Taxane Diterpenoids from Seeds of *Taxus mairei*

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A new 2(3→20) abeotaxane, taxumairone A (1), and a new *cis-p*-coumaroyl myo-inositol have been isolated from the seeds of *Taxus mairei* in addition to taxin B (2), taxinine A, taxuspine X, decinnamoyltaxinine E, 5 α -cinnamoyloxy-9 α ,10 β ,13 α -triaceoxy-taxa-4(20)11-diene and 5 α -cinnamoyloxy-2 α ,9 α ,10 β ,13 α -tetraaceoxy-taxa-4(20)11-diene. The structure of 1 was determined by 2D-NMR spectral analysis and chemical correlation with taxin B (2). Compound 1 exhibited potent cytotoxicity against human colon carcinoma cells with an ED₅₀ of 0.1 μ g/ml.

Key words *Taxus mairei*; Taxaceae; taxane diterpenes; taxumairones

Paclitaxel has proven effective in the treatment of ovarian, breast and lung cancers. However, very little is known about its production and the many steps involved in assembling its unique structure need to be explored.^{1,2)} The rearranged 2(3→20) abeotaxanes such as taxines A and B, 2-deacetyl-taxin B,³⁾ deaminoacyltaxine A⁴⁾ and taxuspines B⁵⁾ and W⁶⁾ were considered to be derived from isomeric verticilladiene precursors. These particular compounds have been found in stem, twigs and needles in tiny amounts from *Taxus baccata*, *T. cuspidata* and *T. yunnanensis*. Although more than 350 taxane diterpenes have been isolated to date,^{7–9)} there are still new taxoids awaiting isolation and structural characterization. The isolation of new taxanes might provide important clues in the biosynthesis of paclitaxel, especially for those compounds related to the intermediates of the verticillene pathway.

In a preliminary study, the crude extract of *T. mairei* seeds was found to contain a high amount of taxoids, which might be the reason for their intoxication to birds, animals and human beings. To search for new and bioactive taxanes, we previously isolated taxumairol M and known taxoids from the seeds of *Taxus mairei*.¹⁰⁾ Continued investigation of the *n*-hexane- and *n*-butanol-soluble fractions of the same material has resulted in the isolation of a new 2(3→20) abeotaxane named taxumairone A (1), and a new myo-inositol of *cis-p*-coumarate ester (4) together with taxin B (2), taxuspine X,¹¹⁾ decinnamoyltaxinine E,¹²⁾ taxinine A,¹³⁾ and a mixture of 5 α -cinnamoyloxy-9 α ,10 β ,13 α -triaceoxy-taxa-4(20)11-diene and 5 α -cinnamoyloxy-2 α ,9 α ,10 β ,13 α -tetraaceoxy-taxa-4(20)11-diene,¹⁴⁾ and the *p*-coumarate ester of myo-inositol. In this communication, we wish to report the isolation and structure determination of the novel compound 1 and the new *cis-p*-coumaroyl myo-inositol (4) from *T. mairei* seeds.

Results and Discussion

The EtOH extract of the seeds of *T. mairei* was partitioned between *n*-hexane, MeOH and H₂O (4:3:1) to give an *n*-hexane soluble and MeOH–H₂O soluble fractions. The MeOH–H₂O soluble fraction was extracted with *n*-butanol to afford an *n*-butanol-soluble fraction. Extensive chromatography of the *n*-hexane-soluble residue by combination of Si gel and Sephadex LH-20 columns as well as normal (Si gel) and reverse-phase (RP-C₁₈) HPLC yielded 1 (0.00036%), 2 (0.0018%), taxuspine X (0.00018%), decinnamoyltaxinine E

(0.0018%), taxinine A (0.00036%), and a mixture of 5 α -cinnamoyloxy-9 α ,10 β ,13 α -triaceoxy-taxa-4(20)11-diene and 5 α -cinnamoyloxy-2 α ,9 α ,10 β ,13 α -tetraaceoxy-taxa-4(20)11-diene (0.057%). The *n*-butanol-soluble residue was chromatographed on Sephadex LH-20 and reverse-phase RP-C₁₈ columns to yield *trans*- and *cis-p*-coumarate esters of myo-inositol. The structures of the known taxoids and *p*-coumarate ester of myo-inositol were identified on the basis of spectral evidence and comparison with authentic samples. The structural elucidation of compounds 1 and 4 is discussed below.

Compound 1, [α] –306° (CHCl₃), had the composition C₂₆H₃₂O₈ as deduced by a combination of low resolution EI

1

2 R = α -OH, β -H
3 R = O

4

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Table 1. ^1H - and ^{13}C -NMR (CDCl_3) Spectral Data of Taxumairone A (**1**)

Position	^{13}C (ppm) ^{a)}	^1H ^{b)}	COSY	HMBC
1	47.8 d	1.80 m		H2, Me16, 17
2	69.4 d	5.76 (dd, 10.5)	H20	
3	35.5 t	2.60 (d, 14)	H3	H20, Me19
		3.08 (dd, 14, 2)		H3
4	134.1 s			H3
5	189.1 s			H3, 7, 20
6	131.4 d	6.29 (d, 10.2)	H7	
7	150.1 d	6.76 (dd, 10.2, 1.8)	H6	H3, Me19
8	52.5 s			H6, Me19
9	202.8 s			H10, Me19
10	80.0 d	6.08 s		
11	130.1 s			H10, Me16
				Me17, 18
12	139.2 s			H10, Me16
13	67.6 d	5.26 (d, 10)	H14	H1, Me18
14	26.8 t	2.67 m (β)	H13, 14	H2
		2.02 m (α)	H13, 14	
15	37.6 s			H10, Me16, Me17
16	25.0 q	1.26 s		Me17
17	33.7 q	1.11 s		Me16
18	17.3 q	1.51 s		
19	28.2 q	1.45 s		H3, 7
20	134.0 d	6.35 (dd, 10.5, 2)	H2	H3
OAc	170.6 s	2.19 s ^{c)}		
OAc	170.0 s	2.17 s ^{c)}		
OAc	169.7 s	2.04 s ^{c)}		

a) Multiplicities and assignments made by the HMQC and HMBC techniques. b) Multiplicities and coupling constants in Hz in parentheses. c) Data interchangeable.

mass (m/z 472), FAB-MS (m/z 495, $\text{M}+\text{Na}$), ^{13}C -NMR spectroscopy and further confirmed by HR-FAB-MS. Its UV and IR bands indicated the presence of conjugated ketone (1674 cm^{-1} , 240 nm) and acetyl (1736 cm^{-1}) groups. This was also supported by fragment ions at m/z 430 ($\text{M}-\text{Ac}$)⁺ and m/z 413 ($\text{M}-\text{OAc}$)⁺ in the EIMS spectrum. The ^1H -NMR spectrum of **1** exhibited proton signals due to four methyls (δ 1.11, 1.26, 1.45 and 1.51), three acetyl methyls (δ 2.04, 2.17 and 2.19) and three olefinic (δ 6.76, 6.29 and 6.35) as well as three oxygenated methine (δ 6.08, 5.76, and 5.26) protons. The ^{13}C -NMR spectrum showed the presence of a ketone carbonyl (δ 202.8), a conjugated carbonyl (δ 189.1) and three olefinic double bonds (δ 130.1, 131.4, 134.0, 134.1, 139.2 and 150.1) in addition to three oxygenated methines (δ 69.4, 80.0 and 67.6). The connectivities of each proton and carbon were established by detailed analysis of ^1H - ^1H COSY and HMBC spectra (Table 1) of **1**. A multiplet at δ 2.67, assigned to H-14 β , correlated with H-14 α (δ 2.02) and a doublet at δ 5.26 (H-13). The H-2 (δ 5.76) showed correlation with a doublet of doublets at δ 6.35 (H-20). Two isolated spin systems were also found. One set was a doublet of doublets at δ 6.76 (H-7) and a doublet at δ 6.29 (H-6). Another characteristic AB spin pattern was located at 3.08 ppm and 2.60 ppm, which were assigned to the C-3 methylene protons. The remaining singlet at δ 6.08 was therefore assigned to H-10. On the basis of COSY experiments, the structure of **1** was similar to that of **2**, which belongs to 2(3 $\rightarrow$ 20) abeo-taxanes.^{3,8)} However, the C ring in compound **1** was an α,β -unsaturated cyclohexenone rather than a cyclohexane as in **2**. In the HMBC spectrum, correlations between Me19 and C-3 (δ 35.5), C-7 (δ 150.1), C-8 (δ 52.5), C-9 (δ 202.8) and between H-3, H-7, H-20 and C-5 (δ 189.1) revealed that **1** possessed an α,β -unsaturated cyclo-

hexenone (ring C). This moiety was corroborated by an UV absorption at 240 nm .¹⁵⁾ HMBC correlations of H-2/Me16/Me17/C-1 (δ 47.8), H-10/Me19/C-9 and H-10/Me16/Me17/Me18/C-11 (δ 130.1) as well as H-2/C-14 (δ 26.8) agreed with a ten membered ring (ring B). Correlations of H-1/Me18/C-13 (δ 67.6), H-10/Me16/C-12 (δ 139.2) and H-10/Me16/Me17/C-15 (δ 37.6) indicated that **1** contained a geminal dimethylcyclohexene moiety (ring A).

The relative stereochemistry of **1** was determined by analysis of NOESY spectrum. Correlations between H-1/H-2, H-13/H-14 β , H-1/H-14 β , H-1/Me17 and Me16/Me17 agreed with a β -configuration for H-2 and H-13. NOESY correlations among H-10/H-7/Me-18 in **1** suggested H-10 was in α -orientation. These findings were consistent with an unusual cage conformation previously reported for taxuspinine B and other taxin B derivatives.³⁻⁵⁾ Thus the configurations in **1** are the same as those of 2(3 $\rightarrow$ 20) abeo-taxanes and its possible conformation is indicated in Fig. 1. To confirm the assigned structure **1**, **2** was oxidized with CrO_3 /pyridine to yield 5-dehydrotaxin B (**3**) and a compound identical with **1**.

Compound **4** was obtained as an amorphous solid. The ^1H -NMR spectrum of **4** resembled that of *trans*-*p*-coumaroyl myo-inositol, suggesting that **4** was an isomer. An AX spin system at δ 6.00 and δ 7.12 (d, $J=12.6\text{ Hz}$) in **4**, rather than the two doublets at δ 6.37 and δ 7.62 (d, $J=16\text{ Hz}$) in *trans*-*p*-coumaroyl myo-inositol, indicated the presence of a *cis*-*p*-coumaroyl moiety in **4**. Compound **4** seems to be unstable and it decomposed after ^1H -NMR measurement.

Taxumairone A is a new 2(3 $\rightarrow$ 20) abeo-taxane having an α,β -unsaturated carbonyl system at C-5, C-6 and C-7 plus an exocyclic double bond at C-4/C-20. This compound was believed to be a natural product because it was also obtained from another batch of ripe seeds of *T. mairei* under a normal

Fig. 1. NOESY and Proposed Conformation of 1

fractionated procedure. Furthermore, taxumairone A exhibited significant cytotoxicity against human colon (COLO-205) and epidermoid carcinoma (KB) cells with an ED_{50} of 0.1 and 1.84 $\mu\text{g}/\text{ml}$, respectively.

Experimental

Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR and UV spectra were recorded with a HORIBA FT-720 and a HITACHI U-3210 spectrophotometer, respectively. EI and FAB-MS spectra were measured with a VG Quattro 5022 mass spectrometer. ^1H - and ^{13}C -NMR spectra were recorded using a Varian FT-300 or a Bruker AM-400 NMR instrument.

Plant material The seeds of *Taxus mairei* were collected in December, 1997, in Tien-shiung, Hua-lein County, Taiwan and were identified by one of the authors (Y. C. S.). A voucher specimen of seeds (TPG8-2) is deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation Unripe fresh seeds (1.1 kg) of *T. mairei* were ground and extracted with EtOH to afford a crude extract (117 g), which was partitioned between *n*-hexane (600 ml) and 25% aq. MeOH (600 ml) to yield an *n*-hexane-soluble fraction (12 g). The 25% aq. MeOH was extracted with *n*-butanol to give an *n*-butanol-soluble fraction (65 g). The *n*-hexane-soluble fraction was chromatographed on a Si gel column (150 g) and eluted with *n*-hexane-EtOAc of increasing polarity to give five fractions, A (mixture of 5 α -cinnamoyloxy-9 α ,10 β ,13 α -triacetox-taxa-4(20)11-diene and 5 α -cinnamoyloxy-2 α ,9 α ,10 β ,13 α -tetraacetox-taxa-4(20)11-diene, 625 mg), B (taxinine, 890 mg), C (0.61 g), D (1 g) and E (0.46 g). Fraction C was rechromatographed on a Si gel column and eluted with *n*-hexane-CHCl₃-MeOH (100:100:1) to yield decinnamoyl-taxinine E and a residue (0.18 g). Further separation of the residue by HPLC column (Si gel, *n*-hexane-CHCl₃-MeOH, 10:10:1) gave taxinine A (4 mg), taxin B (9 mg) and a less polar fraction (74 mg). The latter was separated by PTLC (Si gel) developed with *n*-hexane-CHCl₃-MeOH (10:10:1) to afford two fractions a (16 mg) and b (39 mg). Fraction a was separated by ODS HPLC (LiChrosorb RP-18 column, 90% aq. MeOH) to yield taxumairone A (1, 4 mg) taxinine A and taxuspine X (2 mg). Fraction b was also separated by ODS HPLC (90% aq. MeOH) to provide taxin B (2, 11 mg), taxuspine X and taxezopidine G (16 mg). Part of the *n*-butanol-soluble fraction (5.3 g) was applied to a Sephadex LH-20 and eluted with MeOH to give a residue, which was chromatographed by a reverse-phase C₁₈ column (H₂O/MeOH, 20:1 and 10:1) to yield myo-inositols of *trans*-*p*-coumarate ester (100 mg) of and *cis*-*p*-coumarate ester (4, 20 mg).

Taxumairone A (1): Isolated as an amorphous solid, $[\alpha]_D^{26} -306^\circ$ ($c=0.26$, CHCl₃); FAB-MS: m/z 495 $[M+Na]^+$; UV λ_{max} (log ϵ) (MeOH) nm: 208 (3.71), 240 (3.28); IR (KBr) cm^{-1} : 2929, 1736, 1674, 1666, 1635, 1612, 1439, 1234, 1022, 968, 840, 737, 663; ^1H - and ^{13}C -NMR: Table 1. EIMS m/z (rel. int.): 472 (0.02, M^+), 430 (0.2), 413 (0.2), 371 (0.3), 325 (1), 311 (1), 295 (0.8), 282 (3.6), 264 (10), 249 (9), 239 (2.8), 217 (3.7), 197 (3.5), 175 (1), 160 (17), 145 (18), 133 (17), 121 (25), 105 (17), 91 (15), 83 (21),

77 (13), 69 (11), 55 (19); HR-FAB-MS m/z : 473.2158 $[M+H]^+$ (Calcd for C₂₆H₃₃O₈, 473.2175), m/z : 495.1976 $[M+Na]^+$ (Calcd for C₂₆H₃₂O₈Na, 495.1995).

Oxidation of Taxin B (2): To a solution of 2 (8 mg) in CHCl₃ was added CrO₃ (40 mg) and pyridine (2 drops), and the reaction mixture was stirred for 30 min at room temperature. After filtration to remove the CrO₃, the filtrate was concentrated to give a residue. Purification of the residue by HPLC (LiChrosorb RP-18 column, 80% aq. MeOH) yielded 5-dehydrotaxin B (3, 5 mg): an amorphous solid, $[\alpha]_D^{26} -224^\circ$ ($c=0.9$, CHCl₃); FABMS: m/z 555 $[M+Na]^+$; HR-EI-MS m/z : 532.2309 (Calcd for C₂₈H₃₆O₁₀, 532.2308); ^1H -NMR (300 MHz, CDCl₃) δ : 1.78 (1H, m, H-1), 5.70 (1H, dd, $J=2.2$, 10.2 Hz, H-2), 2.40 (1H, d, $J=14$, 1.5 Hz, H-3 α), 2.91 (1H, d, $J=14$ Hz, H-3 β), 2.80 (1H, d, $J=10$ Hz, H-6 β), 5.42 (1H, dd, $J=3.9$, 12.6 Hz, H-7), 6.50 (1H, s, H-10), 5.28 (1H, d, $J=10.2$ Hz, H-13), 2.70 (1H, m, H-14 β), 1.32 (3H, s, H-16), 1.14 (3H, s, H-17), 1.83 (3H, s, H-18), 1.39 (3H, s, H-19), 5.26 (1H, dd, $J=1.6$, 10.2 Hz, H-20), 2.04, 2.09, 2.17, 2.20 (3H $\times$ 4, s, OCOCH₃); EI-MS m/z (rel. int.): 532 (0.1, M^+), 504 (0.3), 473 (1.7), 430 (1.2), 413 (0.9), 384 (1.1), 371 (2.6), 342 (2.8), 324 (3.1), 300 (2.2), 282 (10), 249 (6), 239 (4), 217 (6.3), 175 (9), 160 (9), 145 (14), 133 (15), 121 (22), 105 (12), 91 (9.4), 79 (5.6), 55 (8.1). Compound 2 (5 mg) in pyridine (1 ml) was reacted with CrO₃ (20 mg)/pyridine (0.033 ml)/CHCl₃ (0.25 ml) and the reaction mixture was stirred for 30 min. Work-up and separation by HPLC as mentioned above (LiChrosorb RP-18 column, 80% aq. MeOH) yielded 5-dehydrotaxin B (3, 1 mg, t_R 11.92 min) and a compound (0.5 mg, t_R 9.96 min), which showed identical spectral data with those of 1.

Cis-*p*-coumaroyl myo-inositol (4): Isolated as an amorphous powder, ^1H -NMR δ (300 MHz, CD₃OD): 3.3–3.8 (5H, m, H-2, 3, 4, 5, 6), 5.50 (1H, m, H-1), 6.00 (1H, d, $J=12.6$ Hz, H-2'), 7.12 (1H, d, $J=12.6$ Hz, H-3'), 6.90 (2H, H-6', 8'), 7.54 (2H, H-5', 9').

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