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NEW TAXANES FROM *TAXUS BREVIFOLIA*, 2.[1]

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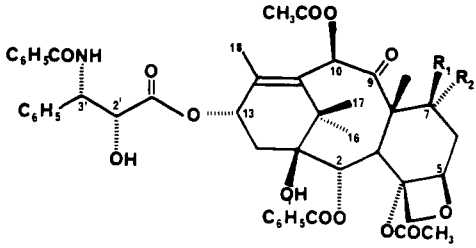
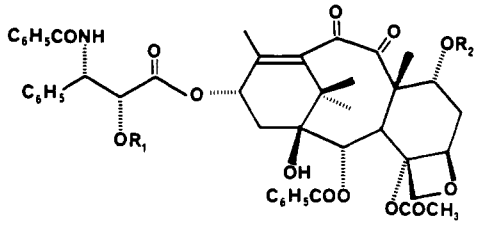
In continuation of our studies on the constituents of *Taxus brevifolia* Nutt. (1), we have investigated a fraction obtained during large-scale extraction and purification of *T. brevifolia* bark (2). This fraction, designated FB 10115-H, was eluted from a silica column before taxol and was found to contain the two taxane diterpenes **1** and **3** as its major constituents.



1 R_1=H, R_2=OH
2 R_1=OH, R_2=H



3 R_1=R_2=H
4 R_1=$COCH_3$, R_2=H
5 R_1=R_2=$COCH_3$

Compound **1** showed ions in the fabms at *m/z* 876 and 854, corresponding to (M^++Na) and (M^++H), respectively, and indicating a molecular weight of 853 corresponding to the composition $C_{47}H_{51}NO_{14}$. Compound **1** is thus isomeric with taxol, and its close relationship to taxol is underscored by its ^{1}H-nmr spectrum (Table 1) which is very similar to that of taxol. The major differences between the ^{1}H-nmr spectra of taxol (**2**) and compound **1** occur in the signals due to the protons at C-7 and C-20. In taxol these occur as a multiplet at 4.33 ppm and as a pair of doublets at 4.17 and 4.27 ppm, respectively; in compound **1** the same signals are observed as a broad doublet at 3.66 ppm and a broad two-proton singlet at 4.36 ppm. Other differences are observed in the chemical shift of the C-10 proton and the coupling constants of the C-5 proton. These changes are almost exactly analogous to those observed in the ^{1}H-nmr spectrum of 7-*epi*-10-deacetyltaxol as compared with that of 10-deacetyltaxol (3) and of other pairs of epimers, such as baccatin III and baccatin V (4,5), and this fact indicates that the new taxane is 7-*epi*-taxol (**1**). Although 7-*epi*-10-deacetyltaxol has been reported previously (3), this is the first report of the isolation of 7-*epi*-taxol itself.

In related work, we investigated the reaction of taxol with the free radical initiator azobis (isobutyronitrile) (AIBN). Heating taxol at 80° in toluene with a catalytic amount of AIBN yielded 7-*epi*-taxol (**1**) as the only diterpenoid product after 30 min; the isolated material was identical in all respects to that obtained from *T. brevifolia*. Similar treatment of 7-acyltaxol derivatives failed to effect any epimerization, which suggests that the driving force for the conversion may well be the known hydrogen bonding between the C-7α-OH group and the C-4α-OAc group (6).

The fact that 7-*epi*-taxol is thermodynamically more stable than taxol raises the question as to whether this

[1]For Part 1, see Kingston *et al.* (1).

TABLE 1. ^{1}H-nmr Chemical Shifts for Taxanes from *Taxus brevifolia*[a]

Protons on:	Compounds				
	1	**2**[b]	**3**	**4**	**5**
C-2	5.71 (d,7)	5.62 (d,7)	5.86 (d,7)	5.84 (d,7)	5.85 (d,7)
C-3	3.89 (d,7)	3.80 (d,7)	4.00 (d,7)	4.01 (d,7)	4.03 (d,7)
C-5	4.89 (dd,4,8)	4.92 (dd,2,8)	4.90 (dd,7,5)	4.91 (t,8)	4.86 (m)
C-6	2.3 (m)		2.3 (m)	2.3 (m)	2.3 (m)
C-7	3.66 (br d,12)	4.33 (m)	3.84 (dt,10,3)	3.84 (dt,10,3)	4.84 (m)
C-10	6.76 (s)	6.26 (s)			
C-13	6.21 (br t,9)	6.15 (t)	6.19 (br t,8)	6.16 (br t,8)	6.22 (br t,8)
C-14	2.3 (m)	2.5 (m)	2.3 (m)	2.3 (m)	2.3 (m)
C-16	1.15 (s)	1.25 (s)	1.17 (s)	1.16 (s)	1.19 (s)
C-17	1.12 (br s)	1.14 (s)	1.09 (s)	1.08 (s)	1.12 (s)
C-18	1.75 (s)	1.78 (s)	1.78 (s)	1.84 (br s)	1.98 (s)
C-19	1.64 (s)	1.67 (s)	1.70 (s)	1.71 (s)	1.82 (s)
C-20	4.36 (br s)	4.17 (d,18)	4.34 (d,8)	4.35 (d,8)	4.32 (d,8)
		4.27 (d,8)	4.40 (d,8)	4.43 (d,8)	4.41 (d,8)
C-2′	4.78 (d,2)	4.71 (d,3)	4.80 (d,3)	5.56 (d,3)	5.51 (d,3)
C-3′	5.77 (dd,2,9)	5.72 (dd,3,9)	5.77 (dd,3,9)	5.87 (dd,3,9)	5.98 (dd,3,9)
N-H	6.99 (d,9)	7.00 (d,9)	6.95 (d,9)	6.85 (d,9)	6.83 (d,9)
OAc	2.16 (s)	2.23 (s)	2.44 (s)	2.12 (s)	2.00 (s)
	2.48 (s)	2.38 (s)		2.50 (s)	2.12 (s)
					2.43 (s)
2-OBz	8.17 (d,7)	8.11 (dd)	8.15 (d,7)	8.14 (d,7)	8.19 (d,7)
	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)	7.4 (m)
3′-NBz	7.69 (d,7)	7.7 (dd)	7.68 (d,7)	7.73 (d,7)	7.74 (d,7)
	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)
Other	3.51 (br s)[c]		3.61 (br s)[c]	4.45 (d,10)[d]	
	4.66 (d,12)[d]		4.43 (d,10)[d]		

[a]Multiplicity and coupling constants in Hz in parentheses.
[b]Data from Kingston *et al.* (1); measured at 200 MHz.
[c]2′-OH proton.
[d]7-OH proton.

substance is a natural product or whether it is formed by isomerization of taxol during the isolation process. Although taxol in our hands does not epimerize in neutral organic solvents in the absence of free radical initiators, we cannot exclude the possibility that a small amount of epimerization might have occurred during the larger-scale workup preceding the preparation of fraction FB10115-H.

The second compound isolated showed molecular ions at *m/z* 832 and 810, corresponding to $(M^+ + Na)$ and $(M^+ + H)$, respectively, and thus to a molecular weight of 809, consistent with a composition $C_{45}H_{47}NO_{13}$. The ^{1}H-nmr spectrum of this compound (**3**) (Table 1) showed the presence of the normal taxol sidechain and normal resonances for all the other protons of taxol except for the absence of the C-10 proton; the presence of at least two hydroxyl groups was indicated by the disappearance of a broad singlet and a doublet when the spectrum was obtained in the presence of D_2O. The exchangeable proton resonating at 4.43 ppm was assigned to the C7-α-OH group because it collapsed from a doublet to a singlet on irradiation of the C7-β-H at 3.84 ppm. The collapse of the signal at 4.43 ppm to a singlet also revealed the two one-proton doublets at 4.40 and 4.34 ppm as a typical AB system with J_{AB} 8 Hz; we assign these signals to the C-20 protons. The chemical shift of the C7-β-H at 3.84 ppm is characteristic of a 7-*epi*-taxol derivative, as discussed in connection with the spectrum of compound **1**. The intramolecular nature of the hydrogen bonding of the C7-α-OH proton was confirmed by measuring the ^{1}H-nmr spectrum at different concentrations; under conditions from 6.9 mg/0.5

ml to 2.0 mg/0.5 ml, the chemical shift of this proton did not change, while that of the C-2′-OH proton moved from 3.61 to 3.47 ppm.[2]

The presence of two acetylatable hydroxyl groups in **3** was confirmed by acetylation under moderate conditions. Two acetate derivatives were obtained and were identified by ^{1}H nmr (Table 1) as the 2′-monoacetate derivative **4** and the 2′,7-diacetate derivative **5**; a similar difference in the reactivity of the 2′- and 7-hydroxyl groups was observed previously (8).

The combination of ^{1}H-nmr and mass spectrometric evidence indicates that the new taxol derivative has the 9,10-diketo structure **3**. Further evidence for this structure was obtained from the ^{13}C-nmr spectrum of **3**. Although the spectrum has not been completely assigned, it showed two downfield absorptions at 207.1 and 188.5 ppm due to ketone carbonyl groups, in addition to ester and amide carbonyl absorptions in the range 165-175 ppm; the carbonyl absorption at 188.5 ppm is new and is not observed in the ^{13}C-nmr spectrum of compound **1** or of baccatin III (9). Assignment of the new resonance to the C-10 carbonyl carbon is made by analogy with the ^{13}C-nmr spectrum of 1-phenyl-1,2-propanedione, which shows carbonyl carbon resonances at 192.5 and 202.5 ppm (10).

Final confirmation of the structure of the new compound was obtained through its preparation from 10-deacetyltaxol by oxidation with MnO_2 in Me_2CO. A 9,10-diketo derivative of a taxane has also been prepared from taxicin-I (11), but this is the first report of a naturally occurring taxane with this structural unit.

The cytotoxicity of the new compounds was determined in KB cell culture. Compound **1** showed an ED_{50} of 3×10^{-5} μg/ml, only slightly less than that of taxol at 1×10^{-5} μg/ml. Compound **3** had an ED_{50} of 2×10^{-2} μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage and are uncorrected. Analytical tlc was performed on silica gel 60 F_{254} plates, 0.20 mm (E. Merck), and preparative tlc was performed on silica gel GF, 1.0 mm Analtech. Hplc was carried out on LiChrosorb RP-8 columns, 25 cm×0.4 cm (analytical) or 25 cm×1 cm (preparative). ^{1}H-nmr spectra were recorded in $CDCl_3$ at 270 MHz on an IBM WP-270 spectrometer; chemical shifts are reported using the residual proton signal at 7.24 ppm as internal standard. ^{13}C-nmr spectra were recorded at 67 MHz on the IBM WP-270 instrument; they are reported using the letters p, s, t, and q to represent primary, secondary, tertiary, and quaternary carbons as determined by the INEPT technique. Mass spectra were obtained by the fab method on a Kratos MS 50 instrument at the Midwest Center for Mass Spectrometry at the University of Nebraska, a National Science Foundation Regional Instrumental Facility (Grant No. CHE 82-11164). Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and the ir spectrum was obtained in KBr on a Perkin-Elmer 710B spectrometer. *T. brevifolia* bark was collected in Oregon and supplied by the National Cancer Institute.

FRACTION FB 10115-H.—*T. brevifolia* bark (8061 lb), B-670549, PR Nos. 57259, 57193, and 57258, was extracted with MeOH to yield 2400 lb of native (wet) extract. This extract was partitioned between CH_2Cl_2 and H_2O to give a CH_2Cl_2 extract (95 kg), which was treated with Me_2CO-hexane (1:1) to give 75 kg of soluble product. This product was chromatographed on Florisil with elution by Me_2CO-hexane (1:1) to give a taxol-rich fraction (13 kg) which was crystallized from aqueous MeOH and from Me_2CO-hexane to give crude taxol (911 g). This crude taxol fraction was chromatographed on silica gel using a gradient of 1.5% to 3% iPrOH in CH_2Cl_2, and fraction FB 10115-H eluted before taxol in this system.

ISOLATION OF COMPOUND **1** AND **3**.—Fraction FB 10115-H (160 Mg) was subjected to preparative tlc on four plates, with development with CH_2Cl_2-MeOH (95:5) to give two uv ab-

[2]The C-2′-OH group could, in principle, also be hydrogen bonded to the amide carbonyl group. Such bonding does not appear to be significant in the solid state for the related side-chain of cephalomannine (7), and, in any event, it would be in competition with hydrogen bonding of the amide NH proton to the ester carbonyl group. A solvent-induced change in chemical shift for the C-2′-OH proton is thus reasonable.

sorping bands at Rf 0.45 and 0.55. The band with Rf 0.55 was eluted to yield 34 mg of material which was homogeneous on tlc but showed two components on analytical hplc (MeCN-H_2O, 60:40, 1.5 ml/min with retention times of 6.35 min (**3**) and 7.75 min (**1**). Preparative hplc (MeOH-H_2O, 70:30, 5.0 ml/min) yielded compounds **1** (11.5 mg) and **3** (6.9 mg) as homogeneous white amorphous solids.

7-*EPI*-TAXOL (**1**).—Mp 168-171°; $[\alpha]^{23}D$ −32.3° (*c* 0.012, MeOH); ms *m/z* 876 (MNa^+, 0.5), 854 (MH^+, 1), 794 (MH^+-AcOH, 1), 776 (MH^+-AcOH-H_2O, 0.3), 613 (0.2), 569 (1), 551 (1), 509 (5), 286 (47), 268 (22), 240 (43), 155 (63), 152 (60), 135 (72), 119 (85), 105 (100); ir 1760, 1720, 1660, 1535, 1500, 1470, 1390, 1260, 1110, 1060, 720 cm^{-1}. ^1H nmr, see Table 1. Partial ^{13}C nmr 14.6 (p), 16.1 (p), 20.6 (p), 21.4 (p), 22.5 (p), 26.0 (p), 35.3 (s), 36.1 (s), 40.4 (t), 54.9 (t), 72.3 (t), 73.2 (t), 78.7 (s), 82.8 (t), 127.0 (t), 128.5-129.2 (approx 4 signals, all t), 130.3 (t), 131.9 (t), 133.7 (t), 138.2 (q), 139.7 (q), 167.2 (q), 169.2 (q), 172.5 (q), 172.8 (q), 207.2 (q).

PREPARATION OF 7-*EPI*-TAXOL (**1**) FROM TAXOL (**2**).—Taxol (19.7 mg) and freshly prepared AIBN (0.9 mg) were dried in vacuo at room temperature for 1 h and then dissolved in 2.0 ml dry toluene. The solution was heated at 80° for 30 min, then cooled, diluted with 6 ml EtOAc and worked up by extraction with aqueous bicarbonate and H_2O allowed by drying ($MgSO_4$) and removal of the solvents under reduced pressure. Purification of the crude product by preparative tlc (EtOAc-hexane, 3:2) yielded 7-*epi*-taxol (**1**) as the major product, yield 16.5 mg (84%). Only a very faint trace of unreacted taxol could be detected on the tlc plate. The isolated **1** was identical to that obtained from *T. brevifolia*. This reaction was repeated successfully with freshly prepared AIBN but failed when an old sample of AIBN was used.

10-DEACETYL-10-OXO-7-*EPI*-TAXOL (**3**).—$[\alpha]^{23}$-60.4 (c 0.002, MeOH); ms *m/z* 848 (MK^+, 0.4), 832 (MNa^+, 4), 810 (MH^+, 0.6), 610 (0.3), 547 (0.6), 509 (14), 390 (13), 286 (30), 240 (23), 122 (32), 105 (100). ^1H nmr, see Table 1. Partial ^{13}C nmr 167.2, 172.2, 172.6, 188.5, 207.1 ppm; ir 3490, 1750 (sh), 1730, 1680 cm^{-1}.

ACETYLATION OF COMPOUND **3**.—Compound **3** (13 mg) in dry pyridine (0.35 ml) was mixed with Ac_2O (250 mg) and 4-dimethylaminopyridine (1.5 mg). The mixture was stirred at room temperature for 14 h, and then diluted with H_2O. The aqueous solution was extracted with CH_2Cl_2, and the extract washed, dried, and evaporated. The crude product was purified by preparative hplc on a 250×4.6 mm RP-8 column; elution with MeCN:H_2O (55:45) at 1.1 ml/min gave retention times of 14.2 and 18.1 min for **4** and **5** which were obtained in yields of 2.2 mg (15.6%) and 2.3 mg (15.4%), respectively. ^1H-nmr spectra, see Table 1.

OXIDATION OF 10-DEACETYLTAXOL.—10-Deacetylaxol[3] (5 mg) in dry Me_2CO (2 ml) was treated with MnO_2 (28 mg) for 18 h, as previously described (12). The product was identical with compound **3** by tlc (EtOAc-hexane, 7:3) and hplc (Waters Associates radialpak C-8 column, MeOH-H_2O, 1:1).

BIOASSAYS.—Cytotoxicity determinations were carried out by the University of Miami School of Medicine according to standard protocols (13). Data on taxol and 7-*epi*-taxol were obtained in the same test with the same control. Data on compound **3** were obtained in a different test.

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