PERGAMON

Phytochemistry 52 (1999) 1571–1575

PHYTOCHEMISTRY

Two novel pseudoalkaloid taxanes from the Chinese yew, *Taxus chinensis* var. *mairei*

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Received 22 January 1999; received in revised form 14 May 1999

Abstract

Two pseudoalkaloid taxane diterpenoids were isolated from seeds of the Chinese yew tree *Taxus chinensis* var. *mairei*. Their structures were established as 2α,9α,10β,13α-tetraacetoxy-5α(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene and 7β,9α,10β,13α-tetraacetoxy-5α(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene, with the aid of NMR spectral and FAB-MS data analysis.

Keywords: *Taxus chinensis* var. *mairei*; Taxaceae; Seeds; Pseudoalkaloid; Taxane diterpenoid

1. Introduction

In the course of our studies on the yew tree, we examined the constituents of the leaves and bark of the Chinese yew, *Taxus chinensis* var. *mairei* (Shi, Oritani, Kiyota & Horguchi, 1998; Shi, Oritani, Sugiyama & Kiyota, 1998a, 1998b). This report now focuses on the constitution of its seeds and the isolation and characterization of two pseudoalkaloid taxane diterpenoids.

2. Results and discussion

The methanolic extract of the seeds of *T. chinensis* var. *mairei* was processed as described in the Experimental Section to produce two pseudoalkaloid taxane diterpenoids.

Thus, compound **1** was isolated as a colorless gummy substance in a yield of 0.0027% by weight of the dry material. FAB-MS produced a protonated molecular ion peak at m/z 682 ($[M+H]^+$). The molecular formula of compound **1**, $C_{38}H_{51}O_{10}N$, was deduced from combined analysis of HR-FAB-MS at m/z 682.3591 ($[M+H]^+$) (Δ−0.0 mmu) and its ^{13}C NMR spectrum (Fig. 1). Intense absorptions at 1740, 1720, and a weak absorption at 3350 cm^{-1} in the IR spectrum implied that **1** possesses ester and amino groups, respectively. The ^1H-NMR spectrum of **1** (Table 1) exhibited proton signals due to four methyl groups at δ 0.86, 1.11, 1.74 and 2.05 ppm, characteristic of the taxane skeleton. Four acetyl methyl groups gave signals at lower field (δ 2.01, 2.05, 2.01 and 2.05 ppm); the acetyl groups were verified by the observation of ^{13}C-NMR signals at δ 169.45, 170.03, 169.92 and 170.62 ppm, respectively. These signals suggest that **1** has a taxane-type skeleton. The connectivities of the protons in the taxane skeleton of **1** were determined by analysis of the ^1H-^1H COSY spectrum. Interpretation of ^1H-, ^{13}C-NMR and HMBC spectra permitted the positional assignment of functional groups. The ^1H-NMR signals at δ 5.32 (1H, br.s), 4.76 (1H, br.s) and 3.16 (1H, br.d, J = 7.13 Hz) are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxa-4(20),11-diene, respectively. Additionally, five oxygen-bearing one-proton signals

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00359-3

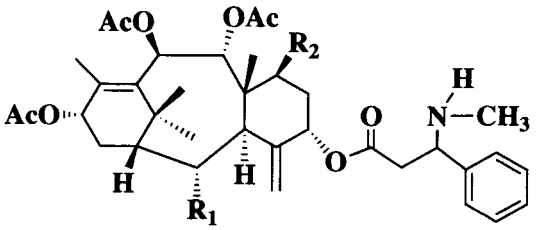

Fig. 1. Pseudoalkaloid taxanes isolated from seeds of *Taxus chinensis* var. *mairei*.

appeared at lower field, of which a pair of one-proton doublets at 5.99 and 5.90 ppm with a coupling constant of 10.71 Hz was assigned to H-10α and H-9β, respectively. The large vicinal coupling constant observed indicates a *trans*-oriented configuration of H-9 and H-10. The spin system derived from 18-CH_3, H-13β, H-14α, and H-14β, H-1β, H-2β, and H-3α was readily interpreted. The signal of three protons as a doublet at δ 2.05 ppm was assigned to 18-CH_3, based on long-range coupling with H-13β; the triplet at δ 5.82 ppm (1H, br.t, J = 8.24 Hz), was assigned to H-13β; the doublet of doublets of doublets at δ 2.50 ppm and the doublet of doublets at δ 1.44 ppm were assigned to the C-14 methylene protons, H-14β and H-14α, respectively, based on their geminal coupling and coupling to H-13β. The signal at 5.27 ppm (1H, br.s) was a characteristic signal of H-5β. All of the proton-bearing carbons were assigned by analysis of the HETCOR spectrum. Five oxygen-containing carbons (C-2, C-5, C-9, C-10, and C-13) were correlated with their corresponding proton signals in the HMQC spectrum. The presence of a nor-Winterstein acid [3-(*N*-methylamino)-3-phenylpropanoyl] moiety in **1** was

Table 1
^{1}H- and ^{13}C-NMR spectral data of **1** and **2**, (300 MHz for ^{1}H, 125 MHz for ^{13}C, $CDCl_3$)

position	**1**				**2**		
	^{13}C	^{1}H	J (Hz)	^{1}H-^{1}H COSY	^{1}1H	J (Hz)	^{1}H-^{1}H COSY
1	48.29	1.92 br.d	9.34	H-2, 14β	1.80 m		H-2, 14β
2	71.71	5.44 dd	2.47, 7.13	H-3	1.84 m		H-1, 3
3	44.07	3.16 d	7.13	H-2,20a	2.81 br.d	9.12	H-2,20a
4	141.81						
5	78.70	5.27 br.s		H-6, 20β	5.3 8 br.s	H-6,20b	
6	27.17	1.58 m		H-5, 7	1.71 m		H-5, 7
7	28.30	1.63 m		H-6	5.45 br.t	9.06	H-6
8	44.04						
9	76.54	5.90 d	10.71	H-10	5.88 d	11.00	H-10
10	72.23	5.99 d	10.71	H-9	6.24 d	11.00	H-9
11	137.12						
12	132.56						
13	70.01	5.82 t	8.24	H14α, 14β, 18	5.87 br.t	8.12	H-14α, 14β, 18
14α	28.00	1.44dd	14.90, 7.97	H-13, 14β	0.96 m	H-13, 14β	
14β		2.50 ddd	14.90, 9.34, 3.60	H-1, 13, 14α	2.55 m		H-1, 13, 14α
15	37.38						
16	31.26	1.11 s			1.10 s		
17	26.89	1.74 s			1.60		
18	15.29	2.05 br.s		H-13	2.13 br.s	H-13	
19	17.80	0.86 s			0.81		
20a	118.28	4.76 br.s		20b	4.94 br.s	H-5, 20b	
20b		5.32 br.s		20a	5.26 br.s	H-3,20a	
1′	171.32						
2′	43.35	2.81 dd	14.83, 5.77	H-2″,3′	2.74 dd	11.27, 6.12	H2″, 3′
2″		2.72 dd	14.83, 8.50	H-2′, 3′	2.65 dd	11.27, 8.79	H-2′, 3′
3′	62.19	3.95 dd	5.77, 8.50	H-2′, 2″	3.95 dd	6.12, 8.79	H2′, 2″
4′	142.11						
5′,9′	128.66	7.34 m			7.32 m		
6′,8′	127.03	7.34 m			7.32 m		
7′	127.76	7.34 m			7.32 m		
2-AcO	21.48, 169.45	2.01 s					
7-AcO					2.02 s		
9-AcO	21.02, 170.03	2.05 s			1.97 s		
10-AcO	20.81, 169.92	2.01 s			2.02 s		
13-AcO	21.02	2.05 s			2.09 s		
N–CH_3	34.48	2.29 s			2.26 s		

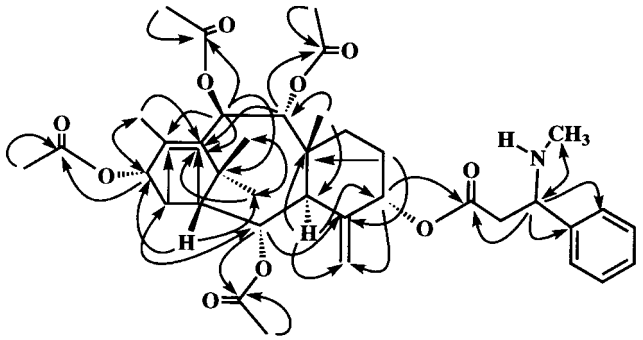

Fig. 2. H–C Long-range correlations observed from the HMBC spectrum of **1** (500 MHz), in which most of the protons are omitted for clarity.

suggested from the signals at δ 2.29 (3H, s), 2.81 (1H, dd, J = 14.83, 5.77 Hz), 2.72 (1H, dd, J = 14.83, 8.50 Hz), 3.95 (1H, dd, J = 5.77, 8.50 Hz), 7.34 (5H, m) ppm in the ^{1}H-NMR spectrum. Further support was provided by the fragment ions in the HR-FAB-MS at m/z 180.1027 and 120.0815 (base peaks) that analyzed for $C_{10}H_{14}O_2N$ (Δ + 0.3 mmu) and $C_8H_{12}N$ (Δ + 0.2 mmu), respectively. It has been reported that removal of one methyl from the dimethylamino group of the Winterstein acid moiety causes a marked upfield shift (ca. 8 ppm) of the remaining methyl in the ^{13}C-NMR spectrum (Appendino et al., 1993a). The signal of N–CH_3 of **1** appears at δ 34.48 ppm, which is in good agreement with this conclusion. The location of the nor-Winterstein acid moiety was deduced to be at C-5 from the HMBC spectrum (Fig. 2). The three-proton signal at δ 2.29 ppm was assigned to N–CH_3. The remaining four three-proton signals at δ 2.01, 2.05, 2.01 and 2.05 ppm were assigned to the acetyl groups connected to C-2, C-9, C-10, and C-13 as deduced from the chemical shifts of the corresponding protons attached to them. The relative stereochemistry of the terpenoid skeleton of **1** was determined from chemical shifts, coupling constants and NOESY experiments. A coupling constant between H-9 and H-10 of 10.71 Hz indicated that the B-ring was in a chair-boat conformation. A NOESY experiment established the relative stereochemistry of **1** as shown in Fig. 3. Thus the structure was determined as 2α,9α,10β,13α-tetraacetoxy-5α-(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene.

Compound **2** was isolated as a colorless gummy substance in a yield of 0.0013% by weight based on dry material. Intense absorptions at 1735, 1720, and a weak absorption at 3330 cm^{-1} implied that **2** possesses ester and amino groups. The ^{1}H-NMR spectrum of **2** also showed characteristic signals of the taxane skeleton. The connection networks of 18-CH_3 → H-13 → 14α → H-14β → H-1 → H_2-2 → H-3 → H-20a → H-20b → H-5 → H_2-6 → H-7, H-9 → H-10 and H-2′ →H-3′ were established by the ^{1}H-^{1}H COSY spectrum. Protonated molecular ion at m/z 682.3589 indicated the composition of **2** was $C_{38}H_{51}O_{10}N$, which the same as that of **1**. Two daughter ions at m/z 502 and 562 clearly indicated the lose of a nor-Winterstein acid group from the side-chains. The base peak at m/z 120.0812 ($C_8H_{10}N$) and a stronger fragment ion at m/z 180.1025 ($C_{10}H_{14}O_2N$) further support that the side-chain is a nor-Winterstein acid. The presence of a nor-Winterstein acid [3-(N-methylamino)-3-phenylpropanoyl] moiety in **2** is also suggested from the signals at δ 2.26 (3H, s), 2.74 (1H, dd, J = 11.27, 6.12 Hz), 2.65 (1H, dd, J = 11.27, 8.79 Hz), 3.95 (1H, dd, J = 6.12, 8.79 Hz), 7.32 (5H, m) ppm in the ^{1}H-NMR spectrum. The location of a nor-Winterstein acid moiety was suggested at C-5 as in the case of all the other taxoids reported (Ando et al., 1997; Appendino, 1995; Appendino et al., 1993a, 1993b; Doss, Carney, Shanks, Williamson & Chamberlain, 1997; Zhang, Fang, Liang & He, 1994). From the above analysis, the structure of **2** was established as 7β,9α,10β,13α-tetraacetoxy-5α(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene. Unfortunately, insufficient sample was available in order to obtain ^{13}C-NMR and HMBC spectra.

In summary, structures of **1** and **2**, both have an unusual nor-Winterstein acid side chain at C-5, which is reported here for the first time. Both EI-MS and FAB-MS spectra can produce strong molecular or protonated molecular ion. Two important daughter ions, usually appearing as base peaks or strong fragment ions, provide useful information about the side chains. The signal of N–CH_3 in the nor-Winterstein acid [3-(*N*-methylamino)-3-phenylpropanoyl] usually resonates at δ 34.5 ppm in the ^{13}C-NMR spectrum, which

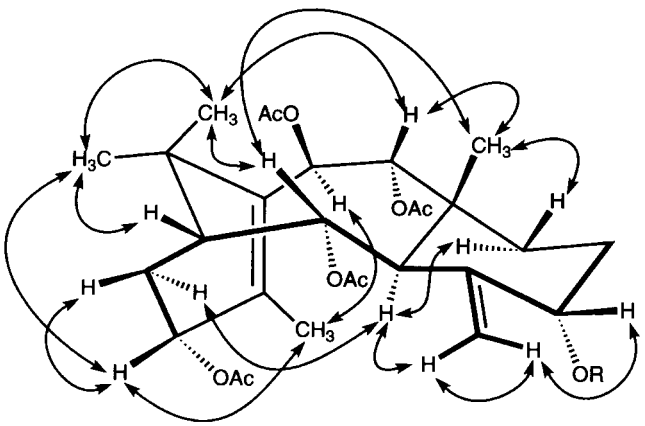

Fig. 3. Relative stereochemistry of **1**, proposed through NOESY experiment (500 MHz).

upfield shifts ca. 8 ppm relative to the Winterstein acid.

3. Experimental

3.1. General

Optical rotations were recorded on a Horiba SEPA-300 digital polarimeter. IR spectra were obtained on a Jasco IR-810 instrument. MS were measured on a Jeol JMS-700 spectrometer using FAB modes. ^{1}H- and ^{13}C-NMR spectra were obtained on Varian GEMINI 2000/300 and Varian Unity Inova 500 spectrometers operating at 300 MHz for ^{1}H, 125 MHz for ^{13}C, in $CDCl_3$ at 20°C, chemical shifts are reported in ppm scale relative to that of tetramethylsilane (TMS, $\delta = 0$) as an internal standard, and coupling constants are given in Hertz. Open column chromatography was performed using Merck silica gel 60 (100–200 mesh). Thin layer chromatography was carried out with the precoated Merck silica gel 60 F_{254} plates. Preparative TLC was performed with plates of 0.85 mm (dried for 24 h at room temperature and activated for 4 h at 120°C) thickness; spots were detected under UV (254 nm) and/or by spraying with 10% sulfuric acid and then heating on a hot plate.

3.2. Plant material

The seeds of *T. mairei* were collected in Jinggangshan, in the south-east of China, in October of 1995. The botanical identification was made by Professor R.L. Liu, Zhangzhou Forestry School, China. A voucher specimen is deposited in our laboratory of Graduate School at the Faculty of Agricultural Science, Tohoku University, Japan.

3.3. Isolation 9 taxanes *1* or *2*

Air dried seeds (1.1 kg) were first crushed and extracted with hexane three times at room temperature, with the hexane solubles discarded. The resulting residue was extracted twice with methanol (MeOH), will the MeOH solubles than evaporated to dryness under reduced pressure. Subsequently this residue was diluted with water and extracted EtOAc (5×). The combined EtOAc solubles were then extracted with 5% HCl. After neutralization, the aqueous layer was extracted with EtOAc (3×), and the combined EtOAc solubles, upon evaporation, yielded 3.0 g of yellowish syrup. This was subjected to preparative TLC repeatedly with different developing solvents, and which finally yielded compounds **1** (3 mg) and **2** (1.4 mg).

3.4. 2α,9α,10β,13α-Tetraacetoxy-5α-(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene (*1*)

Gum, $[\alpha]_D^{24}$ +47° (*c* 0.002, $CHCl_3$). IR (film, $CHCl_3$) ν_{max}: 3350, 3010, 2950, 2850, 2800, 1740, 1720, 1640, 1490, 1440, 1370, 1240, 1030, 1020, 960, 920, 890, 760 and 700 cm^{-1}; FAB-MS: *m*/*z*: 682 ($[M+H]^+$), 622 ($[M+H\text{-}AcOH]^+$), 564 ($[M+H\text{-}AcOH]^+$), 400, 340, 280, 247, 222, 180, 178 ($[HOCOCH_2CH(MeNH)Ph]^+$), 120 ($[MeNH^+=CHPh]^+$) and 43. HR-FAB-MS: 682.3591 (calcd for $C_{38}H_{52}O_{10}N$, 682.3589), 180.1027 (calcd for $C_{10}H_{14}O_2N$, 180.1024), 120.0815 (calcd for $C_8H_{10}N$, 120.0813). EI-MS: 681 ($[M]^+$), 622, 562, 502, 180, 178, 120, and 43. HR-EI-MS: 681.3514 (calcd for $C_{38}H_{51}O_{10}N$, 681.3513, Δ+0.1 mmu), 562.2777 (calcd for $C_{30}H_{42}O_{10}$, 562.2775, Δ−0.1 mmu), 502.2567 (calcd for $C_{28}H_{38}O_8$, 502.2565 Δ+0.0 mmu), 120.0817 (calcd for $C_8H_{10}N$, 120.0813, Δ+0.4 mmu). The ^{1}H-NMR spectral data see Table 1.

3.5. 7β,9α,10β,13α-Tetraacetoxy-5α(3′-methylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene (*2*)

Gum, $[\alpha]_D^{25}$ +10.3° (*c* 0.005, $CHCl_3$); IR (film, $CHCl_3$) ν_{max}: 3330, 3010, 2950, 2905, 2850, 1735, 1720, 1640, 1500, 1370, 1230, 1160, 1100, 1020, and 760 cm^{-1}; FAB-MS: *m*/*z*: 704 ($[M+Na]^+$), 682 ($[M+H]^+$), 622 ($[M+H\text{-}AcOH]^+$), 580, 563, 562, 520, 180, 120, 91, and 43. HR-FAB-MS *m*/*z*: 682.3589 (calcd for $C_{38}H_{52}O_{10}N$, 682.3588), *m*/*z*: 180.1025 (calcd for $C_{10}H_{14}O_2N$, 180.1024), *m*/*z*: 120.0812 (calcd for $C_8H_{10}N$, 120.0813), The ^{1}H-NMR spectral data see Table 1.

Acknowledgements

The authors are grateful to Mrs. Yuhko Sugiyama for measuring the NMR spectra, and to Mr. R.L. Liu for collecting and identifying the plant material for this work. Financial support for the work described here was provided by the Ministry of Education, Science and Culture of Japan through a grant-in-aid for scientific research.

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