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Two bicyclic taxane diterpenoids from the needles of *taxus mairei*

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Abstract

Two new bicyclic taxane diterpenoids were isolated from the needles of the Chinese yew, *Taxus mairei*. Their structures were established as 13-deacetylcanadensene and 7-deacetylcanadensene with the aid of spectroscopic techniques, including 1D and 2D NMR spectroscopies. © 1998 Elsevier Science Ltd. All rights reserved.

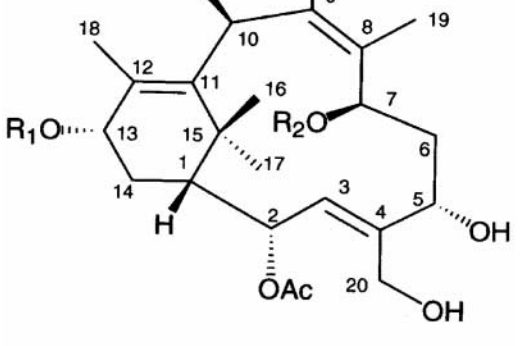
Keywords: Taxus mairei; Taxaceae; Taxoid diterpenes; Bicyclic taxanes

1. Introduction

Taxus mairei, indigenous to China, is an evergreen tall tree and mainly distributed in Jiangxi province and Fujian province, south east of the People's Republic of China. Previous studies have resulted in the isolation of more than 20 new taxanes from this plant, most of which have the normal taxane skeletons with 6/8/6 membered ring system (Liang, Min, Mizuno, Tanaka & Iinuma, 1988a; Mizuno, Tanaka & Iinuma, 1988; Min, Jiang & Liang, 1989; Liang, Min & Niwa, 1988b; Yek, Wang, Liu & Chen, 1988; Liang & Kingston, 1993; Shen, Tai & Chen, 1996; Shen, Tai, Hsieh & Chen, 1996; Shen & Chen, 1997; Liang, Min, Mizuno, Tanaka & Iinuma, 1987; Yang, Fang & Cheng, 1996; Chuang, Chen, Lin & Chen, 1990; Yeh, Waang, Liu & Chen, 1988). In search of new precursors fit for semi-synthesis of taxol analogous, we re-examined the needles of this plant growing in a different area from those examined by other groups. In addition to 10-deacetylbaccatin III (Della, Marcano & Halsall, 1975), 1-dehydroxybaccatin IV (Della et al., 1975), 1-deoxybaccatin VI (Min et al., 1989), taxinine J (Miller, 1980), 2-deacetoxytaxinine J (Liang et al., 1988b), 2-deacetyl-5-decinnamoyltaxinine J (Chen, Zhang & Zhou, 1994), canadensene (Zamir, Zhou, Carron, Nedea, Sauriol et al., 1995), taxachitrienes A and B (Jackson & Pattenden, 1985), two new bicyclic taxanes **1** and **2** were isolated. Bicyclic taxanes have never been reported from this plant. In this paper, we describe the isolation and the structure elucidation of these new compounds.

2. Results and discussion

Compound **1** was isolated as a white amorphous solid in a 0.00035% yield on the dry material. FAB-MS gave ion peaks at *m/z* 575 ($[M + Na]^+$), 553 ($[M + H]^+$), which revealed that its molecular weight was 552. By the combined analysis of FAB-MS, ^1H NMR and ^{13}C NMR data, its molecular formula was



1 : $R_1 = H$, $R_2 = Ac$

2 : $R_1 = Ac$, $R_2 = H$

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proposed as $C_{28}H_{40}O_{11}$. HR-FAB-MS also gave the same formula. The unsaturation number of **1** was calculated as 9. The ^{1}H NMR spectrum of **1** showed the four tertiary methyl groups (δ 1.62, 1.21, 1.10 and 2.13, each 3H), assignable to the 8-CH_3, 15-$(CH_3)_2$, and 12-CH_3 groups, respectively. Four acetyl groups (δ 1.95, 2.04, 2.21 and 2.08, each 3H, *s*) at relatively low field, five oxy-bearing methine groups (δ 5.72, *dd*; 4.63, *br s*; 5.09, *d*; 6.98, *d* and 4.16, *br d*), one oxy-bearing methylene group (δ 4.56, 1H, *d*, $J = 12.64$ Hz; 3.48, 1H, *d*, $J = 12.64$ Hz), one trisubstituted olefin (δ 6.16, 1H, *dd*, $J = 11.26$, 1.37 Hz) were also involved. Two tetrasubstituted olefins (δ 123.30, 144.18, 133.96 and 138.34) were deduced from the ^{13}C NMR spectrum. Since seven out of nine unsaturations were accounted for, only two unsaturation equivalents were left. The carbon skeleton of **1** was thus deduced to contain two rings only. Its ^{1}H NMR and ^{13}C NMR spectra closely resembled to those of canadensene (Zamir et al., 1995) with the exception of one signal upfield to δ 4.16 in ^{1}H NMR spectrum and an acetyl group disappeared both in ^{1}H NMR and ^{13}C NMR spectra. With the help of ^{1}H-^{1}H COSY and HMBC spectra (Fig. 1), the structure of **1** was proposed as 13-deacetyl canadensene. The relative stereochemistry of **1** was deduced from the nuclear Overhauser effect spectroscopy (NOESY) spectrum as shown in Fig. 2. The regiochemistry of the 12-membered ring part was determined to be 3*E* and 8*E* from observation of NOEs between H-2 and H-20b, H-7 and H-10, respectively. The NOESY correlation between H-13 and 16-CH_3 revealed that the ring A had a boat conformation and H-13 was β-oriented, while the correlations between H-2 and 17-CH_3, H-5 and H-20b indicated the β-orientation of H-2 and H-5. NOESY cross-peaks between H-7 and 18-CH_3, H-10 and 18-CH_3 revealed that the relative configurations of H-7 and H-10 were α-oriented.

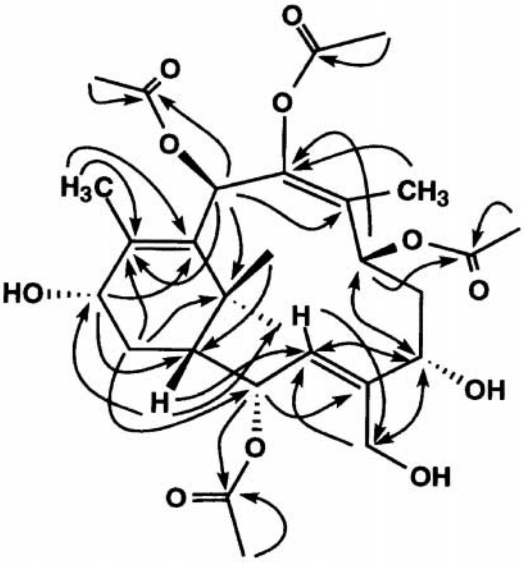

Fig. 1. Selected C/H correlations obtained by HMBC spectrum of **1** (most protons are not shown for clarity).

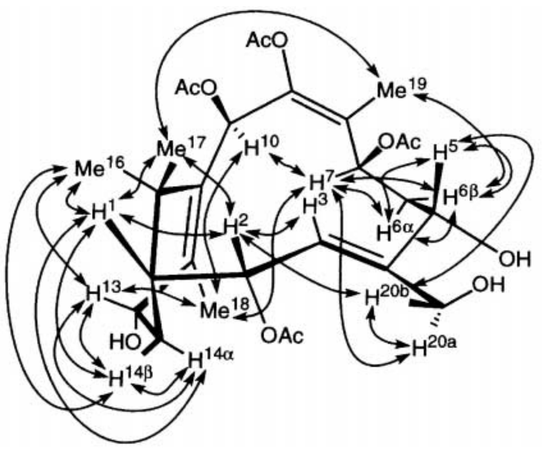

Fig. 2. Relative stereochemistry of **1**, the arrows denote NOESY correlations.

Compound **2** was obtained as a white amorphous solid, FAB-MS yielding ion peaks at *m/z* 575 ($[M + Na]^+$) and 591 ($[M + K]^+$), which revealed that its molecular weight was 552. By the combined analyses of the FAB-MS and ^{13}C NMR data, its molecular formula was proposed as $C_{28}H_{40}O_{11}$, which was the same formula as **1**. The ^{1}H NMR and ^{13}C NMR spectra of **2** also closely resembled those of canadensene (Zamir et al., 1995) with the exception of one signal upfield at δ 4.30 in the ^{1}H NMR spectrum and an acetyl group which disappeared both in the ^{1}H NMR and ^{13}C NMR spectra. Detailed analyses of the 1D and 2D NMR spectra led us to propose the structure of **2** as 7-deacetyl canadensene.

Bicyclic taxanes, which were proposed by Jackson and Pattenden (1985) and Begley, Jackson and Pattenden (1985) as the precursor of taxane biosynthesis, were discovered recently as new type taxanes with 6/12 membered rings. Among more than 200 taxanes discovered, only 6 are bicyclic taxanes (Zamir et al., 1995; Fang, Fang, Liang, Lu & Zheng, 1995; Shigemori, Wang, Yoshida & Kobayashi, 1997; Hosoyama, Inubushi, Katsui, Shigemori & Kobayashi, 1996; Fang, Fang & Liang, 1996). The occurrence of bicyclic taxane in yew tree is of great interest from the viewpoint of biosynthetic significance.

3. Experimental

3.1. General

Silica gel 60 (Merck 100–200 mesh) was used for conventional CC. Kieselgel 60 F_{254} silica gel for prep.

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of compounds **1** and **2** in $CDCl_3$

	1			**2**		
position	^1H	J(Hz)	^{13}C	^1H	J(Hz)	^{13}C
1	1.75 *br t*	4.95	47.10	1.75 *m*		46.69
2	5.72 *dd*	11.26,4.66	71.03	5.73 *dd*	11.3,4.3	68.39
3	6.16 *dd*	11.26,1.37	122.74	5.99 *br.d*	11.3	121.13
4			141.22			133.56
5	4.63 *br s*		68.23	4.63 *br.s*		70.70
6α	2.03 *m*		37.30	1.90–2.00 *m*		35.83
6β	2.71 *ddd*	15.93, 8.52, 3.02		2.56 *m*		
7	5.09 *d*	8.52	68.48	4.30 *d*	8.3	68.04
8			123.30			128.12
9			144.18			143.45
10	6.98 *d*	1.10	67.05	6.96 *br.s*		67.91
11			133.96			136.42
12			138.34			134.72
13	4.16 *br d*	8.79	68.75	5.31 *br.d*	9.3	69.32
14α	2.35 *br d*	16.48	27.54	1.90–2.00 *m*		27.82
14β	2.45 *ddd*	16.48, 1.65,8.79		2.52–2.59 *m*		
15			35.98			38.93
16	1.21 *s*		24.67	1.22 *s*		25.43
17	1.10 *s*		34.01	1.12 *s*		33.85
18	2.13 *s*		17.35	1.99 *s*		16.84
19	1.62 *d*	1.10	12.69	1.58 *d*	1.0	12.27
20a	4.56 *d*	12.64	57.73	4.51 *d*	12.5	57.56
20b	3.48 *d*	12.64		3.43 *d*	12.5	
2-AcO	2.04 *s*		20.49	2.02 *s*		20.85
			171.86			168.47
7-AcO	2.08 *s*		21.41			
			171.68			
9-AcO	2.21 *s*		21.81	2.22 *s*		21.76
			168.14			171.67
10-AcO	1.95 *s*		20.87	1.97 *s*		20.37
			168.06			164.86
13-OAc				2.13 *s*		21.26
						170.55

TLC. Mps were measured on a MRK micro-melting point apparatus and are uncorr. Optical rotations were recorded on a Horiba-SEPA-300 polarimeter. MS were obtained on a JMS-700 mass spectrometer, using the FAB method and *m*-nitrobenzyl alcohol as a matrix. NMR spectra were taken on Varian GEMINI 2000/300 (300 MHz for ^1H) and Varian Unity INOVA 500 (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometers in $CDCl_3$ at 20°. ^1H chemical shifts were recorded in ppm from internal TMS.

3.2. Plant material

The needles of *T. mairei* were collected from Jiangxi province, south-east of the People's Republic of China, in the summer of 1995. The material was authenticated by Mr. Liu of Zhangzhou Forestry School, and where a voucher specimen has been deposited.

3.3. Extraction and isolation

Air dried needles (7.1 kg) were extracted with MeOH 2× at room temp. The MeOH extracts were concd to residue under red. pres. This residue was diluted with H_2O and the aq. soln was extracted 3× with EtOAc. The combined EtOAc extract, upon evaporation, yielded 88 g of dark syrup. Part of the syrup (65 g) was subjected to a silica gel CC, eluted with hexane and a gradient of hexane–EtOAc (2:1, 1:1, 1:2, 1:4 and EtOAc), six fractions was obtained. Fr. 2 was repeatedly chromatographed on silica gel column and plates, eluted or developed with hexane-acetone and $CHCl_3$-MeOH, finally afforded compounds **1** (25 mg) and **2** (8 mg).

3.4. Compound (1)

White amorphous solid, mp 98–99°; $[\alpha]_D^{24}$ +4.52° (c 0.003, $CHCl_3$). IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3450, 3010, 2930, 2875, 1740, 1735, 1718, 1665, 1580, 1520, 1435, 1364, 1240, 1200, 1100, 1042 and 1020. FAB-MS: *m*/*z*: 575 ($[M + Na]^+$), 553 ($[M + H]^+$), 535 ($[M + H - H_2O]^+$), 493 ($[M + H - AcOH]^+$), 475 ($[M + H - H_2O - AcOH]^+$), 433 ($[M + H - 2AcOH]^+$), 415 ($[M + H - H_2O - 2AcOH]^+$), 373 ($[M - 3AcOH - COCH_3]^+$), 313 ($[M + H - 4AcOH]^+$), 295 ($[M + H - H_2O - 4AcOH]^+$), 185, 93, 43. HR-FAB-MS: 553.2617 ($[M + H]^+$ $C_{28}H_{41}O_{11}$ calcd for 553.2646). ^{1}H NMR (300 and 500 MHz) and ^{13}C NMR (125 MHz) data: see Table 1.

3.5. Compound (**2**)

White amorphous solid, mp 95–96°; $[\alpha]_D^{25}$ +5.33° (*c* 0.003, $CHCl_3$); IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3410, 3010, 2935, 2875, 1737, 1735, 1718, 1660, 1370, 1240, and 1020. FAB-MS: *m*/*z*: 591 ($[M + K]^+$), 575 ($[M + Na]^+$), 553 ($[M + H]^+$), 535 ($[M + H - H_2O]^+$), 493 ($[M + H - AcOH]^+$), 475 ($[M + H - H_2O - AcOH]^+$), 451 ($[M + H - AcOH - COCH_3]^+$), 433 ($[M + H - 2AcOH]^+$), 415 ($[M + H - H_2O - 2AcOH]^+$), 373 ($[M - 3AcOH - COCH_3]^+$), 313 ($[M + H - 4AcOH]^+$), 295 ($[M + H - H_2O - 4AcOH]^+$), 185, 93, 43. ^{1}H NMR (300 MHz) and ^{13}C NMR (125 MHz) data: see Table 1.

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