New Taxanes with an Opened Oxetane Ring from the Roots of *Taxus mairei*

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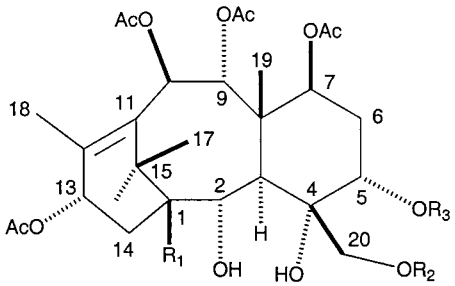
Two novel taxoids, taxumairols N (**1**) and O (**2**), have been isolated from extracts of the roots of *Taxus mairei*. The structures of **1** and **2** were identified as 7*β*,9α,10*β*,13α-tetraacetoxy-2α,4α,5α,20-tetrahydroxytax-11-ene and 7*β*,9α,10*β*,13α-tetraacetoxy-1*β*,2α,4α,5α,20-pentahydroxytax-11-ene on the basis of 1D and 2D NMR techniques including COSY, HMQC, HMBC, and NOESY experiments.

Approximately 100 taxoids from *Taxus* species (Taxaceae) were discovered before 1992, and over 250 new taxane diterpenoids have been isolated and characterized between 1992 and 1999.[1−3] *Taxus mairei* (Lemee & Levl.) S. Y. Hu is an evergreen shrub growing in the medium to high altitudes of the northern and central parts of Taiwan. This plant has been developed for ornamental, horticultural, and pharmaceutical purposes. The taxoids in the leaves, twigs, bark, and heartwood of *T. mairei* have been investigated extensively in the past decade.[3] As a result, 100 new taxane diterpenoids have been isolated and structurelly determined from *T. mairei*. Nevertheless, many new diterpenoids continue to be isolated from this species.[4−6]

We have reported previously the isolation of taxumairols A−F, K, and M and many known taxoids from *T. mairei*.[7] As part of our continuing research on taxane diterpenoids, we have focused on the isolation of polyhydroxylated taxoids in the medium-polarity fractions of *T. mairei* roots. During this investigation, much effort has been made to eliminate the large quantities of lignans present, which mask the polar taxoids in many chromatographic systems. Methylation of taxoid/lignan-containing fractions has facilitated the isolation of taxanes without change or destruction of their structures. Because lignans usually contain phenolic groups, their methylated products may be easily removed. In this paper, we wish to report the isolation and structural elucidation of two additional novel taxanes with an opened oxetane ring system.

As described in the Experimental Section, extensive column chromatography of the $CHCl_3$/MeOH- and *n*-hexane/EtOAc-soluble fractions in the roots of *T. mairei* and subsequent separation and purification of taxanes by passing over a Sephadex LH-20 column, and normal- and reversed-phase HPLC furnished taxumairols N (**1**, 0.00015%, dry wt) and O (**2**, 0.00025%).

Taxumairol N (**1**), [α] +52.2°($CHCl_3$), had the composition $C_{28}H_{42}O_{12}$, as deduced by a combination of negative-ion HRFABMS (*m/z* 569.2569, [M − H^-]) and low-resolution positive-ion FABMS (*m/z* 593, [M + Na^+]). Its UV and IR bands indicated the presence of hydroxyl (3433, 3398, 3363, and 3302 cm^{-1}) and acetyl (1738 cm^{-1}) groups. This was also supported by a major fragment ion at *m/z* 552 $(M - H_2O)^+$ in the EIMS. The characteristic resonances in the ^1H and ^{13}C NMR spectra (Table 1) of **1**, such as peaks for four methyl and four acetyl groups as well as the



1 R_1 = H, R_2 = H, R_3 = H
2 R_1 = OH, R_2 = H, R_3 = H

oxymethylene signal (*δ* 66.5), indicated that this compound is a 6/8/6 taxane with an opened oxetane ring skeleton.[8] This was corroborated by detailed analysis of the $^1H-^1H$ COSY and HMBC spectra of **1** (Table 1). The proton at *δ* 2.94, assigned to H-3, correlated with a proton at *δ* 3.65 (H-2). The H-2 signal also showed a correlation with a multiplet at *δ* 1.90 (H-1). A doublet of doublets at *δ* 5.59 was assigned to the C-7 proton, correlating with the C-6 methylene protons at *δ* 1.95 and 1.70, which in turn coupled with the C-5 methine proton at 4.27 ppm. The overlapping C-13 proton (*δ* 5.65) correlated with C-14 protons at *δ* 2.60 and 2.00 in addition to correlating with the Me-18 signal at *δ* 2.19. Two isolated AB spin systems with doublets between *δ* 5.67 and 6.10 and between *δ* 3.67 and 4.22 indicated the usual C-9 and C-10 methine and C-20 methylene protons, respectively. These findings indicated clearly that taxumairol N (**1**) has four hydroxyl groups at C-2, C-4, C-5, and C-20. Detailed comparison of the ^1H and ^{13}C NMR spectral data of **1** with those of taxumairol A (**3**) suggested that the benzoyl moiety at C-20 and the acetyl group at C-5 in **3** were missing in **1**. Strong evidence came from analysis of its HMBC data (Table 1), which fully supported the structure of **1**. In the HMBC spectrum, long-range correlations of H-10, Me-16, Me-17, and Me-18 to C-11 (*δ* 134.0), H-14*β* to C-12 (*δ* 138.3) and C-13 (*δ* 69.8), and Me-16 and Me-17 to C-1 (*δ* 50.2) and C-15 (*δ* 37.2) indicated that **1** contained a geminal dimethylcyclohexene moiety. The cross-peaks between H-3, H-5, H-20, and C-4 (*δ* 78.2) and between H-3, H-20, and C-5 (*δ* 69.5) revealed that **1** possesses an opened oxetane ring with C-4 being oxygenated and fully substituted. HMBC correlations of H-2/H-3/H-7/H-9/C-8 (*δ* 45.8) and of H-10/Me-19/C-9 (*δ* 75.5) and H-10/C-9/C-11, as well as H-3/C-2 (*δ* 70.4), agreed with an eight-membered ring (ring B) and cyclohexane moiety (ring C) being present. Other

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Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) Spectral Data of Taxumairol N (**1**)

position	^{13}C[a]	^{1}H[b]	COSY	HMBC
1	50.2 d	1.90 m	H-2	Me-16, Me-17, H-3
2	70.4 d	3.65 (brs)	H-1, H-3	H-3
3	43.1 d	2.94 (d,4.5)	H-2	H-5, δ 4.89
4	78.2 s			H-3, H-5, H-7, H-20
5	69.5 d	4.27 (brs)	H-6	H-3, H-20
6	32.0 t	1.95 m, 1.70 m	H-5, H-7	H-7
7	68.9 d	5.59 (dd,11.4,4.8)	H-6	H-5, H-9, Me-19
8	45.8 s			H-2, H-3, H-7, H-9
9	75.5 d	5.67 (d,11)	H-10	H-10, Me-19
10	71.8 d	6.10 (d,11)	H-9	H-9
11	134.0 s			H-10, Me-16 Me-17, Me-18
12	138.3 s			H-10, H-14β, Me-18
13	69.8 d	5.65 (overlapping)	H-14	H-14
14	27.2 t	2.60 m(β), 2.00 m	H-13	H-2
15	37.2 s			H-10, Me-16, Me-17
16	26.1 q	1.60 s		Me-17
17	32.7 q	1.00 s		Me-16
18	15.8 q	2.19 s	H-13	
19	13.8 q	1.00 s		H-3, H-7, H-9
20 A	66.5 t	4.22 (d,10.8)	H-20	H-3
20 B		3.67 (d,10.8)	H-20	
OAc-7	169.4 s	2.09 s[c]		H-7
	21.0 q			
OAc-9	170.1 s	2.03 s[c]		H-9
	21.1 q			
OAc-10	169.0 s	2.00 s[c]		H-10
	20.8 q			
OAc-13	170.3 s	1.94 s[c]		H-13
	21.4 q			
OH		2.80, 3.13, 4.89 brs		

[a] Assignments made using the HMQC and HMBC techniques. [b] Multiplicities and coupling constants in Hz in parentheses. [c] Data interchangeable.

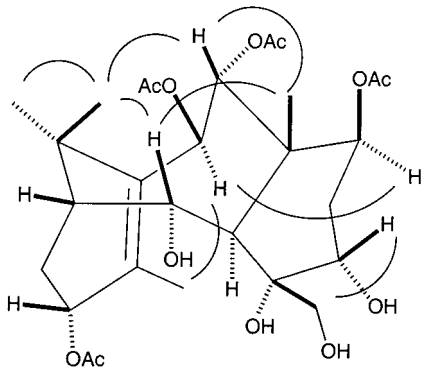

Figure 1. Selective NOESY correlations and proposed conformation for taxumairol N (**1**).

correlations such as four acetoxyl groups attached at C-7, C-9, C-10, and C-13 were also observed in the HMBC study of **1**.

The configurations of the acetoxyl and hydroxyl groups at C-2, C-5, C-7, C-9, C-10, and C-13 were determined to be α, α, β, α, β, and α, respectively, on the basis of a NOESY study of **1** (Figure 1) and comparison of the observed coupling constants with those of taxumairol A, isolated previously from *T. mairei*.[9] The broad singlets of H-2 and H-5 agreed with α-configurations for the hydroxyl groups at C-2 and C-5 in the ^{1}H NMR spectrum of **1**. The coupling pattern of H-7 and the coupling constant between H-9 and H-10 (J = 11.4 Hz) of **1** were similar to those of taxumairol A, indicating that both compounds have identical chiral

Table 2. ^{1}H and ^{13}C NMR Spectral Data of Taxumairol O (**2**)

position	^{13}C[a] (CD_3COCD_3)	^{1}H[b] ($CDCl_3$)	COSY[d]	HMBC[d]
1	76.0 s			Me-16, Me-17
2	73.4 d	4.00 (brs)	H-3	H-3
3	44.1 d	2.58 (d, 3.0)	H-2	H-5, H-20
4	78.4 s			H-3, H-5, H-20
5	69.7 d	4.21 (brs)	H-6	H-20
6	32.7 t	2.00 m, 1.73 m	H-5	
7	69.3 d	5.57 (dd, 10.8, 3.9)	H-6	H-9, Me-19
8	47.0 s			H-3, H-9
9	75.8 d	5.78 (d, 11)	H-10	H-10, Me-19
10	71.4 d	6.13 (d, 11)	H-9	H-9
11	135.9 s			H-10, Me-16 Me-17, Me-18
12	140.0 s			H-10, Me-18
13	70.4 d	5.84 (d, 9.0)	H-14	Me-18
14	36.8 t	2.40 m	H-13	H-13
15	42.7 s			H-10 Me-16, Me-17
16	28.7 q	1.58 s		Me-17
17	32.7 q	1.37 s		Me-16
18	14.8 q	2.12 s	H-13	
19	13.9 q	1.11 s		H-3, H-7
20 A	67.3 t	4.30 (d, 10.4)	H-20	H-3
20 B		3.94 (d, 10.4)	H-20	
OAc-7	168.5 s	2.08 s[c]		H-7
	20.1 q			
OAc-9	169.8 s	2.07 s[c]		H-9
	20.9 q			
OAc-10	168.5 s	2.05 s[c]		H-10
	20.1 q			
OAc-13	170.1 s	1.98 s[c]		H-13
	21.1 q			

[a] Assignments made using the HMQC and HMBC techniques. [b] Multiplicities and coupling constants in Hz in parentheses. [c] Data interchangeable. [d] Correlation data observed in DMSO-d_6.

centers. The hydroxymethyl group at C-4 in **1** was established to be β from the NOESY correlations between H-5 and H-20. NOESY correlations of H-7/H-10 and of H-2/Me-19/H-9 also indicated the C-7 and C-10 acetoxyls to be in the β-configuration, with the C-9 acetoxyl and the C-2 hydroxyl in the α-disposition. Thus, the structure of taxumairol N (**1**) was elucidated as 7β,9α,10β,13α-tetraacetoxy-2α,4α,5α,20-tetrahydroxytax-11-ene. The ^{1}H NMR coupling constants for H-9/H-10 suggested that the conformation of ring B is in the twist-boat conformation with H-9β and H-10α pseudoaxial.

Taxumairol O (**2**), [α] +51.3°(MeOH), had the composition $C_{28}H_{42}O_{13}$ as determined by low-resultion FABMS, consistent with ^{1}H and ^{13}C NMR spectral data. Analysis of the ^{1}H and ^{13}C NMR spectra of **2** revealed that it was an analogue of **1**. Characteristic peaks included four methyls (δ 1.11, 1.37, 1.58, and 2.12), four acetyls (δ 1.98, 2.05, 2.07, and 2.08), and six oxygenated methine protons as well as two oxygenated methylene protons (H-20) at δ 4.30 and 3.94. Detailed comparison of the ^{1}H NMR data with those of **1** suggested that compound **2** contains an additional hydroxyl group at C-1, since the chemical shifts of H-2, H-3, and H-13 in **2** appeared at δ 4.00, 2.58, and 5.84 relative to δ 3.65, 2.94, and 5.65 in **1**. This finding was supported by the ^{13}C NMR spectrum of **2**, which showed nine oxygenated carbons, while compound **1** had only eight. Thus, the major difference between the two compounds was reflected by the chemical shifts of C-1, C-2, C-14, and C-15. In compound **2** these were at 76.0, 73.4, 36.8, and 42.7 ppm, respectively, while they occurred at 50.2, 70.4, 27.2, and 37.2 ppm in compound **1**. The structure of **2** was confirmed by COSY, HMQC, and HMBC studies (Table 2). The stereochemistry represented by **2** was

assigned on the basis of direct comparison with compound **1**.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. The ^{1}H, ^{13}C NMR, HMQC, HMBC, and NOESY spectra were recorded either on a Varian Inova 500 or a Bruker Avance 500 NMR spectrometer. EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer. The high-resolution FABMS data were collected from a JEOL JMS-HX 110 mass spectrometer.

Plant Material. The roots of *Taxus mairei* (Lemee & Levl.) S. Y. Hu were purchased in Kaohsiung, Taiwan, in October 1995. A voucher specimen (TPG8-1) was deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction And Isolation. Dried roots (60 kg) of *T. mairei* were extracted as previously described.[10] Fraction 2 (1.0 g), separated from a taxoid-containing fraction (85 g), was chromatographed on a reversed-phase C_{18} column (35 g) using mixtures of MeOH/H_2O with decreasing polarity (7:3, 7:4, 7:5, 7:6, and 1:1, each 300 mL) to afford six fractions: fractions I (taxumairol B, 11 mg),[9] II (taxumairol C, 29 mg),[10] III (190 mg), IV (taxumairol E, 6 mg),[10] V (33 mg), and VI (18 mg). Part of fraction III (80 mg) was methylated with CH_2N_2 (freshly prepared; Diazald, 1 g) to give a residue, which was separated on a Si gel column (3 g) using mixtures of *n*-hexane/$CHCl_3$/MeOH of increasing polarity (30:30:1, 20:20:1, and 10:10:1, each 30 mL) to give six fractions: fraction a (1β-hydroxy-9-deacetylbaccatin I[3] and 7-deacetyl-1β-hydroxybacctin I,[3] 2.3 mg), fraction b (13 mg), fraction c (taxumairol E, 24 mg),[10] fraction d (21 mg), fraction e (9 mg), and fraction f (6 mg). Separation of fraction d (21 mg) by HPLC (silica gel) using *n*-hexane/$CHCl_3$/MeOH (5:5:1) as the solvent system afforded taxumairol F (2.5 mg)[11] and a fraction (13 mg), which was further purified by reversed-phase HPLC (RP-C_{18}, MeOH/H_2O; 3:2) to yield compound **1** (6 mg).

Another batch of the dried roots (90 kg) was ground and repeatedly extracted with EtOH (300 L) at room temperature. The combined extracts were concentrated to a brown tar (9.5 kg), which was extracted with a solvent mixture of *n*-hexane/EtOAc according to the following ratios and volumes (2:1, 45 L; 1:1, 48 L; 1:2, 45 L; and EtOAc, 12 L) to give four portions: A (900 g), B (1080 g), C (1500 g), and D (2500 g). Part of portion B (100 g) was applied on a Si gel column (1 kg) and eluted with mixtures of $CHCl_3$/Me_2CO of increasing polarity to provide 1β-dehydroxybaccatin VI,[12] 1β-dehydroxybaccatin IV,[12] baccatin VI,[13] and (−)-secoisolariciresinol.[14] Then, the Si gel column was washed with acetone to yield a fraction (4 g). Part of this (2 g) was methylated with CH_2N_2 and the reaction mixture applied on a Sephadex LH-20 column (30 g) eluted with MeOH (500 mL) to give a residue (0.8 g). This was chromatographed on a silica gel column and eluted with $CHCl_3$/*n*-hexane/MeOH according to the following ratios and volumes (40:40:1, 20:20:1, 10:10:1, and 4:4:1, each 500 mL) to yield taxumairol C and a fraction (100 mg). Purification of this fraction by HPLC (RP-C_{18}) using MeOH/H_2O/CH_3CN (5:4:1) as the solvent system afforded compound **2** (10 mg).

Taxumairol N (**1**): amorphous solid; $[\alpha]^{26}_D$ +52.2° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3433, 3398, 3363, 3302, 1738, 1649, 1512, 1375, 1240, 1024 cm^{-1}, ^{1}H and ^{13}C NMR ($CDCl_3$), in Table 1; EIMS *m*/*z* 552 (0.2), 536 (0.2), 518 (0.1), 476 (0.1), 382 (0.3), 368 (4.3), 255 (4.9), 229 (6.9), 221 (6.9), 211 (6.1), 193 (4.7), 185 (6.2), 163 (8.1), 149 (12), 135 (14), 121 (16), 109 (18), 97 (30), 83 (34), 69 (37), 57 (38), 55 (41); FABMS *m*/*z* 593 [M + Na]$^+$; HRFABMS *m*/*z* 569.2569 (calcd for $C_{28}H_{41}O_{12}$, 569.2572).

Taxumairol O (**2**): amorphous powder; $[\alpha]^{25}_D$ +51.3 (*c* 0.2, MeOH); IR (neat) ν_{max} 3417, 1735, 1643, 1257, 1037, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR (CD_3COCD_3) in Table 2; ^{1}H NMR (CD_3COCD_3) δ 6.12 (1H, d, J = 11 Hz, H-10), 5.86 (1H, d, J = 11.1 Hz, H-9), 5.84 (1H, m, H-13), 5.67 (1H, dd, J = 4.2, 12 Hz, H-7), 5.17 (1H, d, J = 4.2, OH), 4.22 (1H, d, J = 4.2, H-2), 4.20 (1H, H-20A), 4.17 (1H, m, H-5), 3.95 (1H, H-20B), 2.68 (1H, d, J = 4.2 Hz, H-3), 2.47 (1H, dd, J = 15.6, 6.9 Hz, H-14β), 2.11 (3H, s, H-18), 1.95, 2.03, 2.05, 2.09 (3H x 4, s, $OCOCH_3$), 1.55 (3H, s, H-16), 1.38 (3H, s, H-17), 1.11 (3H, s, H-19); ^{13}C NMR (DMSO-d_6) δ 75.4 (s, C-1), 72.5 (d, C-2), 43.3 (d, C-3), 78.2 (s, C-4), 67.6 (d, C-5), 32.6 (t, C-6), 69.2 (d, C-7), 46.6 (s, C-8), 75.2 (d, C-9), 70.9 (d, C-10), 135.3 (s, C-11), 139.2 (s, C-12), 70.2 (d, C-13), 36.3 (t, C-14), 42.4 (s, C-15), 28.6 (q, C-16), 32.6 (q, C-17), 14.8 (q, C-18), 14.1 (q, C-19), 65.9 (t, C-20), 168.8, 169.0, 169.3, 170.1, 170.3 (s, O*C*OCH$_3$), 21.5, 21.4, 20.7, 20.6 (q, OCO*C*H$_3$); FABMS *m*/*z* 587 [M + H], 609 [M + Na]$^+$.

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