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TAXANES FROM *TAXUS MAIREI*

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Key Word Index—*Taxus maire*; Taxaceae; taxane; twigs; diterpenoids.

Abstract—Four new taxane diterpenes, 9α-hydroxy-14β-(2-methylbutyryl)oxy-2α,5α,10β-triacetoxytaxa-4(20), 11-diene, 2α,5α,9α,10β,14β-pentaacetoxytaxa-4(20),11-diene, 5α-(cinnamoyl)oxy-7β-hydroxy-9α,10β-13α-triacetoxytaxa-4(20),11-diene and 5α-hydroxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene, along with 12 known taxa-4(20),11-dienes, have been isolated from twigs of *Taxus mairei* and their structures determined by spectral methods. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The chemical constituents of the plants of the *Taxus* genus have been extensively investigated [1–3], partly due to the finding of an antitumour agent, taxol [4]. *Taxus mairei* is the only species belonging to the genus *Taxus* found in Taiwan. A few taxane derivatives have been isolated from the heartwood of this plant [5, 6]. We report herein 16 taxane diterpenes having C4(20)-exocyclic double bonds isolated from the twigs of *T. mairei.*

RESULTS AND DISCUSSION

A concentrated acetone extract of the twigs of *T. mairei* was diluted with water and extracted with ethyl acetate. The ethyl acetate soluble material was chromatographed to give 16 taxanes (**1**–**16**), 12 of which were known: 2α,5α,10β,14β-tetraacetoxytaxa-4(20), 11-diene (**1**) [7], 14β-(isobutyryl)oxy-2α,5α,10β-triacetoxytaxa-4(20),11-diene (**2**) [7], 14β-(2-methylbutyryl)oxo-2α,5α,10β-triacetoxytaxa-4(20),11-diene (**3**) [7], yunnanxane (**4**) [8], taxuyunnanine B (**7**) [9], 5α-(cinnamoyl)oxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene (**8**) [10, 11], 2α-deacetoxytaxinine J (**10**) [12], taxinine J (**11**) [6], taxacin (**12**) [13], taxusin (**13**) [14, 15], 5α,7β,9α,10β,13α-pentaacetoxytaxa-4(20),11-diene (**15**) [16] and 5α-hydroxy-2α,7β,9α,10β,13α-pentaacetoxytaxa-4(20),11-diene (**16**) [17]. Compounds **1**–**3** have been found in cell cultures of *T. chinensis* var. *mairei* [7].

Compound **5** gave rise to a molecular ion $[M]^+$ at *m/z* 562.312 consistent with a molecular formula $C_{31}H_{46}O_9$. The ^1H NMR spectrum (Table 1) showed resonances of four methyl groups at δ 0.87 (*s*), 1.12 (*s*), 1.58 (*s*) and 2.12 (*s*), which are characteristic of taxane diterpenes. In addition to the resonances for three acetyl groups at δ 169.8 (*s*), 169.9 (*s*), 170.5 (*s*), 21.3 (*q*), 21.3 (*q*) and 21.9 (*q*), the ^{13}C NMR spectrum (Table 2) displayed a signal at δ 175.6 (*s*) attributable to an ester group. The α-methylbutyrate moiety was indicated by the proton resonances at δ 0.85 (*t*, $J = 7.2$ Hz, CH_3), 1.11 (*d*, $J = 7.2$ Hz, CH_3), 1.46 (*m*, CH_2) and 2.34 (*m*, CH). The proton resonances at δ 4.84 (*br s*) and 5.28 (*br s*) as well as the carbon signals at δ 141.8 (*s*) and 117.5 (*t*) suggested the presence of a methylene group. The structure of **5** was assigned as 9α-hydroxy-14β-(2-methylbutyryl)oxy-2α,5α,10β-triacetoxytaxa-4(20), 11-diene. The methylbutyrate group was located at C-14 by ^1H–^1H COSY, HMBC, and HMQC. The corresponding H-14 signal occurred at δ 4.93 (*dd*, $J =$ 4.4 and 8.8 Hz). The absolute configuration of the side chain was tentatively assigned to have the (*S*)-configuration based on the literature [7, 8]. The 2β-, 5β- and 10α-protons geminal to the acetoxy groups had chemical shifts [δ 5.23 (*dd*, $J = 2.6$ and 6.4 Hz), 5.29 (1H, *br s*) and 5.80 (*d*, $J = 10$ Hz)] close to those values in taxuyunnanine B. Compound **5** had a hydroxyl group at C-9 as its H-9β signal occurred at δ 4.19 (*d*, $J = 10$ Hz) whereas the H-9β signal in taxuyunnanine B occurred at δ 5.77. The ^1H NMR spectrum also exhibited a characteristic signal of H-3α at δ 2.92 (1H, *d*, $J =$ 6 Hz).

Compound **6** was assigned the molecular formula $C_{30}H_{42}O_{10}$ ($[M]^+ = m/z$ 562.276). The ^1H and 13C NMR spectra (Tables 1 and 2) were similar to those of compound **7** except that the signals of the methylbutyrate group in **7** were replaced with the signals of an acetyl group in **6**. The structure of **6** was assigned as 2α,5α,9α,10β,14β-pentaacetoxytaxa-4(20), 11-diene. From the ^1H–^1H COSY spectrum the reso-

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Table 1. ^{1}H NMR spectral data for taxanes **5**, **6**, **9** and **14** ($CDCl_3$)

H	**5**	**6**	**9**†	**14**
1	1.82 (*d*, 2.4)*	190 (*d*, 2.4)		1.76 (*m*)
2	5.32 (*dd*, 2.4, 6.4)	5.38 (*dd*, 2.4, 6.3)		
3	2.92 (*d*, 6.4)	2.93 (*d*, 6.3)	2.93 (*d*, 3.9)	3.26 (*d*, 4.5)
5	5.29 (br, s)	5.24 (*br*, *s*)	5.55 (*br s*)	4.26 (*br s*)
7			4.32 (*dd*, 4.8, 11.1)	
9	4.19 (*d*, 10.0)	5.76 (*d*, 10.2)	6.05 (*d*, 11.1)	5.73 (*d*, 10.2)
10	5.80 (*d*, 10.0)	6.03 (*d*, 10.2)	6.25 *d*, 11.1)	6.07 (*d*, 10.2)
13	2.87 (*dd*, 9.2, 19.2) 2.32 (*m*)	2.83 (*dd*, 9.0, 19.2) 2.40 (*m*)	5.77 (*br t*, 7.8)	5.67 (*dd*, 3.3, 7.8)
14	4.93 (*dd*, 4.4, 8.8)	4.93 *dd*, 4.8, 9.0)		1.12 (*dd*, 4.5, 15.3) 2.78 (*m*)
16	1.58 (*s*)	1.68 (*s*)	1.73 (*s*)	1.55 (*s*)
17	1.12 (*s*)	1.09 (*s*)	1.01 (*s*)	0.97 (*s*)
18	2.12 (*s*)	2.15 (*s*)	2.22 (*s*)	2.08 (*s*)
19	0.87 (*s*)	0.82 (*s*)	0.81 (*s*)	0.68 (*s*)
20	4.84 (*br s*) 5.28 (*br s*)	4.84 (*br s*) 5.30 (*br s*)	4.97 (*br s*) 5.35 (*br s*)	4.71 (*br s*) 5.09 (*br s*)
2′	2.34 (*m*)			
3′	1.46 (*m*)			
4′	0.85 (*t*, 7.2)			
5′	1.11 (*d*, 7.2)			

*The values in parentheses are coupling constants (Hz).

†Cinnamoyl data: δ 7.74 (*d*, 16.0), 6.52 (*d*, 16.0) and 7.37–7.48 (5H).

Table 2. ^{13}C NMR spectral data for taxanes **5**, **6**, **9** and **14** ($CDCl_3$, δ in ppm)

C	**5**	**6**	**9***	**14**
1	59.0	58.4	40.0	39.5
2	70.0	70.2	69.9	32.3
3	44.1	44.2	37.3	36.1
4	141.8	141.3	146.2	153.3
5	78.6	78.3	78.2	77.4
6	28.5	28.3	34.5	26.4
7	25.8	27.2	70.5	29.0
8	44.8	44.8	46.2	43.4
9	76.2	76.7	76.5	74.3
10	76.0	72.4	71.6	72.8
11	133.2	132.7	134.9	136.1
12	137.0	137.2	137.1	137.2
13	39.7	39.4	70.0	70.2
14	69.9	69.8	31.8	26.0
15	36.8	36.9	39.3	38.7
16	26.2	25.8	27.1	27.4
17	31.5	31.6	31.0	32.3
18	21.0	21.0	13.1	15.8
19	22.4	17.3	15.2	17.1
20	117.5	118.3	115.9	111.1
1′	175.6			
2′	41.0			
3′	26.7			
4′	11.5			
5′	13.9			

*Cinnamoyl data: δ 166.0, 118.3, 145.6, 134.0, 128.0, 128.9 and 130.5.

nance at δ 4.93 (*dd*, $J = 4.8$ and 9.0 Hz) was assigned to H-14α. No coupling was observed between H-1β and H-14α, because these two protons were nearly orthogonal.

Compound **9** ($C_{35}H_{44}O_9$) gave rise to a molecular ion $[M]^+$ at *m/z* 608.293 (calc. 608.297). The IR spectrum showed absorptions at 1730, 1715 and 1630 cm^{-1} attributable to normal and conjugated ester groups. In the ^{1}H NMR spectrum, three acetoxy groups appeared at δ 1.82 (*s*), 2.00 (*s*) and 2.06 (*s*). The resonances at δ 7.74, 6.52 (AB quartet, $J = 16$ Hz) and 7.37–7.48 (5H) indicated the presence of a cinnamoyl group. By comparison of the ^{1}H and ^{13}C NMR spectra of **9** with those of **8** and 2α-deacetoxytaxinine J, the structure of **9** was assigned as 5α-(cinnamoyl)oxy-7β-hydroxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene. The chemical shifts of the 5β-, 9β- and 10α-protons [δ 5.55 (*br s*), 6.05 (*d*, $J = 11.1$ Hz) and 6.25 (*d*, $J = 11.1$ Hz)] were close to the values in **8** and **10**. Because the dihedral angle between H-10α and H-9β was nearly 180°, these protons exhibited a large coupling constant (11.1 Hz). The H-7α in **9** was geminal to a hydroxyl group and resonated at δ 4.32 (*dd*, $J = 4.8$ and 11.1 Hz), whereas the H-7α in **10** geminal to an acetoxy group appeared at a lower field (δ 5.70).

Compound **14** ($C_{26}H_{38}O_7$), $[M]^+$ at *m/z* 462.260, gave ^{1}H an d^{13}C NMR spectra similar to those of **13** ($C_{24}H_{36}O_8$). Compound **14** had three acetoxy groups as shown by the signals at δ_H 2.02 (*s*), 2.05 (*s*) and 2.07

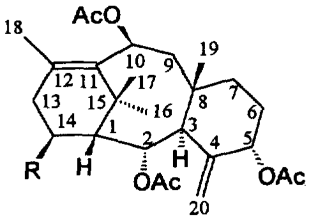
(*s*), whereas compound **13** had four acetoxy groups. The structure of **14** was assigned as 5α-hydroxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene. The proton resonances at δ 5.09 (1H, *br s*) and 4.71 (1H, *br s*) were attributable to the methylene group at C-4. The 9β- and 10α-protons appeared as an AB system at δ 5.73 and 6.07 with a large coupling constant (10.2 Hz). From the ^{1}H–^{1}H COSY spectrum, H-5β and δ 4.26 geminal to a hydroxyl group was correlated with H-6α (at δ 1.75), h-6β (at δ 1.65) and H-20 (at δ 5.09). The signal at δ 5.67 (H-13β) showed correlations with H-14α (δ 1.12), H-14β (δ 2.78) and H-18 (δ 2.08).

In summary, the twigs of *T. mairei* contain various taxane diterpenes. Isolation of taxanes **1–7** is significant since taxanes having substituents at C-14 are rare.

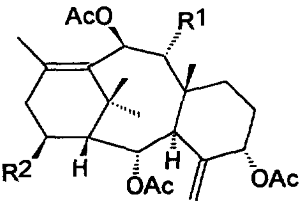
EXPERIMENTAL

General. Mp: uncorr; ^{1}H NMR: 300 MHz; ^{13}C NMR 75 MHz; HPLC: Hibar Lichrosorb Si 60 column (10 μm or 7 μm, 25 cm × 1 cm i.d.).

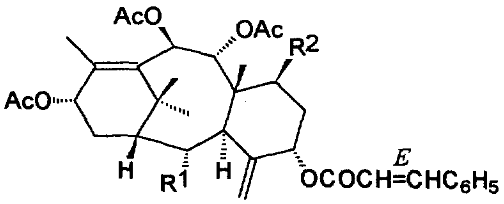
Plant material. Twigs (1.2 kg) of *T. mairei*, collected in the remote mountains at an elevation of *ca* 2100 m (Tong-Shi, Taichung county), were exhaustively extracted with Me$_2$CO (7 l × 3). The Me$_2$CO extract was concd to give 100 g of residue, which was diluted with H$_2$O and extracted (×3) with EtOAc. The combined EtOAc extracts were concd to give an oil (75 g), which was absorbed with 110 g silica gel and then subjected to CC on a column packed with 650 g silica gel. Elution was with gradients of hexane, EtOAc and Me$_2$CO. The portion obtained from elution with EtOAc–hexane (30–



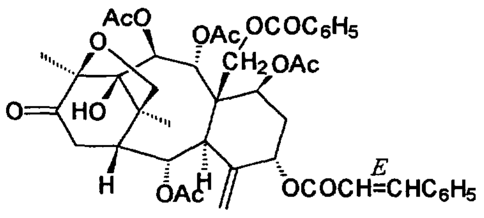
	R
1	OAc
2	$O\overset{1'}{C}O\overset{2'}{C}HMe_2$
3	$O\overset{1'}{C}O\overset{2'}{C}H\overset{5'}{Me}\overset{3'}{C}H_2\overset{4'}{Me}$
4	OCOCHMeCH(OH)Me



	R^1	R^2
5	OH	OCOCHMeCH$_2$Me
6	OAc	OAc
7	OAc	OCOCHMeCH$_2$Me



	R^1	R^2
9	H	H
10	H	OH
11	H	OAc
12	OAc	OAc



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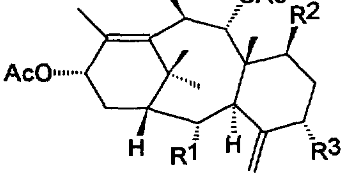

	R^1	R^2	R^3
13	H	H	OAc
14	H	H	OH
15	H	OAc	OAc
16	OAc	OAc	OH

60%) was further subjected to flash chromatography and HPLC with elution with EtOAc–CH_2Cl_2 (20–40%) or Me_2CO–hexane (30–50%) to give **2** (12 mg), **3** (80 mg), **1** (75 mg), **7** (28 mg), **13** (21 mg), **6** (55 mg), **10** (90 mg), **11** (10 mg), **15** (79 mg), **4** (22 mg), **5** (15 mg), **14** (11 mg), **8** (15 mg), **9** (30 mg), **12** (13 mg) and **16** (12 mg) in ascending order of polarity.

9α-*Hydroxy*-14β-(2-*methylbutyryl*)*oxy*-2α,5α,10β-*triacetoxytaxa*-4(20),11-*diene* (**5**). Amorphous solid, $[\alpha]_D^{23}$ +65.8° ($CHCl_3$; *c* 0.55). FABMS (NBA) *m/z* (rel. int.): 585 $[M+Na]^+$ (15), 503 (4), 461 (10), 443 (6), 401 (6), 341 (16), 281 (30), 57 (100). IR ν_{max}^{KBr} cm^{-1}: 3456, 1725, 1620.

2α,5α,9α,10β,14β - *Pentaacetoxytaxa* - 4(20),11 - *diene* (**6**). Needles, mp 132–133°, $[\alpha]_D^{25}$ +26° ($CHCL_3$; *c* 2.4). FABMS (NBA) *m/z* (rel. int.): 562 $[M]^+$ (3), 503 (5), 443 (14), 383 (9), 323 (12), 263 (40), 135 (100). IR ν_{max}^{KBr} cm^{-1}. 2929, 1730, 1234, 1021.

5α - (*Cinnamoyl*)*oxy* - 7β - *hydroxy* - 9α,10β,13α - *triacetoxytaxa*-4(20),11-*diene* (**9**). Yellow gum. $[\alpha]_D^{25}$ +20° ($CHCl_3$; *c* 0.5). FABMS (NBA) *m/z* (rel. int.): 608 $[M]^+$ (20), 590 (13), 548 (100), 488 (30), 428 (16). IR ν_{max}^{neat} cm^{-1}: 3469, 1730, 1631, 1234, 1159, 1021.

5α - *Hydroxy* - 9α,10β,13α - *triacetoxytaxa* - 4(20),11 - *diene* (**14**). Needles, mp 204–206°, $[\alpha]_D^{26}$ +266° ($CHCl_3$; *c* 0.9). FABMS (NBA) *m/z* (rel. int.): 462 $[M]^+$ (6), 444 (20), 403 (70), 343 (63), 283 (100), 265 (58). IR ν_{max}^{KBr} cm^{-1}: 3485, 1734, 1235, 1019, 898.

REFERENCES

1. Appendino, G. (1995) *Nat. Prod. Rep.* 349.
2. Kingston, D. G. I., Molinero, A. A. and Rimoldi, J. M. (1993) *Progress in the Chemistry of Organic Natural Products*, Vol. 61 (Herz, W., Kirby, G. W., Moore, R. E., Steglich, W. and Tamm, Ch., eds), p. 1. Springer, New York.
3. Chen, W.-M. (1990) *Acta Pharm. Sin.* **25**, 227.
4. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. and McPhail, A. T. (1971) *J. Am. Chem. Soc.* **93**, 2325.
5. Yeh, M.-K., Wang, J.-S. and Chen, F.-C. (1988) *J. Chin. Chem. Soc.* **35**, 309.
6. Min, Z.-D., Jiang, H. and Liang, J. Y. (1989) *Acta Pharm. Sin.* **24**, 673.
7. Ma, W., Stahlhut, R. W., Adams, T. L., Park, G. L., Evans, W. A. Blumenthal, S. G., Gomez, G. A., Nieder, M. H. and Hylands, P. J. (1994) *J. Nat. Prod.* **57**, 1320.
8. Chen, W.-M., Zhang, P.-L., Wu, B. and Zheng, Q.-T. (1991) *Acta Pharm. Sin.* **26**, 747.
9. Zhang, H., Takeda, Y., Minami, Y., Yoshida, K., Matsumoto, T., Xiang, W., Mu, O. and Sun, H. (1994) *Chem. Letters* 957.
10. Yeh, M.-K., Wang, J.-S. and Chen, F.-C. (1988) *Phytochemistry* **27**, 1534.
11. Zhang, Z. and Jia, Z. (1991) *Phytochemistry* **30**, 2345.
12. Liang, J.-Y., Min, Z.-D. and Niwa, M. (1988) *Acta. Chem. Sin.* **46**, 1053.
13. Yoshizaki, F., Fukuda, M., Ishida, T. and In, Y. (1988) *Chem. Pharm. Bull.* **36**, 2098.
14. Erdtmam, H. and Tsuno, K. (1969) *Phytochemistry* **8**, 931.
15. Ho, T.-I., Lee, G.-H., Peng, S.-M., Yeh, M.-K., Chen, F.-C. and Yang, W.-L. (1987) *Acta Cryst.* **C43**, 1378.
16. Della Casa De Marcano, D. P. and Halsall, T. G. (1969) *J. Chem. Soc., Chem. Commun.* 1282.
17. Kingston, D. G. I., Hawkins, D. R. and Ovington, L. (1982) *J. Nat. Prod.* **45**, 466.