A New Taxane Diterpenoid from *Taxus mairei*

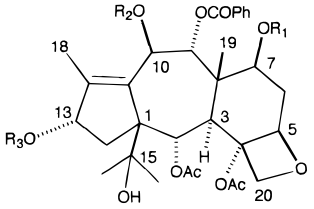
Ya-Ching Shen,*,† Ching-Yeu Chen,† and Yao-Haur Kuo‡

Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Road, Kaohsiung, Taiwan, Republic of China, and National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China

Received December 26, 1997

A new 11(15→1)-abeotaxane diterpene, taxumairol K (**1**), has been isolated from the ethanolic extract of the roots of Formosan *Taxus mairei* (Lemee & Levl.) S. Y. Hu. The structure of **1** was determined as 9α-(benzoyloxy)-2α,4α-diacetoxy-5*β*,20-epoxy-1*β*,7*β*,10*β*,13α-tetrahydroxy-11(15→1)-abeotaxane on the basis of spectral analysis. Taxumairol K (**1**) exhibited mild cytotoxicity against HeLa tumor cells.

The discovery of paclitaxel as a clinically useful antitumor agent has stimulated great interest in searching for practical and renewable sources of paclitaxel and other taxoids.[1−8] *Taxus mairei* (Lemee & Levl.) S. Y. Hu (Taxaceace) is an evergreen shrub distributed over mountainous regions of the northern and central parts of Taiwan. The roots of *T. mairei* have been used in Chinese folk medicine for thetreatment of diabetes.[9] Previous studies on the diterpenoids in the roots of *T. mairei* have resulted in the isolation of taxumairols A−F together with many known taxoids.[10−13] Taxumairols A−F have a 6/8/6-membered ring skeleton. Continued investigation of *T. mairei* has resulted in the identification of a novel abeotaxane with a 5/7/6-membered ring system from the roots of this species. Herein, we wish to report the isolation, structure elucidation, and cytotoxicity of taxumairol K (**1**).



1 $R_1 = R_2 = R_3 = H$
2 $R_1 = R_2 = R_3 = Ac$

Extensive chromatography of the ethanolic extract of *T. mairei* roots on Sephadex LH-20, Si gel, and RP-C18 gave a residue, which was finally purified by HPLC to furnish taxumairol K (**1**). Taxumairol K (**1**), [α] −8.5° (MeOH), had the composition $C_{31}H_{40}O_{11}$ as derived by a combination of EIMS and DEPT spectroscopy. Its UV and IR bands indicated the presence of benzoyl (229 nm), hydroxyl (3475, 3444, 3421 cm^{-1}), and acetyl (1722 cm^{-1}) groups. This was also supported by the base peak at *m*/*z* 105 due to the benzoyl ion and peaks at *m*/*z* 447 and *m*/*z* 405 arising from loss of benzoic acid, 2 mol of H_2O, and acetic acid from the molecular ion. The presence of one benzoyl and two acetyls as well as four hydroxyls was verified from the ^1H and ^{13}C NMR spectral data of **1** (Table 1). A taxene skeleton was inferred from the observation of characteristic resonances, such as four methyl singlets [*δ* 1.02 (17-Me), 1.23 (16-Me), 1.74 (19-Me), and 1.80 (18-Me)] and the corresponding ^{13}C NMR signals.[1,2] The ^1H NMR spectrum of **1** consisted of several overlapped, broad lines, and spectral analysis was difficult.[11] Fortunately, the relationship between each proton such as H-2/H-3, H-6a/H-6b/H-7, and H-9/H-10 in **1** could be observed from the COSY spectrum. In accord with this, the overlapped H-2 (*δ* 5.97) and H-9 (*δ* 5.98) signals were distinguished by their correlations with H-3 (*δ* 3.19) and H-10 (*δ* 4.70), respectively. The H-7 signal was assigned at *δ* 4.47 because it correlated with signals at *δ* 1.75/2.60 (H-6). Although correlations between H-13/H-14a and H-13/H-14b could not be observed, cross-peaks between H-14a/H-14b appeared at *δ* 1.58 and *δ* 2.20. The remaining H-20 signals (*δ* 4.44/4.46) were overlapped with those of H-13 and H-7. Detailed analysis of the ^1H and ^{13}C NMR spectra revealed that compound **1** is an isomer of an 11(15→1)-abeotaxane,[14,15] namely, 7,9-dideacetyltaxayuntin, isolated from *T. yunnanensis*.[16] These isomeric compounds had similar ^1H and ^{13}C NMR spectra. Detailed comparison of the ^1H and ^{13}C NMR spectrum of **1** with those of taxayuntin F further suggested that the benzoate was at C-9 and the acetates at C-2 and C-4. This was further backed by the observation that the chemical shift difference between the diastereotopic C-20 protons was small, as expected for the presence of a C-2 acetate, while a C-2 benzoate would have led to a larger difference.[14,17−22] An HMBC study of **1** failed to correlate the signals between H-10 and C-12 and between H-9 and benzoyl carbon via long-range coupling due to low sensitivity.

Upon acetylation, compound **1** yielded a pentaacetate **2** ($C_{37}H_{46}O_{14}$), which is an isomer of 10,13-diacetyltaxayuntin E.[23] Compound **2** showed the H-7 signals at *δ* 5.55, H-10 at *δ* 6.44, and H-13 at *δ* 5.62 in its ^1H NMR spectrum, indicating that two of the three hydroxyls occur at the C-7 and C-13 positions in **1**. The remaining hydroxyl group was assigned to either the C-9 or the C-10 position. To confirm the position of the benzoyloxy group and to assign the ^1H and ^{13}C NMR signals of **2**, COSY, HMQC, and HMBC experiments were performed. The connectivities between H-10 (*δ* 6.44) and C-12 (*δ* 147.1) and between H-9 (*δ* 6.32) and

* To whom correspodance should be addressed. Tel.: (886) 7-525-2000 ext 5058. Fax: (886) 7-525-5020.
† National Sun Yat-sen University.
‡ National Research Institute of Chinese Medicine.

S0163-3864(97)00573-9 CCC: $15.00
Published on Web 06/05/1998

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

position	^{13}C	carbon type[a]	^{1}H[b]	COSY	HMBC
1	66.6	s			H-2
2	70.2	d	5.97 (overlap)	H-3	H-3
3	43.7	d	3.19 (brs)	H-2	H-2
4	79.7	s			H-20
5	85.4	d	4.92 (d, 6.6)		
6	34.7	t	2.60 (m) 1.75 (m)	H-7	
7	70.2	d	4.47 (m)	H-6	H-5[c]
8	43.4	s			Me-19
9	80.8	d	5.98 (overlap)	H-10	
10	68.7	d	4.70 (brs)	H-9	
11	137.9	s			Me-18[c]
12	146.2	s			H-10,[c] Me-18[c]
13	77.6	d	4.49 (m)		Me-18[c]
14	39.5	t	2.20 (m, β), 1.58 (m)		
15	76.6	s			Me-16, Me-17
16	25.6	q	1.23 s		Me-17[c]
17	27.5	q	1.02 s		Me-16[c]
18	11.3	q	1.80 s		
19	14.0	q	1.74 s		
20A	75.1	t	4.44 (overlap)	H-20	
20B			4.46 (overlap)	H-20	
OAc	170.4	s			
	21.7	q	2.00 s		
OAc	171.3	s			
	22.4	q	2.16 s		
$OCOC_6H_5$	167.7	s			H-9,[c] *o*-C_6H_5
i	130.4	s			
o	129.8	d	8.00(d, 6.0)	*m*-C_6H_5	*p*-C_6H_5, *m*-C_6H_5
m	128.3	d	7.43 (m)	*o*,*p*-C_6H_5	
p	133.0	d	7.53 (m)	*m*-C_6H_5	*o*-C_6H_5
OH-7			3.44 (brs)	H-7	
OH-10			3.85 (brs)	H-10	
OH-13			1.50 (overlap)	H-13	

[a] s = C, d = CH, t = CH_2, q = CH_3. Multiplicities and assignments made by the DEPT and HMBC. [b] Multiplicities and coupling constants in Hz in parentheses. [c] Data taken from taxumairol K pentaacetate (**2**).

the benzoyl carbonyl (δ 167.5) unambiguously established the benzoyl moiety at the C-9 position. The stereochemistry of **1** and **2** was tentatively determined by comparison of their coupling constants in the ^{1}H NMR spectra and chemical shifts of ^{13}C NMR spectra with those of known compounds.

Biological study on tumor cytotoxicity revealed that taxumairol K (**1**) showed weak activity toward cervical carcinoma (HeLa) with an IC_{50} value of 9.2 μg/mL. Taxumairol K (**1**) was inactive against hepatoma (Hep-3B) tumor cells (IC_{50} 40 μg/mL). Compound **1** is a new compound having the rearranged 11(15→1)-abeotaxane skeleton. The broad and poorly resolved NMR spectra might be explained by the existence of slow interconversion of two conformations (twist-chair and twist-boat) of **1**. The first occurrence of the 5/7/6-membered structure in *T. mairei* is of significance from a biogenetic point of view.

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 V-3210 spectrophotometers, respectively. EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer. The ^{1}H and ^{13}C NMR and DEPT spectra were recorded on a Varian FT-300 instrument and HMQC and HMBC spectra on a FT-400 AMX spectrometer.

Plant Material. The roots of *T. mairei* (Lemee & Levl.) S. Y. Hu were purchased in Kaohsiung, Taiwan, in October 1993. A voucher specimen has been deposited at the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation. Dried roots (60 kg) were extracted as previously described.[12] A portion (1.5 kg) was obtained from the $CHCl_3$/MeOH-soluble fraction. Part of the residue (200 g) was applied to a Sephadex LH-20 column (1 kg) and eluted with MeOH to afford a taxoid-containing fraction (85 g). The taxoid fraction was chromatographed on a silica gel column (850 g) and eluted with a solvent mixture of $CHCl_3$−Me_2CO by increasing the polarity to provide seven fractions. Fraction F (9 g) was applied to a LH-20 column and eluted with MeOH to give a residue (2.83 g). Rechromatography of the residue on a silica gel column and elution with *n*-Hex/$CHCl_3$/MeOH according to the following ratios and volumes (7:7:1, 6:6:1, 5:5:1, 4:4:1, 3:3:1, 2:2:1, and 1:1:1 each 1 L) yielded four fractions, fractions 1 (0.42 g), 2 (1.07 g), 3 (0.16 g), and 4 (0.61 g). Separation of fraction 2 (1.07 g) on a reversed phase C18 column (35 g) using solvent mixtures of MeOH/H_2O of decreasing polarity (7:3, 7:4, 7:5, 7:6, and 1:1, each 300 mL) afforded five fractions; fractions A (taxumairol B, 18 mg), B (taxumairol C, 7 mg), C (taxumairol E, 250 mg), D (baccatin III, 30 mg), and E (65 mg). Purification of fraction E by HPLC (silica gel) using *n*-hexane−$CHCl_3$−MeOH (5:5:1) as solvent system yielded taxumairol K (**1**, 35 mg).

Taxumairol K (1): amorphous solid; $[\alpha]^{25}_D$ −8.5° (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.22) nm; IR (neat) ν_{max} 3475, 3444, 3421, 2981, 1722, 1369, 1238

cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data are listed in Table 1; EIMS (70 eV) *m/z* 465 (0.1), 447 (2), 405 (1), 345 (1), 297 (11), 183 (5), 123 (9), 105 (100).

Acetylation of taxumairol K (1). Acetylation (Ac_2O−Pyr 1:1; room temperature) of **1** (5 mg) gave a solid (6 mg) after workup: $[\alpha]^{25}_D$ −33° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.17) nm; IR (neat) ν_{max} 3451, 2952, 1735, 1436, 1234 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 7.6 Hz, H-2), 2.98 (1H, d, J = 6.7 Hz, H-3), 4.97 (1H, d, J = 7.6 Hz, H-5), 1.80 (1H, m, H-6a), 2.66 (1H, m, H-6b), 5.54 (1H, t, J = 7.1 Hz, H-7), 6.32 (1H, d, J = 10.8 Hz, H-9), 6.43 (1H, d, J = 10.8 Hz, H-10), 5.62 (1H, t, J = 6.9 Hz, H-13), 1.70 (1H, m, H-14a), 2.28 (1H, m, H-14b), 1.19 (3H, s, H-16), 1.16 (3H, s, H-17), 1.87 (3H, s, H-18),1.76 (3H, s, H-19), 4.50 (1H, d, J = 7.3 Hz, H-20a), 4.41 (1H, d, J = 7.3 Hz, H-20b), 2.21, 2.01, 1.82, 1.64 (3H × 5, s, $OCOCH_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 67.6 (s, C-1), 68.2 (d, C-2), 44.0 (d, C-3), 79.0 (s, C-4), 85.0 (d, C-5), 34.7 (t, C-6), 70.5 (d, C-7), 43.7 (s, C-8), 78.7 (d, C-9), 67.9 (d, C-10), 136.0 (s, C-11), 147.1 (s, C-12), 78.7 (d, C-13), 46.9 (t, C-14), 75.5 (s, C-15), 25.0 (q, C-16), 27.5 (q, C-17), 11.7 (q, C-18), 13.3 (q, C-19), 74.7 (t, C-20), 168.0, 169.0, 170.0, 170.2, 170.6 (s, $OCOCH_3$), 129.6 (s, *i*-C_6H_5), 129.9 (d, *o*-C_6H_5), 128.5 (d, *m*-C_6H_5), 133.4 (d, *p*-C_6H_5), 167.5 (s, $OCOC_6H_5$), 22.7, 21.9, 21.6, 21.0, 20.6 (q, $OCOCH_3$); EIMS (70 eV) *m/z* 595 (0.1), 537 (2), 476 (1), 432 (1), 372 (3), 354 (1), 339 (6), 330 (1), 312 (2), 297 (3), 252 (7), 180 (2), 105 (100), 77 (23), 59 (41), 43 (73); FABMS *m/z* 737 $[M + Na]^+$, 715 $[M + H]^+$, 655 $[M - OAc]^+$; HRFABMS *m/z* $[M + H]^+$ 715.2956 ($C_{37}H_{47}O_{14}$ requires 715.2966).

Cytotoxicity Assay. A bioassay against HeLa (cervical carcinoma) and Hep-3B (hepatoma) tumor cells was based on reported procedures.[24−26] The cells for assay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37 °C. The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. In summary, samples and control standard drugs were prepared at a concentration of 1, 10, 40, and 100 μg/mL. After seeding 2880 cells/well in a 96-well microplate for 3 h, 20 mL of sample or standard agent was placed in each well and incubated at 37 °C for 3 days. After the medium was removed from the microplates, the cells were fixed with 10% formal saline for 30 min and then dyed with 1% (w/v) methylene blue in 0.01 M borate−buffer (100 μL/well) for 30 min. The 96-well plate was dipped into a 0.01 M borate−buffer solution four times in order to remove the dye. Then, 100 μL/well ethanol−0.1M HCl (1/1) was added as a dye-eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The IC_{50} value was defined by comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance. Mytomycin C and actinomycin D were used as standard compounds, which both exhibited IC_{50} values of 0.01 μg/mL under the above conditions.

Acknowledgment. We thank Ms. Chao-lein Ho and Ms. Shiu-ching Yu of the NSC Southern NMR and MS Instrument Center at National Sun Yat-sen University and Ms. Ru-ron Wu, Department of Chemistry, National Cheng Kung University, for measurements of NMR and MS spectral data. The authors thank the National Science Council and the National Institute of Health, Republic of China, for financial support (Grant Nos. NSC86-2732-B110-002 and DOH 87-HR-509).

References and Notes

(1) Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: New York, 1993; Vol. 62, pp 1−188.
(2) Appendino, G. *Nat. Prod. Rep.* **1995**, *12*, 349−360.
(3) Fuji, K.; Tanaka, K.; Li, B.; Shingu, T.; Yokoi, T.; Sun, H.; Taga, T. *Tetrahedron* **1995**, *51*, 10175−10188.
(4) Tong, X. J.; Fang, W. S.; Zhou, J. Y.; He, C. H.; Chen, W. M.; Fang, Q. C. *J. Nat. Prod.* **1995**, *58*, 233−238.
(5) Yang, S. J.; Fang, J. M.; Cheng, Y. S. *Phytochemistry* **1996**, *43*, 839−842.
(6) Morita, H.; Gonda, A.; Wei, L.; Yamamura, Y.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1997**, *60*, 390−392.
(7) Hall, A. M.; Tong, X. J.; Chang, C, J. *Nat. Prod. Lett.* **1997**, *60*, 390−392.
(8) Shrestha, T. B.; Khatri, C.; Banskota, A. H.; Manandhar, M. D.; Taylor, W. C. *J. Nat. Prod.* **1997**, *60*, 820−821.
(9) Kan, W. S. *Pharmaceutical Botany*; National Research Institute of Chinese Medicine: Taipei, 1975; p 140.
(10) Shen, Y. C.; Tai, H. R.; Chen C. Y. *J. Nat. Prod.* **1996**, *59*, 173−176.
(11) Shen, Y. C.; Tai, H. R.; Hsieh, P. W.; Chen, C. Y. *Chin. Pharm. J.* **1996**, *48*, 207−217.
(12) Shen, Y. C.; Chen C. Y. *Phytochemistry* **1997**, *44*, 1527−1533.
(13) Shen, Y. C.; Chen C. Y. *Planta Med.* **1997**, *63*, 569−570.
(14) Yue, Q.; Fang, Q. C.; Liang, X. T.; He, C. H. *Phytochemistry* **1995**, *39*, 871−873.
(15) Appendino, G.; Barboni, L.; Gariboldi, P.; Bombardelli, E.; Gabetta, B.; Viterbo, D. *J. Chem. Soc., Chem. Commun.* **1993**, 1587−1589.
(16) Zhong, S. Z.; Hua, Z. X.; Fan, J. S. *J. Nat. Prod.* **1996**, *59*, 603−605.
(17) Zhang, S.; Lee, C. T. L.; Kashiwada, Y.; Chen, K.; Zhang, D. C.; Lee, K. H. *J. Nat. Prod.* **1994**, *57*, 1580−1583.
(18) Zhang, H.; Tadeda, Y.; Sun, H. *Phytochemistry* **1995**, *39*, 1147−1151.
(19) Chen, R.; Kingston, D. G. I. *J. Nat. Prod.* **1994**, *57*, 1017−1021.
(20) Rao, K. V.; Bhakuni, R. S.; Hanuman, J. B.; Davies, R.; Johnson, J. *Phytochemistry* **1996**, *41*, 863−866.
(21) Barboni, L.; Gariboldi, P.; Torregiani, E.; Appendino, G.; Gabetta, B.; Zini, G.; Bombardelli, E. *Phytochemistry* **1993**, *33*, 145−150.
(22) Chattopadhyay, S. K.; Sharma, R. P.; Appendino, G.; Gariboldi, P. *Phytochemistry* **1995**, *39*, 869−870.
(23) Appendino, G.; Gravotto, G.; Enriu, R.; Jakupovic, J.; Gariboldi, P. *Phytochemistry* **1994**, *36*, 407−411.
(24) Chen, C. F.; Hwang, J. M. H.; Wu, C. H.; Chen, C. S.; Chen, K. Y. *Chin. Med. J. (Taipei)* **1990**, *46*, 7−16.
(25) Elliott, W. M.; Auersperg, N. *Biotech. Histochem.* **1993**, *68*, 29−35.
(26) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem.* **1984**, *139*, 272−277.

NP970573Y