Two New Taxane Diterpenoids from Taxus mairei

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TWO NEW TAXANE DITERPENOIDS FROM *TAXUS MAIREI*

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ABSTRACT.—Two new taxane diterpenoids were isolated from the heartwood of *Taxus mairei* and identified as 5α,7β,9α,10β,13α-pentaacetoxy-2α-benzoyloxy-4α,20-dihydroxytax-11-ene [**2**] and 7β,9α,10β,13α,20-pentaacetoxy-2α-benzoyloxy-4α,5α-dihydroxytax-11-ene [**3**]. The structures of **2** and **3** were determined primarily on the basis of analysis of their ^{1}H-nmr, ^{13}C-nmr, ^{1}H-^{1}H 2D COSY, ^{1}H-^{13}C 2D COSY, DEPT, and mass spectra. This is the first report of naturally occurring taxoids lacking a D ring but oxygenated at the 4, 5, and 20 positions.

The discovery that the diterpenoid natural product taxol [**1**] (1) is clinically active against ovarian and breast cancer (2) has stimulated a renewed interest in the isolation of related compounds from various *Taxus* species. These compounds could in some cases serve as potential sources of taxol through appropriate chemical transformations, but they can also serve as sources for taxol analogues, thus extending our understanding of structure-activity relationships in this area. A recent review (3) lists over 100 taxane diterpenoids (or taxoids), and new ones are being described on a regular basis.

Previous studies on the diterpenoids in the bark and the heartwood of *Taxus mairei* (Lemee et Levl.) S.Y. Hu (Taxaceae) have resulted in the isolation of various diterpenoids (4,5) and many new and known taxoids (6–13). In a continuation of the investigation of *T. mairei* by one of us (4–8), we now report the isolation and structure elucidation of two new taxoids from the heartwood of this plant.

Extraction of the heartwood of *T. mairei* with EtOH, followed by extraction of the dried EtOH extract with $CHCl_3$, gave a residue which was purified by cc on Si gel and by preparative tlc to yield the two new taxoids **2** and **3**.

Compound **2** had the composition $C_{37}H_{48}O_{14}$ as deduced by a combination of low-resolution ms and ^{13}C-nmr spectroscopy. Its ^{1}H-nmr spectrum (Table 1) showed the presence of four C-methyl groups and five acetate methyl groups, together with one benzoyl group. The presence of a taxene skeleton was inferred from the observation of characteristic resonances, such as the broad singlet at 2.30 ppm for the C-18 methyl group, and from the source of the material; this conclusion was corroborated by detailed analysis of the ^{1}H- and ^{13}C-nmr spectra (Table 1).

A one-proton multiplet at 1.92 ppm was correlated by ^{1}H-^{1}H COSY with protons resonating at 5.82 and 2.66 ppm, and was thus assigned to C-1. The proton at 5.82 ppm, assigned to C-2, correlated with one at 3.12 ppm which must therefore be C-3. The C-3 proton appeared as a doublet (J=4.9 Hz), indicating that the C-4 position is fully substituted. The C-5 proton appeared as a broad singlet at 5.21 ppm, and it could be correlated with a signal at 1.94, assigned to one of the C-6 protons. The other C-6 proton is assigned to a multiplet at 2.65 ppm, but the correlations of this proton were complicated by overlapping signals, and the assignment remains tentative. The C-7 proton was assigned to a doublet of doublets at 5.55 ppm, correlating with the C-6 proton at 1.94 ppm. The C-9 and C-10 protons appeared as a pair of doublets at 5.97 and 6.19 ppm, respectively, and the C-13 proton appeared as a multiplet at 5.80 ppm. This signal correlated with the C-14 protons at 2.41 and 2.66 ppm. An isolated spin system with signals at 3.43 and 3.71 ppm was assigned to C-20. The ^{13}C-nmr spectrum of **2**

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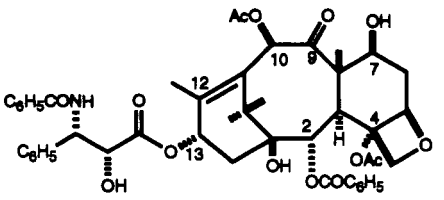

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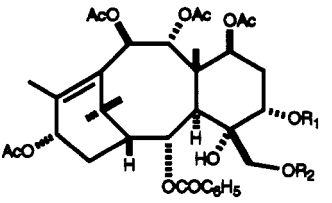
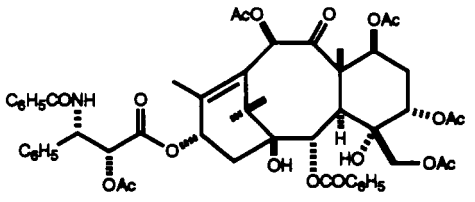


2 R_1=Ac, R_2=H
3 R_1=H, R_2=Ac



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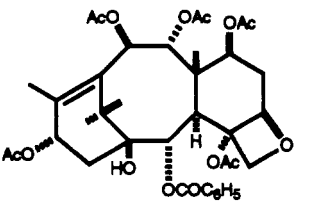

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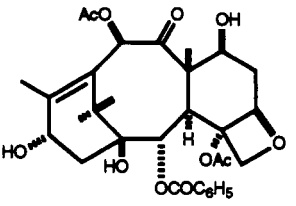

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(Table 1) fully supports the assigned structure. In particular, a singlet at 75.42 could only be assigned to C-4, indicating this position to be fully substituted and oxygenated.

Strong support for the assignment of structure **2** came from a comparison of the ^{1}H-nmr spectrum with that of the acetate **4** formed by acetylation of the reaction product of taxol and Meerwein's reagent (14). The ^{1}H-nmr spectra of the two compounds are very similar, with the major differences being in the chemical shifts of the C-3, C-16, and C-20 protons. The shift in the C-20 protons is expected because the C-20 position is acetylated in compound **4**, and the shift of the C-16 protons has been observed in many similar situations (3). The shift of the C-3 proton, from 3.12 ppm in **2** to 4.04 ppm in **4**, may be attributed to the shielding effect of the 9-keto group in **4**. Thus the C-3 proton of baccatin VI [**5**] resonates at 3.18 ppm, while that of baccatin III [**6**] resonates at 3.84 ppm (3).

The stereochemistry of **2** was assigned on the basis of analogy with related compounds and on the observed coupling constants. The C-2/C-3 coupling is consistent with an α configuration for the benzoyloxy group, and the narrow triplet at 5.21 ppm observed for the C-5 proton indicates an α configuration for the 5-acetoxyl group; the corresponding proton in **4** appeared as a broad singlet. The C-7 acetoxyl group is assigned the β configuration on the basis of the observed coupling constants, which are similar to those for the 7β-hydroxyl group of taxol. The C-9 and C-10 acetoxyl groups are assigned as α and β, respectively, on the basis of an observed coupling constant of 11

TABLE 1. ^{13}C- and ^{1}H-nmr Spectra of 5α,7β,9α,10β,13α-Pentaacetoxy-2α-benzoyloxy-4α,20-dihydroxytax-11-ene [**2**].

Position	^{13}C	Carbon type[a]	^{1}H[b]	^{1}H-^{13}C COSY[c]	^{1}H-^{1}H COSY
1	48.08	D	1.92 (1H, m)	*	C-2, C-14
2	71.99	D	5.82 (1H, m)	*	C-3, C-1
3	45.87	D	3.12 (1H, d, 4.9)	*	C-2
4	75.42	S	—		
5	73.00	D	5.21 (1H, t, J=2)	*	C-6
6	30.90	T	194 (1H, m)		
			2.65 (1H, m)		C-7, C-5
7	68.62	D	5.55 (1H, dd, 11.8, 4.8)	*	C-6
8	46.07	S	—		
9	75.20	D	5.97 (1H, d, 11.0)	*	C-10
10	71.59	D	6.19 (1H, d, 11.0)	*	C-9
11	134.80	S	—		
12	137.98	S	—		
13	70.71	D	5.80 (1H, m)	*	C-14α, C-14β
14	27.67	T	2.41 (1H, dd, 15.7, 5.2, αH)		C-13, C-14β
			2.66 (1H, ddd, 15.7, 8.7, 8.6, βH)		C-13, C-1, C-14α
15	38.04	S	—		
16	26.04	Q	1.81 (3H, s)	*	
17	31.91	Q	1.07 (3H, s)	*	
18	15.50	Q	2.30 (3H, brs)		
19	14.48	Q	1.00 (3H, s)	*	
20	64.48	T	3.43 (1H, d, 11.1)		C-20a
			3.71 (1H, d, 11.1)		C-20b
OAc	20.90	Q	1.99 (3H, s)		
	169.24	S			
OAc	21.09	Q	2.04 (3H, s)		
	169.76	S			
OAc	21.55	Q	2.00 (3H, s)		
	170.23	S			
OAc	21.81	Q	2.32 (3H, s)		
	170.80	S			
OAc	22.03	Q	2.33 (3H, s)		
	172.50	S			
$OCOC_6H_5$	165.32	S	—		
i	129.85	S	—		
o	129.89	D	8.07 (2H, d, 7.4)	*	meta-Ar
m	128.82	D	7.46 (2H, m)	*	ortho-Ar
p	133.66	D	7.59 (1H, m)	*	

[a]S=C, D=CH, T=CH_2, Q=Me. Assignments made by the ADEPT technique.
[b]Multiplicity and coupling constant (s) in Hz in parentheses.
[c]An asterisk indicates that a ^{1}H-^{13}C correlation was observed in a HETCOR spectrum.

Hz, comparable to values observed for these protons in molecules such as baccatin VI [**5**] (3). Finally, the C-13 acetoxyl group is assigned an α configuration on the basis of analogy with all the other derivatives of this type that have been isolated. Based on the above data, the new taxoid is assigned the structure and stereochemistry as illustrated in **2**.

The second compound obtained was isomeric with **2** and had similar ^{1}H- and ^{13}C-nmr spectra (Table 2). The major difference between the two compounds was in the

TABLE 2. ^1H- and ^{13}C-nmr Spectra of 7β,9α,10β,13α,20-Pentaacetoxy-2α-benzoyloxy-4α,5α-dihydroxytax-11-ene [**3**].

Position	^{13}C	Carbon type[a]	^1H[b]	^1H-^{13}C COSY[c]	^1H-^1H COSY
1	48.04	D	1.87 (1H, m)	*	C-14α, C-2
2	72.39	D	5.89 (1H, m)	*	C-1, C-3
3	43.31	D	3.31 (1H, d, 4.9)	*	C-2
4	75.80	S	—		
5	70.70	D	3.70 (1H, br.s)	*	C-6
6	31.46	T	1.80 (1H, m)		C-7
			1.95 (1H, m)		C-7, C-5
7	68.39[d]	D	5.68 (1H, dd, 11.8, 4.8)	*	C-6α, C-6β
8	46.20	S	—		
9	75.48	D	5.87 (1H, d, 10.9)	*	C-10
10	72.13	D	6.23 (1H, d, 11.0)	*	C-9
11	134.47	S	—		
12	139.20	S	—		
13	69.99[d]	D	5.68 (1H, m)	*	C-18, C-14
14	27.52	T	2.55 (1H, dd, 15.5, 4.4, αH)		C-1
			2.65 (1H, ddd, 15.5, 7.8, 3.9, βH)		C-13, C-1,
15	37.72	S	—		
16	25.91	Q	1.75 (3H, s)	*	
17	32.50	Q	1.01 (3H, s)	*	
18	15.94	Q	2.26 (3H, br.s)	*	C-13
19	14.52	Q	1.03 (3H, s)	*	
20	65.43	T	4.02 (1H, d, 11.9)		C-20′
			4.23 (1H, d, 11.9)		C-20
OAc	20.45	Q	1.78 (3H, s)	*	
	169.18	S			
OAc	20.92	Q	2.04 (3H, s)	*	
	169.51	S			
OAc	21.11	Q	1.98 (3H, s)	*	
	169.99	S			
OAc	21.27	Q	2.15 (3H, s)	*	
	170.08	S			
OAc	21.56	Q	2.05 (3H, s)	*	
	170.23	S			
$OCOC_6H_5$	165.13	S	—		
C_6H_5, i	129.80	S	—		
o	129.90	D	7.99 (2H, d, 7.0)	*	*m*-C_6H_5
m	128.74	D	7.47 (2H, m)	*	*o*- C_6H_5
p	133.64	D	7.60 (1H, m)	*	

[a]S=C, D=CH, T=CH_2, Q=Me. Assignments made by the ADEPT technique.

[b]Multiplicity and coupling constant (s) in Hz in parentheses.

[c]An asterisk indicates that a ^1H-^{13}C correlation was observed in a HETCOR spectrum.

[d]These signals could not be distinguished by the HETCOR spectrum; assignment is based on analogy to compound **2**.

chemical shifts of the C-5 and C-20 protons. In compound **2** these were at 5.21 and 3.43/3.71 ppm, respectively, while in compound **3** they occurred at 3.70 and 4.02/4.23 ppm. These chemical shift changes clearly indicate that compound **3** has a free hydroxyl group at C-5 and an acetoxyl group at C-20. The structure and stereochemistry represented by **3** are thus assigned to this second compound.

Compounds **2** and **3** are the first naturally occurring examples of a taxoid oxygenated at C-4, C-5, and C-20 but lacking a D ring. Compounds of this type have previously been formed by opening of the oxetane ring of taxol (14,15) and have also been prepared as intermediates in a synthesis of a baccatin IV derivative from a taxicin-I derivative (16). Compounds of this type have also been proposed as intermediates in the biosynthesis of the oxetane ring system of baccatin III and taxol (17), and the isolation of compounds **2** and **3** thus provides additional support for this proposal.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Varian Unity 400 spectrometer in $CDCl_3$ at ambient temperature. ^1H chemical shifts are recorded in ppm from internal TMS, and ^{13}C shifts are based on the $CHCl_3$ signal at 77.0 ppm. Ms were obtained on a VG 7070 mass spectrometer, using the ci method. Ir spectra were recorded as KBr pellets on an Perkin-Elmer 283B spectrometer, and uv spectra were recorded on a Perkin-Elmer 330 spectrometer. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Melting points were determined on a Thermolyne hot stage and are uncorrected.

ISOLATION OF COMPOUNDS **2** AND **3**.—Plant material was collected in Fujian Province, People's Republic of China, and a voucher specimen is deposited in the Department of Phytochemistry, China Pharmaceutical University. A 16.6 kg sample of heartwood of *T. mairei* was air-dried, ground, and extracted with EtOH. The EtOH extract was evaporated to dryness and then re-extracted with $CHCl_3$ in a Soxhlet extractor to give 60 g of $CHCl_3$-soluble material. This material was subjected to twofold chromatography on Si gel with elution by CH_2Cl_2/hexanes. After the second separation the fractions that eluted immediately after 1-dehydroxybaccatin VI were subjected to preparative tlc [Si gel F_{254}, 20×20 cm, EtOAc-hexanes (5:3)] to give **2** (17.6 mg) and **3** (46.7 mg).

5α,7β,9α,10β,13α-Pentaacetoxy-2α-benzoyloxy-4α,20-dihydroxytax-11-ene [**2**].—Mp 114–115° (Me_2CO); $[\alpha]^{20}D$ −30° (*c*=0.1, $CHCl_3$); uv λ max (EtOH) 228 (ε 4643), 270 (542); ir (KBr) ν max 3400, 1720, 1705, 1360, 1200, 1020, 700 cm^{-1}; ^1H and ^{13}C nmr see Table 1; cims *m/z* $[MH-H_2O]^+$ 699 (2), $[MH-HOAc]^+$ 657 (10), $[MH-H_2O-HOAc]^+$ 639 (10), 597 (15), 579 (5) 535 (25), 517 (15), 492 (10), 475 (25), 457 (15), 433 (15), 415 (50), 347 (30), 373 (30), 355 (60), 337 (40), 313 (35), 295 (80), 123 (80), 105 (100).

7β,9α,10β,13α,20-Pentaacetoxy-2α-benzoyloxy-4α,5α-dihydroxytax-11-ene [**3**].—Mp 122–123° (Me_2CO); $[\alpha]^{20}D$ −37° (*c*=0.1, $CHCl_3$); uv λ max (EtOH) 228 (6386), 270 (1214); ir (KBr) 3400, 1720, 1705, 1360, 1220, 1010, 700 cm^{-1}; ^1H and ^{13}C nmr see Table 2; cims *m/z* $[MH-H_2O]$ 699 (3), $[MH-HOAc]^+$ 657 (18), $[MH-H_2O-HOAc]^+$ 639 (15), 597 (15), 579 (10), 535 (25), 517 (15), 492 (10), 475 (30), 457 (15), 433 (12), 415 (60), 347 (40), 373 (25), 355 (70), 337 (45), 313 (30), 295 (100), 277 (100), 123 (40), 105 (60).

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