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Taxane Diterpenes from *Taxus mairei*

Ya-Ching Shen[1,2], and Ching-Yeu Chen[1]

[1] Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Rd., Kaohsiung, Taiwan, R.o.C.
[2] Address for correspondence

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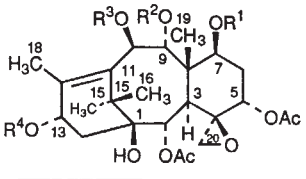
Abstract: **In addition to 7-*O*-deacetyl-1β-hydroxybaccatin I and 9-*O*-deacetyl-1β-hydroxybaccatin I, a new taxane taxumairol F has been isolated from the ethanolic extracts of the roots of *Taxus mairei*. Their structures were determined on the basis of spectral evidence and chemical correlation with 1-β-hydroxybaccatin I.**

The investigation of phytochemical constituents from various parts of *Taxus* species has led to the discovery of many new taxoids (1–4). *T. mairei*, an evergreen shrub was developed for sculptural materials and ornamental purposes. The diterpenoids in the bark, the heartwood and the twigs of *T. mairei* have been extensively investigated (5, 6). The roots of *T. mairei* have been used in Chinese folk medicine to treat diabetes (7). Recently, we reported the isolation of taxumairols A, B, C (**4**), D (**5**), and E, and known taxoids from the roots of this species (8–10). Continued investigation on the minor constituents of *Taxus mairei* has resulted in the isolation of an additional new taxane, named taxumairol F (**3**) together with compounds **1** and **2**. Herein, we wish to report the isolation and structure elucidation of compound **3**.



	R^1	R^2	R^3	R^4
1	Ac	H	Ac	Ac
2	H	Ac	Ac	Ac
3	Ac	Ac	Ac	H
4	H	H	H	Ac
5	Ac	Ac	H	Ac
6	Ac	Ac	Ac	Ac

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The extract of *T. mairei* roots gave compounds **1**–**3**. Compounds **1** and **2** were determined as 9-*O*-deacetyl-1β-hydroxybaccatin I and 7-*O*-deacetyl-1β-hydroxybaccatin I, respectively, by comparison of their spectral data (EIMS and ^{1}H-NMR) with those of reported in the literature (11) and the stereochemistry was confirmed by acetylation of **1** and **2** to yield 1β-hydroxybaccatin I (**6**). The structure of compound **3** was established as follows.

Taxumairol F (**3**) had the composition $C_{30}H_{42}O_{13}$ as derived from FABMS, EIMS, and ^{13}C-NMR data. The characteristic resonances of four methyl singlets (δ 1.08, 1.23, 1.59 and δ 2.11) suggested that compound **3** is a taxane analog. A pair of doublets at δ 2.34 (J = 5.4 Hz) and 3.61 (J = 5.4 Hz) was indicative of the C-20 methylene protons of the epoxide ring. The presence of five acetyl and two hydroxy groups was verified by the observation of ^{1}H- and ^{13}C-NMR spectral data. A COSY spectrum established not only the connectivity of each proton but also located the acetyl moieties at the C-2, C-5, C-7, C-9, and C-10 positions. The C-13 proton was observed at δ 4.80, suggesting that C-13 was hydroxylated. The stereochemistry of **3** was determined by comparison of the observed chemical shifts and coupling constants with those of compounds **1** and **2**, and chemical correlation with a known compound. The coupling constant between H-2 and H-3 agreed with α-configurations for the C-2 acetoxy and for H-3. The coupling pattern of H-7 and H-5 were similar to those of compounds **1** and **2**. The J value (10.5 Hz) of the isolated AB system at δ 6.00 (H-9) and δ 6.21 (H-10) was also indicative of a *trans*-oriented configuration.

Acetylation of **3** yielded a hexaacetate identical to 1β-hydroxybaccatin I (**6**). Because the stereochemistry of **6** was established by X-ray crystallographic analysis (10), the structures of compounds **1**–**3** were confirmed unequivocally. Therefore, the new taxumairol F (**3**) was established as 13α-deacetyl-1β-hydroxybaccatin I.

Materials and Methods

IR and UV spectra were measured on Hitachi T-2001 and on Hitachi V-3210 spectrophotometers, respectively. EI and FAB mass spectra were recorded on a VG Quattro 5022 mass spectrometer. The ^{1}H-NMR, COSY, ^{13}C-NMR, and DEPT spectra were recorded on Varian FT-300 and FT-400 spectrometers. Sephadex LH-20, silica gel 60, and RP-C18 were used for CC and HPLC, and pre-coated silica gel plates (Kieselgel 60 F_{254}, 1 mm) were used for preparative TLC.

Plant materials: The roots of *Taxus mairei* were purchased in Kaohsiung, Taiwan, 1993. A voucher specimen (TPG8-1) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation: Dried roots (60 kg) were extracted as previously described (10). A portion (1.5 kg) was obtained from the $CHCl_3$/MeOH soluble fraction. Extensive separation by LH-20, silica gel and RP-C18 chromatography gave fraction c (85 mg) and fraction d (420 mg). Fraction c was rechromatographed on a LH-20 column (*n*-hexane/$CHCl_3$/MeOH, 1 : 1 : 2) to give a residue (70 mg), which was further purified by HPLC (silica gel, *n* hexane/$CHCl_3$/MeOH, 5 : 5 : 1) to obtain a mixture of compounds **1** and **2** (14 mg). Fraction d was separated by the same procedures as described in fraction c to yield taxumairols F (**3**, 6 mg) and D (**4**), 40 mg) as well as a mixture of **1** and **2** (6 mg). Compounds **1** and **2** showed identical spectral data (^{1}H-, ^{13}C-NMR, and EIMS) to those reported (11).

Taxumairol F (**3**): Isolated as an amorphous solid, $[\alpha]_D^{25}$: +13° (*c* 0.09, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.50 (1H, m, H-2), 3.20 (1H, d, J = 3.3 Hz, H-3), 4.23 (1H, m, H-5), 1.80 (2H, m, H-6), 5.50 (1H, m, H-7), 6.00 (1H, d, J = 10.5 Hz, H-9), 6.21 (1H, d, J = 10.5 Hz, H-10), 4.80 (1H, m, H-13), 1.98 (1H, m, H-14α), 2.56 (1H, m, H-14β), 1.59 (3H, s, H-16), 1.23 (3H, s, H-17), 2.11 (3H, s, H-18), 1.08 (3H, s, H-19), 3.61 (1H, d, J = 5.4 Hz, H-20a), 2.34 (1H, d, J = 5.4 Hz, H-20b), 2.17, 2.09, 2.07, 2.05, 2.00 (3Hk × s, $OCOCH_3$); ^{13}C-NMR (75.4 MHz, $CDCl_3$): δ = 75.5 (s, C-1), 71.4 (d, C-2), 42.1 (d, C-3), 58.3 (s, C-4), 77.8 (d, C-5), 31.1 (t, C-6), 71.7 (d, C-7), 46.8 (s, C-8), 75.3 (d, C-9), 68.8 (d, C-10), 144.5 (s, C-11), 135.1 (s, C-12), 69.2 (d, C-13), 41.5 (t, C-14), 42.8 (s, C-15), 16.2 (q,C-16), 15.7 (q, C-17), 28.6 (q, C-18), 13.3 (q, C-19), 50.2 (t, C-20), 169.3, 169.4, 169.8, 169.9, 170.1 (s, $OCOCH_3$), 21.8, 21.5, 21.4, 20.9, 20.8 (q, $OCOCH_3$); EIMS: *m/z* (rel. int.) = 466 (0.1), 448 (0.2), 406 (0.4), 388 (1.0), 373 (1.0), 241 (2.0), 211 (2.1), 149 (34), 105 (19), 91 (15), 55 (15), 43 (100).

Acetylation of compounds **1**, **2** *and taxumairol F* (**3**): Acetylation (Ac_2O : Py; 1 : 1; rt) of **1**, **2** (3 mg) and **3** (2 mg), respectively, gave a product which showed identical ^{1}H-NMR, [α], EIMS data and Rf value with those of 1β-hydroxybaccatin I (**6**).

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