	6.91 (d, 9)	6.88 (d, 8)	7.06 (d, 8)	7.04 (d, 9)	6.90 (d, 9)
OAc	2.55 (s)	2.41 (s)	2.40 (s)	2.42 (s)	2.33 (s)	2.13 (s)
	2.21 (s)	2.16 (s)	2.20 (s)	2.15 (s)	2.15 (s)	2.47 (s)
		2.14 (s)	2.14 (s)			
2-OBz	8.11 (d, 8)	8.09 (d, 7)	8.09 (d, 7)	8.05 (d, 8)	8.09 (d, 8)	8.12 (d, 7)
	7.5 (m)	7.60 (t, 7)	7.61 (t, 7)	7.4 (m)	7.4 (m)	7.60 (t, 7)
		7.4 (m)	7.4 (m)			7.4 (m)
3′-NBz	7.71 (d, 8)	7.73 (d, 7)	7.78 (d, 7)	7.72 (d, 8)	7.76 (d, 8)	7.74 (d, 7)
	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)
3′-Ph	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)
Other	−0.29 (3H, s)[b]	3.09 (s)[c]	3.07 (s)[c]	3.09 (s)[c]	3.08 (s)[c]	4.80 (d, 12)[d]
	−0.04 (3H, s)[b]					5.01 (d, 12)[c]
	0.80 (9H, s)[b]					4.62 (d, 12)[e]

TABLE 1. Continued.

Proton	Compound					
	11	**12**	**13**	**14**	**15**	**16**
H-2	5.65 (d, 7)	5.65 (d, 7)	5.64 (d, 7)	5.67 (d, 7)	5.62 (d, 7)	5.67 (d, 7)
H-3	3.93 (d, 7)	3.94 (d, 7)	3.77 (d, 7)	3.79 (d, 7)	3.71 (d, 7)	3.78 (d, 7)
H-5	4.90 (br d, 9)	4.91 (dd, 9, 1)	4.88 (br d, 8)	4.96 (d, 7)	4.91 (br d, 9)	4.96 (br d, 9)
H-6	2.38 (m)	2.5 (m)	2.1 (m)	2.2 (m)	1.8, 2.4 (m)	2.2 (m)
H-7	5.56 (m)	5.57 (dd, 7, 11)	4.39 (dd, 4, 11)	4.43 (dd, 6, 10)	4.36 (dd, 7, 10)	4.48 (dd, 6, 11)
H-10	6.34 (s)	6.33 (s)	6.25 (s)	6.28 (s)	6.27 (s)	6.27 (s)
H-13	6.33 (br t, 9)	6.31 (br t, 9)	6.33 (br t, 9)	6.24 (br t, 9)	6.06 (br t, 9)	6.21 (br t, 8)
H-14	2.27 (d, 9)	2.24 (d, 9)	2.5 (m)	2.4 (m)	1.8, 2.1 (m)	2.2 (m)
H/16	1.24 (s)	1.22 (s)	1.24 (s)	1.19 (s)	1.16 (s)	1.20 (s)
H-17	1.17 (s)	1.13 (s)	1.13 (br s)	1.10 (s)	1.09 (s)	1.11 (s)
H-18	2.00 (s)	2.01 (s)	1.91 (s)	1.91 (s)	1.84 (s)	1.90 (s)
H-19	1.79 (s)	1.79 (s)	1.64 (s)	1.55 (s)	1.62 (s)	1.69 (s)
H-20	4.11 (d, 8)	4.11 (d, 8)	4.26 (d, 8)	4.18 (d, 8)	4.14 (d, 8)	4.17 (d, 8)
	4.28 (d, 8)	4.28 (d, 8)	4.31 (d, 8)	4.29 (d, 8)	4.27 (d, 8)	4.30 (d, 8)
H-2′	4.94 (d, 6)	4.82 (d, 6)	4.94 (d, 6)	5.46 (d, 3)	5.61 (d, 5)	5.51 (d, 3)
H-3′	5.71 (d, 6)	5.07 (br d, 6)	5.73 (d, 6)	6.00 (dd, 3, 9)	5.91 (dd, 5, 8)	5.97 (dd, 3, 9)
NH	—	5.70 (br s)	—	7.4 (d, 9)	8.28 (d, 8)	7.07 (d, 9)
OAc	2.14 (s)	2.10 (s)	1.94 (s)	2.18 (s)	2.16 (s)	2.20 (s)
	1.97 (s)	2.13 (s)	2.22 (s)	2.44 (s)	2.39 (s)	2.43 (s)
2-OBz	8.04 (d, 8)	8.06 (d, 7)	8.04 (d, 7)	8.17 (d, 7)	8.09 (d, 7)	8.10 (d, 8)
	7.4 (m)	7.62 (t, 7)	7.45 (m)	7.4 (m)	7.4 (m)	7.4 (m)
		7.4 (m)				
3′-NBz	7.71 (d, 8)		7.69 (d, 8)	7.79 (d, 7)	7.76 (d, 8)	7.73 (d, 8)
	7.4 (m)		7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)
3′-Ph	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)
Other	5.01 (d, 12)[c]	5.00 (d, 12)[c]		3.4–3.6 (m)[f]	3.2 (m)[f]	2.6 (m)[k]
	4.62 (d, 12)[c]	4.62 (d, 12)[c]		2.6 (m)[f]	2.8 (m)[f]	
				5.17 (br t, 6)[g]	5.2 (br s)[g]	
				4.87 (s)[h]	8.08 (s)[j]	
				7.4 (m)[i]		

[a]Spectra obtained in $CDCl_3$, chemical shifts in ppm from internal TMS, and coupling constants in Hz. Assignments of the methyl groups are based on analogy with related compounds and are not rigorously established.

[b]CH_3 protons of the *t*-butyldimethylsilyl group.

[c]CH_3 protons of the 7-mesyl group.

[d]CH_2 protons of the 2′-troc group.

[e]CH_2 protons of the 7-troc group.

[f]CH_2CH_2 protons of the β-alanyl group.

[g]NH protons of the β-alanyl group ($[NH_3]^+$ for **15**).

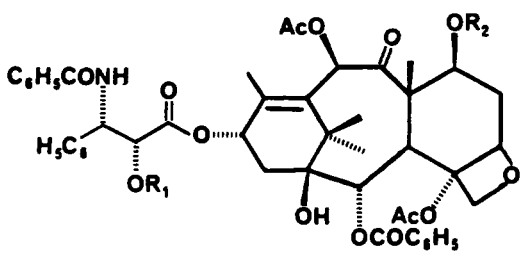
[h]Benzylic CH_2 protons of the CBZ group.

[i]Phenyl protons of the CBZ group.

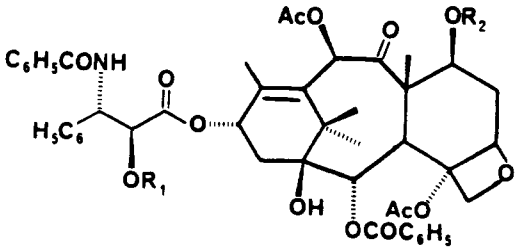
[j]HCOOH proton.

[k]CH_2CH_2 protons of the succinate group.

cluded any meaningful interpretation of the effect of the stereochemistry at the 2′ position on biological activity. We thus investigated the effects of base treatment with DBU on 2′,7-di(2,2,2-trichloroethyloxycarbonyl)taxol [**10**], with the expectation



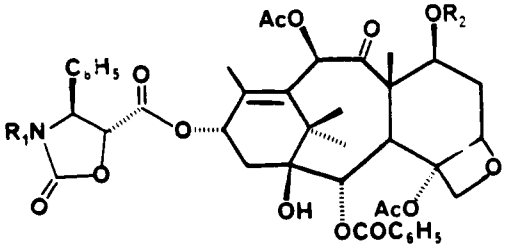
5 $R_1 = Si(Me)_2C(Me)_3$, $R_2 = H$
6 $R_1 = Ac$, $R_2 = SO_2Me$
9 $R_1 = H$, $R_2 = SO_2Me$
10 $R_1 = R_2 = COOCH_2CCl_3$



7 R_1=Ac, R_2=SO_2Me
8 R_1=H, R_2=SO_2Me

that the 2,2,2-trichloroethyloxycarbonyl (troc) group could be removed with Zn and HOAc (7) to yield 2′-*epi*-taxol. In the event, matters did not turn out as expected.

Treatment of taxol with 2,2,2-trichloroethylchloroformate in MeCN and pyridine at 0° yielded the 2′,7-di(troc) derivative **10** in excellent yield and purity. Treatment of the reaction product with DBU did not yield any of the desired 2′ epimer, however. Instead, rapid conversion to a single major product occurred. This product was identified as the oxazolone **11** on the basis of spectroscopic data. The ^{1}H-nmr spectrum of **11** showed sharp doublets at 4.94 and 5.71 ppm (J=6 Hz) for the protons at 2′ and 3′, respectively, together with the absence of the doublets at 4.80 and 4.73 ppm for the methylene unit of the troc group at the 2′ position. The fabms of **11** showed a protonated molecular ion peak at *m/z* 1054, and exact mass measurement of this peak indicated a composition of $C_{51}H_{50}NCl_3O_{17}$. The ir spectrum showed a new peak at 1820 cm^{-1} corresponding to the carbonyl absorption of a cyclic urethane. These data leave no doubt that the structure of the new product is that of the oxazolone **11**.



11 R_1=C_6H_5CO, R_2=$COOCH_2CCl_3$
12 R_1=H, R_2=$COOCH_2CCl_3$
13 R_1=C_6H_5CO, R_2=H

The ready cyclization of compound **10** to the oxazolone **11** must occur through deprotonation of the amide nitrogen by DBU followed by intramolecular displacement on the troc carbonyl group. This process takes place much more rapidly than epimerization of the 2′ position, leading to cyclized unepimerized product as the only detectable product of the reaction.

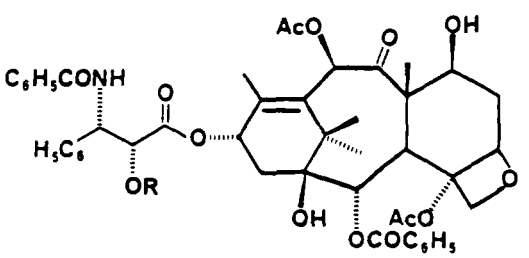
Treatment of the oxazolone **11** with dilute HCl during work-up also gave a small amount of the debenzoyl product **12**. The structure of **12** followed from its ^{1}H-nmr spectrum and mass spectrum. In its ^{1}H-nmr spectrum the doublet at 4.82 ppm for the 2′ proton was coupled to a broad doublet at 5.07 ppm for the 3′ proton, which was in turn coupled to a broad singlet at 5.70 ppm due to the amide proton. The mass spectrum of compound **12** showed its $[MH]^+$ ion at *m/z* 950, with a composition $C_{44}H_{46}NO_{16}Cl_3$ corresponding to the loss of a benzoyl group from **11**.

Removal of the troc group from **11** by reaction with Zn dust in methanolic HOAc yielded the unprotected oxazolone **13**. The spectral data of **13** were consistent with its assigned structure; in particular, the ^{1}H-nmr signal for the 7 proton shifted upfield to 4.39 ppm, and the carbonate carbonyl stretching frequency of 1775 cm^{-1} was absent from its ir spectrum.

A final series of reactions was carried out in attempts to prepare active H_2O-soluble derivatives of taxol. Taxol itself suffers from very low solubility in H_2O, making formulation very difficult, and any active derivative showing increased H_2O solubility would thus be a very desirable compound.

In the design of H_2O-soluble congeners of taxol, we took advantage of the fact that acyl groups at the 2′ position are readily hydrolyzed, as already noted, and, thus, a suitable 2′-acyltaxol would serve as a prodrug form of taxol itself. We, therefore, prepared a number of 2′ derivatives of taxol, two of which are discussed here.

Acylation of taxol with *N*-carbobenzyloxy-β-alanine (*N*-CBZ-β-alanine) was achieved by the use of *N,N*′-dicyclohexylcarbodiimide as the coupling agent (8) to yield 2′-(*N*-CBZ-β-alanyl)taxol **14** as the product in high yield; the spectroscopic data of **14** were totally consistent with the assigned structure. Deprotection of **14** was effected using 5% Pd/C or 5% Pd/Al_2O_3 as catalyst and HCO_2H as hydrogen source. The product was obtained as 2′-(β-alanyl)taxol formate on filtration and evaporation of the solvent, and its spectroscopic data were consistent with the assigned structure. Alternate deprotection methods, such as hydrogenation in MeOH or alternate *N*-protecting groups such as the *t*-butyloxycarbonyl group, failed to give product in satisfactory yield.



14 R=$COCH_2CH_2NHCOOCH_2C_6H_5$
15 R=$COCH_2CH_2NH_3^+HCOO^-$
16 R=$COCH_2CH_2COOH$

Although 2′-(β-alanyl)taxol formate **15** showed promising P-388 in vivo activity and an improved solubility in H_2O as compared with taxol, it proved to be too easily hydrolyzed to be suitable as a prodrug form of taxol. The solubility of **15** in H_2O was determined by an hplc method to be 2.2 mg/ml, but dissolution in H_2O was accompanied by a slow decomposition to taxol, with approximately 10% decomposition occurring over a 24-h period at room temperature. At 37° in a pH 6.6 buffer, decomposition was complete at 20 h. This instability precluded its development as a prodrug for taxol, but more stable analogs would perhaps be suitable replacements.

The second compound prepared was 2′-succinyl taxol [**16**]. Treatment of taxol with succinic anhydride yielded **16** in excellent yield and purity; its spectroscopic data were consistent with the assigned structure. Regrettably the initial bioassay results on this substance indicated that it had a much diminished P-388 in vivo activity as compared with taxol, and further efforts were, thus, concentrated on other derivatives.

The biological assay data on the various modified taxols discussed in this paper are summarized in Tables 2 and 3. Table 2 lists the cytotoxicity of various taxol derivatives.

TABLE 2. Cytotoxicity of Taxol and Modified Taxols.[a]

Compound	ED_{50}, μg/ml
Taxol [**1**]	1×10^{-5}
2′-Acetyltaxol [**2**]	2×10^{-5}
7-Acetyltaxol [**3**]	4×10^{-3}
2′,7-Diacetyltaxol [**4**]	2×10^{-1}
2′-(*t*-Butyldimethylsilyl)taxol [**5**]	3×10^{-1}
Oxazolone derivative **13**	2×10^{-1}
2′-(*N*-CBZ-β-alanyl)taxol [**14**]	1×10^{-4}
2′-(β-Alanyl)taxol formate [**15**]	2×10^{-4}
2′-Succinyltaxol [**16**]	1×10^{-2}

[a]Cytotoxicity in the KB cell culture system, determined by standard NCI protocols (9).

The lack of activity of 2′-(*t*-butyldimethylsilyl)taxol [**5**] as compared with taxol supports the hypothesis that the 2′-hydroxyl group is essential for biological activity. The activity of the 2′-acyl taxols such as **2**, **14**, **15**, and **16** can then best be explained by their hydrolysis to taxol under the conditions of the bioassay. The lack of activity of the oxazolone derivative **13** is not surprising, inasmuch as this derivative lacks a 2′-acyl group and is not readily hydrolyzed.

Results of the P-388 in vivo assay are given in Table 3. These data show that 2′-acyltaxols retain some in vivo activity. It should be recognized, however, that the T/C ratios are quite variable, as indicated by the different values obtained for **15** on two different occasions, and too much stress should not be placed on the actual numerical values. The major conclusion from these data is thus that 2′-acyl taxols do show reproducible in vivo activity. The lack of activity of 2′,7-diacetyltaxol may be due to its lack of solubility, since both 2′-acetyl and 7-acetyltaxol show reproducible in vivo activity.

TABLE 3. P-388 in vivo Activity of Taxol and Modified Taxols.

Compound	Dose (mg/kg)	T/C
Taxol [**1**]	20	104
	10	137
	5	156
	2.5	147
2′-Acetyltaxol [**2**]	20	140
	10	130
	5	127
	2.5	127
7-Acetyltaxol [**3**]	26	130
	13	127
	6.5	124
	3.25	128
2′,7-Diacetyltaxol [**4**]	40	104
	20	100
	10	105
2′-(β-Alanyl)taxol formate [**15**]	40	140, 153
	20	138, 130
	10	122, 117
2′-Succinyltaxol [**16**]	44	129
	22	122
	11	112

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General procedures have been previously described (4). The designation $[M-RCOOH]^+$ in the listing of mass spectral data indicates an ion formed by loss of the C-13 ester side chain as an acid.

2′-(*t*-BUTYLDIMETHYLSILYL)TAXOL [**5**].—Taxol (5 mg) was treated with 25 μl (5 eq) of a silylating solution consisting of 10.2 g *t*-butyldimethylsilyl chloride and 9.3 g imidazole dissolved in 18 ml dry DMF. After 12 h at room temperature, the reaction was worked up by standard methods and the product isolated by preparative hplc (MeOH-H_2O, 85:15). 2′-(*t*-Butyldimethylsilyl)taxol [**5**] had the following properties: $[\alpha]^{23}D$ −28.3 (*c*=0.004, MeOH); fabms *m/z* $[MNa]^+$ 990 (3), $[MH]^+$ 968 (33), $[MH-RCOOH]^+$ 569 (18), $[MH-RCOOH-H_2O]^+$ 551 (17), $[MH-RCOOH-AcOH]^+$ 509 (62), $[RCOOH_2]^+$ 400 (24), 354 (17), 177 (20), 149 (12), $[C_6H_5CO]^+$ 105 (100); ir 1745, 1670, 1535, 1510, 1490, 1385, 1260, 1120, 1085, 850, 790, 720 cm^{-1}; ^1H nmr see Table 1.

2′-ACETYL-7-MESYL TAXOL [**7**].—2′-Acetyltaxol [**2**] (3) (30 mg) and 4-dimethylaminopyridine (3.6 mg) were dissolved in pyridine-d_5 (0.5 ml) and treated with mesyl chloride (0.09 ml). The reaction was followed by ^1H nmr and was complete after 3 h. Normal work-up yielded 2′-acetyl-7-mesyltaxol [**6**] as a homogeneous compound in 95% yield; $[\alpha]^{23}D$ −25.1° (*c*=0.005, MeOH); fabms *m/z* $[MNa]^+$ 996 (55), $[MH]^+$ 974 (100), 914 (20), 587 (80), $[C_6H_5CO]^+$ 105 (100); *m/z* 974.3065 ($[MH]^+$; $C_{50}H_{56}NO_{17}S$ requires 974.3268); ir 1775, 1740, 1680, 1575–1440, 1395, 1360, 1285, 1260, 1200, 1150–1090 cm^{-1}; ^1H nmr see Table 1.

REACTION OF 2′-ACETYL-7-MESYL TAXOL [**6**] WITH DBU.—2′-Acetyl-7-mesyltaxol (50 mg) was dissolved in dry CH_2Cl_2 (2.0 ml), and DBU (50 μl) was added. The reaction was allowed to proceed for 26 h, and was then worked up by standard methods. Preparative tlc (Si gel, CH_2Cl_2-2-butanone, 80:20) yielded four bands with R_f 0.80 (8 mg), 0.55 (5 mg), 0.38 (20 mg), and 0.70 (2 mg). The band with R_f 0.70 was identified as starting material.

2′-*epi*-ACETYL-7-MESYLTAXOL [**7**].—The band from the previous experiment with R_f 0.80 was identified as the *epi* derivative **7** on the basis of the following data: fabms *m/z* $[MNa]^+$ 996 (7), $[MH]^+$ 974 (52), $[MH-RCOOH-CH_2SO]^+$ 587 (5), 310 (30), 268 (18), 240 (20), 210 (13), 155 (20), 135 (17), 119 (58), $[C_6H_5CO]^+$ 105 (100); ir 1740, 1670, 1535, 1505, 1470, 1390–1350, 1290, 1250, 1195, 1115, cm^{-1}; ^1H nmr see Table 1.

2′-*epi*-7-MESYLTAXOL [**8**].—The band from the DBU experiment with R_f 0.38 was a mixture and was purified by preparative hplc (MeOH-H_2O, 85:15). The major component of the mixture was tentatively identified as **8** on the basis of its ^1H-nmr spectrum (see Table 1).

7-MESYLTAXOL [**9**].—The band from the DBU experiment with R_f 0.55 was identified as 7-mesyltaxol [**9**]: fabms *m/z* $[MNa]^+$ 954 (18), $[MH]^+$ 932 (90), $[MH-AcOH]^+$ 872 (17), $[MH-CH_2SO_2]^+$ 854 (20), $[MH-RCOOH-AcOH]^+$ 587 (8), 286 (32), 240 (30), 135 (28), 119 (68), $[C_6H_5CO]^+$ 105 (100); ^1H nmr see Table 1.

2′,7-DI(2,2,2-TRICHLOROETHYLOXYCARBONYL)TAXOL [**10**].—A solution of taxol (50 mg) in dry CH_2Cl_2 (1 ml) at 0° was treated with dry pyridine (25 μl) and 2,2,2-trichloroethyl chloroformate (25 μl). After 5 min at 0° the mixture was worked up by standard methods to yield homogeneous 2′,7-di(troc)taxol **11**, R_f 0.67 (EtOAc-hexane, 1:1). $[\alpha]^{23}D$ −26.8 (*c*=0.004, MeOH); fabms *m/z* $[MH]^+$ 1202 (30), $[MH-AcOH]^+$ 1142 (5), $[MH-AcOH-H_2O]^+$ 1124 (7), $[MH-RCOOH-AcOH]^+$ 684 (3), $[RCOOH_2]^+$ 460 (12), $[RCO]^+$ 442 (21), 210 (20), $[C_6H_5CO]^+$ 105 (100); *m/z* 1202.1406 ($[MH]^+$; $C_{53}H_{54}NO_{18}Cl_6$ requires 1202.1475); ^1H nmr see Table 1.

REACTION OF 2′,7-DI(2,2,2-TRICHLOROETHYLOXYCARBONYL)TAXOL [**10**] WITH DBU.—2′,7-Di(troc)taxol (**10**, 112 mg) in 1.5 ml dry CH_2Cl_2 was treated with DBU (10 μl) for 5 min, after which time two products (R_f 0.43 and 0.55) were detected by tlc. Work-up by standard methods (including a wash with 1.0 N HCl to remove DBU) yielded an additional product, R_f 0.20. Isolation by preparative tlc (EtOAc-hexane, 2:3) gave two pure products with R_f 0.43 and 0.20; the product with R_f 0.55 was not obtained pure. Yield: R_f 0.43, 38 mg; R_f 0.20, 13.5 mg.

OXAZOLONE **11**.—The product with R_f 0.43 was identified as the oxazolone **11**: $[\alpha]^{23}D$ −52.7° (*c*=0.003, MeOH); fabms *m/z* $[MH]^+$ 1054 (58), $[MH-AcOH]^+$ 994 (20), $[MH-AcOH-H_2O]^+$ 976 (22), $[MH-RCOOH]^+$ 743 (3), $[MH-RCOOH-AcOH]^+$ 683 (28), $[C_6H_5CO]^+$ 105 (100); *m/z* 1054.2277 ($[MH]^+$; $C_{51}H_{51}NO_{17}Cl_3$ requires 1054.2274); ir 1820, 1775, 1750, 1720, 1665, 1475, 1390, 1260, 1195, 1110, 1080, 1040–1060 cm^{-1}; ^1H nmr see Table 1.

DEBENZOYL OXAZOLONE **12**.—The product with R_f 0.20 was identified as the debenzoyloxazolone **12**: fabms *m/z* $[MK]^+$ 988 (22), $[MNa]^+$ 972 (6), $[MH]^+$ 950 (100), $[MH-AcOH]^+$ 890 (20), $[MH-RCOOH-C_2H_2O]^+$ 701 (45), $[MH-RCOOH-AcOH]^+$ 683 (62), $[MH-RCOOH-2AcOH]^+$ 623 (32), $[C_6H_5CO]^+$ 105 (100); *m/z* 950.1867 ($[MH]^+$; $C_{44}H_{47}NO_{16}Cl_3$ requires 950.1962); ir 1810 (inf), 1780, 1760 (inf), 1740 (inf), 1460, 1390, 1280, 1260, 1240, 1100, 1080, 995, 720 cm^{-1}; ^1H nmr see Table 1.

DEPROTECTION OF OXAZOLONE **11**.—Oxazolone **11** (as part of the crude reaction product from the reaction of **10** with DBU) was treated with Zn dust (200 mg) in MeOH-HOAc, 9:1 (2.0 ml) at room temperature for 30 min. Purification by preparative tlc (EtOAc-hexane, 1:1) yielded the deprotected oxazolone **13** in 28% yield as the only isolable product from **10**; fabms *m/z* $[MH]^+$ 880 (22), 858 (20), $[MH-RCOOH]^+$ 569 (12), $[MH-RCOOH-AcOH]^+$ 509 (48), 449 (23), 387 (33), 369 (23), $[C_6H_5CO]^+$ 105 (100); ir 1815, 1760 (inf), 1740, 1720 (inf), 1670, 1465, 1390, 1255, 1200, 1095 cm^{-1}; ^1H nmr see Table 1.

2′-(*N*-CARBOBENZYLOXY-β-ALANYL)TAXOL [**14**].—Taxol (209 mg), *N,N′*-dicyclohexyl-carbodiimide (400 mg), and *N*-carbobenzyloxy-β-alanine (200 mg) were dissolved in dry MeCN (10 ml) and the mixture stirred at room temperature for 15 h. The precipitated dicyclohexylurea was removed by filtration and the product purified by preparative tlc (EtOAc-hexane, 55:45) to yield 2′-(*N*-carbobenzyloxy-β-alanyl)taxol [**14**] (234 mg) as a homogeneous white solid: $[\alpha]^{23}D$ −27.3° (*c*=0.10, MeOH); fabms *m/z* $[MNa]^+$ 1081 (4), $[MH]^+$ 1059 (20), $[MH-AcOH]^+$ 999 (3), $[MH-RCOOH]^+$ 569 (5), $[MH-RCOOH-AcOH]^+$ 509 (17); $[C_6H_5CO]^+$ 105 (100); ir 1760, 1745, 1725, 1680, 1545, 1485, 1395, 1285, 1265, 1200, 1090, 1070 cm^{-1}; ^1H nmr see Table 1.

2′-(β-ALANYL)TAXOL FORMATE [**15**].—The protected β-alanyltaxol **14** (159 mg) was treated with 5% Pd/C (98 mg) in HCO_2H-MeOH, 40:60 (25 ml) with stirring at room temperature. The reaction was monitored by hplc and was complete after 75 min. The solution was filtered, the solvent evaporated, and the glassy product dissolved in CH_2Cl_2 and treated with hexane to yield 2′-(β-alanyl)taxol formate [**15**] (131 mg), as a white solid with a purity >99% as judged by hplc; the minor impurity was identified as taxol. The product **15** had the following properties: fabms *m/z* $[MH]^+$ 925 (62), $[MH-RCOOH-AcOH-H_2O]^+$ 509 (6), 357 (28), 296 (54), 225 (48), 171 (68), 135 (38), 119 (57), $[C_6H_5CO]^+$ 105 (100); ir 3200–3600, 1720, 1660, 1540, 1380, 1250, 1190, 1080 cm^{-1}; ^1H nmr see Table 1; on addition of D_2O to the sample dissolved in $CDCl_3$, peaks for the amine protons at 8.28 and 5.2 ppm disappeared, and the resonance for the proton at C-3′ collapsed from an apparent triplet to a doublet.

2′-SUCCINYLTAXOL [**16**].—Taxol (205 mg), 4-dimethylaminopyridine (2.9 mg), and succinic anhydride (49 mg) were dried in vacuo for 2 h and then dissolved in 2.0 ml dry pyridine and the solution stirred at room temperature for 2.5 h. Work-up by standard methods yielded homogeneous product **16** (221 mg). The product had the following properties: $[\alpha]^{23}D$ −39.5° (*c*=0.006, MeOH); fabms *m/z* $[MNa]^+$ 976 (20), $[MH]^+$ 954 (68), $[MH-RCOOH]^+$ 569 (4), $[MH-RCOOH-H_2O]^+$ 551 (6), $[MH-RCOOH-AcOH]^+$ 509 (12), $[RCOOH_2]^+$ 386 (35), $[RCO]^+$ 368 (29), $[C_6H_5CO]^+$ 105 (100); ir 3400, 1740, 1720 (inf), 1670, 1530, 1390, 1260, 1185, 1095, 1065 cm^{-1}; ^1H nmr see Table 1.

BIOLOGICAL ACTIVITY DATA.—Cytotoxicities in the KB cell culture system were determined at the University of Miami by Dr. Wolf Lichter and his co-workers according to standard NCI protocols (9). In vivo testing in the P-388 system was carried out by Southern Research Institute according to standard NCI protocols (9).

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