Taxane Diterpenoids from Seeds of *Taxus mairei*

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Received February 24, 2000; accepted May 26, 2000.

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, 5α-cinnamomoyloxy-9α,10β,13α-triacetoxyl-taxa-4(20)11-diene and 5α-cinnamomoyloxy-2α,9α,10β,13α-tetraacetoxyl-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 ED$_{50}$ of 0.1 μg/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 deaminacetyltaxine 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 5α-cinnamomoyloxy-9α,10β,13α-triacetoxyl-taxa-4(20)11-diene and 5α-cinnamomoyloxy-2α,9α,10β,13α-tetraacetoxyl-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 H$_2$O (4:3:1) to give an *n*-hexane soluble and MeOH→H$_2$O soluble fractions. The MeOH→H$_2$O 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-C$_{18}$) HPLC yielded 1 (0.00036%), 2 (0.0018%), taxuspine X (0.00018%), decinnamoyltaxinine E (0.0018%), taxinine A (0.00036%), and a mixture of 5α-cinnamomoyloxy-9α,10β,13α-triacetoxyl-taxa-4(20)11-diene and 5α-cinnamomoyloxy-2α,9α,10β,13α-tetraacetoxyl-taxa-4(20)11-diene (0.057%). The *n*-butanol-soluble residue was chromatographed on Sephadex LH-20 and reverse-phase RP-C$_{18}$ 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]$_D$ -306° (CHCl$_3$), had the composition C$_{26}$H$_{32}$O$_8$ as deduced by a combination of low resolution EI
mass (m/z 472), FAB-MS (m/z 495, M+Na), 13C-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 (M–Ac)+ and m/z 413 (M–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 13C-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-14β, correlated with H-14α (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→20) abeoatanes.34 However, the C ring in compound 1 was an αβ-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 αβ-unsaturated cyclohexene (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-14β, H-1/H-14β, H-1/Me17 and Me16/Me17 agreed with a β-configuration for H-2 and H-13. NOE-SY 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 taxusprine B and other taxin B derivatives.3-5 Thus the configurations in 1 are the same as those of 2(3→20) abeo-taxanes and its possible conformation is indicated in Fig. 1. To confirm the assigned structure 1, 2 was oxidized with CrO3/pridine 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 Hz) in 4, rather than the two doublets at δ 6.37 and δ 7.62 (d, J=16 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.

Taxumaireone A is a new 2(3→20) abeo-taxane having an αβ-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
fractionated procedure. Furthermore, taxaimure A exhibited significant cytotoxicity against human colon (COLO-205) and epidermoid carcinoma (KB) cells with an ED$_{50}$ of 0.1 and 1.84 µg/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. El and FAB-MS spectra were measured with a VG Quattro 5022 mass spectrometer. $^1$H- 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-shing, Hua-Tei County, Taiwan and were identified by one of the authors (Y. C. S.). A voucher specimen of seeds (TPGS-2) is deposited in the Institute of Marine Resources, National Sun Yat-sen University.

**Extraction and Isolation**
Unripe fresh seeds (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 5α-cinna-11α-oxo-one (40 mg) and 5α-cinna-11α-oxo-one (20 mg) was reacted with CrO$_3$ (20 mg) and pyridine (0.033 ml) CHCl$_3$ (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 5dehydrotaxol (31 mg, 91.92% yield) and a compound (0.5 mg, $t_R$ 9.96 min), which showed identical spectral data with those of I.

**Cis-5-coumaroyl myo-inositol (4):** Isolated as an amorphous powder, $^1$H-NMR (300 MHz, CDCl$_3$): $d$ 0.26, 0.28, 0.75, 0.80, 2.36, 2.64 (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 C$_{38}$H$_{56}$O$_{11}$, 473.2175, m/z: 495.1976 [M+Na$^+$] (Calcd for C$_{38}$H$_{53}$ONa, 495.1995).

**Acknowledgements**

The authors thank Dr. Yao-hau Kuo, National Research Institute of Chinese Medicine for providing the cytotoxicity tests. The National Institute of Health, Republic of China (NIRIT-GE-EX989809L) is acknowledged for financial support.

**References**