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TAXAYUNTIN H AND J FROM *TAXUS YUNNANENSIS*

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Key Word Index—*Taxus yunnanensis*; Taxaceae; taxoids; diterpenoids; taxayuntin H; taxayuntin J.

Abstract—The structures of two new 11(15 → 1)abeotaxanes (taxayuntin H and J) isolated from the barks of *Taxus yunnanensis* have been elucidated by means of 1D and 2D NMR spectroscopic method. Taxayuntin J belongs to the rare group of taxanes with C-4 unfunctionalised.

INTRODUCTION

The natural taxane diterpenoid Taxol® [1], first isolated from the barks of *Taxus brevifolia*, has been approved by the FDA for the treatment of ovarian and breast cancers. It has stimulated a great interest in the isolation of secondary metabolites from *Taxus* species. In our search for bioactive taxoids and precursors of semisynthesis taxol, we have isolated many taxoids from the barks of *T. yunnanensis* Cheng *et* L. K. Fu [2]. In this paper we report on the isolation of two new taxoids, named taxayuntin H (**1a**) and J (**2**), from this source.

RESULTS AND DISCUSSION

Taxayuntin H (**1a**) showed a $[MH]^+$ ion peak at *m/z* 611 in its FAB-MS. The FAB-MS and ^{13}C NMR spectra suggested a formula $C_{30}H_{42}O_{13}$. IR absorptions at 3550 and 1730 cm^{-1} indicated the presence of hydroxy and ester groups respectively. The ^1H NMR spectrum of **1a** (Table 1) contained the signals of four tertiary methyl groups (δ 1.05, 1.13, 1.66 and 1.94), five acetyl groups (δ 1.95, 2.01, 2.02, 2.08 and 2.15), and five oxymethine groups (δ 4.47, 5.45, 5.97, 6.02 and 6.24). The presence of an oxetane ring was suggested by the presence of a pair of doublets at δ 4.37 and 4.51 (J = 7.6 Hz). The signal of Me-16 in normal taxane diterpenoids which lack a carbonyl group at C-9 appears at lower field (δ 1.43 ~ 1.86) [3]. For compound **1a** the chemical shift of Me-16 was at δ 1.13, suggesting that **1a** possessed a 5/7/6 membered ring skeleton [3]. This was supported by the chemical shift of C-1 (δ_C 67.57) [4] and the ion peak at *m/z* 59 produced by the hydroxyisopropyl fragment in FAB-MS. The ^1H NMR spectra of **1a** and taxayuntin (**1b**) [5] were very similar except for the presence of an acetyl group in **1a** and a benzoyl group in **1b**, and the signal of H-10α (δ 6.24 in **1a** and δ 6.53 in **1b**). The downfield shift of H-10α in **1b** was attributed to the deshielded effect of a benzoyl group substituted at C-10β [3]. Thus the structure of taxayuntin H is assigned to be **1a**.

Taxayuntin J (**2**) gave FAB-MS ion peaks at *m/z* 651 and 635, corresponding to $[MK]^+$ and $[MNa]^+$ respectively. Its molecular formula, $C_{30}H_{44}O_{13}$, was deduced by FAB-MS, ^1H and ^{13}C NMR spectroscopy. IR absorptions at 3450 and 1740 cm^{-1} implied that **2** possessed hydroxy and ester groups respectively. In addition to four methyl groups of the taxane type, five acetyl groups, six oxymethine groups and one oxymethylene group were observed in the ^1H NMR spectrum (Table 1). The 5/7/6 membered ring skeleton was deduced from the upfield resonance of Me-16 at δ 1.31, the absence of a carbonyl group signal at C-9 and the signal of C-1 at δ_C 69.07 [3–4]. The large value of $J_{20a,b}$ (11.2 Hz) and the C-20 oxymethylene carbon resonance at δ 63.81 in the ^{13}C-^1H COSY spectrum instead of ca δ 75 in usual taxane diterpenoids with an oxetane ring between C-5 and C-20 suggested the presence of ring-opened oxetane moiety [3, 6, 7]. The signals of H-20 at δ 4.41 and 4.06 correlated to the carbonyl carbon resonance at δ 171.49, which was further correlated with the acetyl methyl signal at δ 2.07 in the HMBC spectrum. This observation indicated the presence of an acetyl group at C-20, thus confirming the presence of the ring-opened oxetane moiety. The ^1H NMR spectrum revealed the signals of H-3 (δ 2.54, J = 8.2, 4.7 Hz), H-20a (J = 11.2, 1.8 Hz) and H-20b (J = 11.2, 9.7 Hz) as doublet of doublets instead of the usual doublet found in many

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Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1a** and **2** (δ, $CDCl_3$)

	1		**2**		
Position	^1H	^{13}C	^1H	^{13}C	NOE
1		67.57		69.07	
2	6.02 *d* (7.7)	68.00	4.67 *d* (8.2)	65.73	H-9, 16, 19
3	3.09 *d* (7.7)	43.85	2.54 *dd* (8.2, 4.7)	40.44	H-4, 7, 10, 14α
4		80.05		41.09	
5	4.92 *dd* (7.8, 1.2)	84.99	5.05 *dd* (2.7, 5.1)	70.20	H-4, 20b
6α	1.85 *bt* (15.8, 8.0)	34.72	1.89 *m*	29.48	
6β	2.52 *dt* (15.8, 8.0)		1.89 *m*		
7	5.45 *t* (8.0)	70.27	5.33 *dd* (9.5, 6.9)	69.24	
8		43.76		43.51	
9	5.97 *d* (10.6)	76.59	5.61 *d* (10.5)	76.42	
10	6.24 *d* (10.6)	68.74	6.23 *d* (10.5)	69.07	
11		134.09		134.39	
12		150.60		150.16	
13	4.47 *t* (7.4)	77.20	4.58 *d* (7.1)	76.58	H-14β, 17
14α	1.57 *dd* (15.0, 7.4)	39.69	1.74 *dd* (7.3, 14.6)	39.74	
14β	2.23 *dd* (15.0, 7.4)		2.32 *dd* (7.3, 14.6)		
15		75.05		76.42	
16	1.13 *s*	25.05	1.31 *s*	26.98	
17	1.05 *s*	27.33	1.26 *s*	27.48	
18	1.94 *d* (1.3)	11.68	1.97 *s*	11.72	
19	1.66 *s*	12.73	1.02 *s*	14.06	H-2, 6β, 9, 20a,b
20a	4.51 *d* (7.6)	75.07	4.41 *dd* (1.8, 1.2)	63.81	H-4, 20b
20b	4.39 *d* (7.6)		4.06 *dd* (9.7, 11.2)		H-5, 19, 20a
COMe	2.15	22.33	2.07 *s*	21.39	
	2.08	21.35	2.07 *s*	21.05	
	2.02	20.71	2.04 *s*	20.92	
	2.01	21.54	1.98 *s*	20.70	
	1.95	20.69	1.96 *s*	20.70	
COMe		171.71		171.49	
		170.20		170.07	
		169.72		169.94	
		169.60		169.41	
		167.67		167.91	

natural taxoids with an oxetane ring [3], suggesting that one proton was present at C-4. This was supported by the correlation of the one proton multiplet at δ 2.30 with H-3 and H-20a,b in ^1H-^1H COSY spectrum. The location of the acetyl groups at C-9 and C-10 was established by the observation of a pair of doublets at δ 5.61 and 6.22 (J = 10.5 Hz). The signals at δ 5.05 and 5.33 correlated with the C-6 protons as a multiplet at δ 1.89 in the ^1H-^1H COSY spectrum, and thus were attributed to H-5 and H-7, indicating the presence of acetyl groups at C-5 and C-7. A doublet at δ 4.67 coupled with H-3 (J = 8.2 Hz) and a triplet at δ 4.58 correlated with the C-14 protons (δ 2.32 and 1.74) and C-18 methyl (δ 1.97) in the ^1H-^1H COSY suggested that C-2 and C-13 were hydroxylated. In the HMBC spectrum, the signals of H-5, H-7, H-9 and H-10 showed correlations with the carbonyl resonances at δ 170.07, 169.41, 169.94 and 167.91, which confirmed the location of the other four acetyl groups at the C-5, C-7, C-9 and C-10 positions.

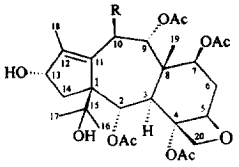
The relative stereochemistry of **2** was elucidated by the NOE data. The NOE of H-2 to H-9, H-16 and H-19 indicated that a hydroxy and an acetyl groups were connected at C-2α and C-9α respectively. The β-orientation of the acetyl groups at H-7 and H-10 were deduced by the NOE between H-3, H-7 and H-10. NOE between H-19 and H-20a,b, and H-3 and H-4 suggested that an acetoxymethylene group was substituted at C-4β. The configuration of the substituents at C-5 and C-13 was α, based on the NOE of H-20a,b to H-5, and H-13 to H-14β.

Based on the spectral analysis described above, the structure and stereochemistry of taxayuntin J was thereby established as **2**.

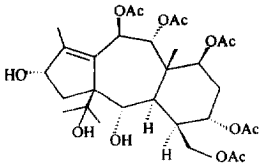
EXPERIMENTAL

General

Mp: uncorr; ^1H and ^{13}C NMR: Bruker AM 500 spectrometer in $CDCl_3$, chemical shift are reported in



1a R=OAc
1b R=OBz



2

δ (ppm) using TMS as int. standard; FAB-MS: JMS-SX 102; EI mass spectra: ZAB-2F.

Plant material

The bark of *T. yunnanensis* Cheng *et* L. K. Fu was collected in 1987 in Yunnan province, People's Republic of China, and identified by Prof Yu-Heng Chen. A voucher specimen is deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China.

Extraction and isolation

Dried powdered bark was extracted with EtOH. The extract after evaporation was suspended in H_2O and extracted with petrol, CH_2Cl_2 and EtOAc successively. The CH_2Cl_2 extract was dissolved in Et_2O. The Et_2O-insoluble part was then developed on silica gel by dry-CC using CH_2Cl_2–MeOH and cut into 25 equal frs which were individually eluted with MeOH. Frs 10–17 were repeatedly rechromatographed on silica gel columns and plates to furnished 65 mg of **1a**. The Et_2O-soluble part was subjected to dry-CC on silica gel and rechromatographed by column and prep. TLC repeatedly to give 30 mg of **2**.

Taxayuntin H (**1a**). Colourless prism, mp 249–250° (MeOH), $[\alpha]$ −66.70° (*c* 0.04, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 204 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3550, 1730, 1440, 1375, 1220 ~ 1250, 1044, 1030, 980, 950, 900; FAB-MS *m/z* (rel. int.): 611 $[MH]^+$ (50), 593 $[MH-H_2O]^+$ (6), 551 $[MH-HOAc]^+$ (100), 533 $[MH-HOAc-H_2O]^+$ (48), 491 $[MH-2\times HOAc]^+$ (30), 431 $[MH-3\times HOAc]^+$ (26), 373 $[MH-3\times HOAc-Me_2CO]^+$ (83), 355 $[MH-4\times HOAc-H_2O]^+$ (25), 311 $[MH-5\times HOAc]^+$ (56), 293 $[MH-5\times HOAc-H_2O]^+$ (13), 253 $[MH-5\times HOAc-Me_2CO]^+$ (56), 59 $[Me_2COH]^+$ (30); ^1H and ^{13}C NMR: Table 1.

Taxayuntin J (**2**). White powder, mp 125–127°, $[\alpha]$ −54° (*c* = 0.05, MeOH); UV λ_{max}^{EtOH} nm (log ε): 207 (3.42); IR ν_{max}^{KBr} cm^{-1}: 3456, 1740, 1647, 1372, 1248, 1070, 1032, 984, 905; FAB-MS *m/z*: 651 $[MK]^+$, 635 $[MNa]^+$; EI-MS (70 eV) *m/z* (rel. int): 594 $[M-H_2O]^+$ (0.5), 534 $[M-H_2O-HOAc]^+$ (0.8), 516 $[M-2\times H_2O-HOAc]^+$ (1.8), 492 $[M-2\times HOAc]^+$ (4.6), 416 $[M-2\times HOAc-Me_2CO-H_2O]^+$ (14), 374 $[M-3\times HOAc-Me_2CO]^+$ (32), 356 $[M-3\times HOAc-Me_2CO-H_2O]^+$ (54), 296 $[M-4\times HOAc-Me_2CO-H_2O]^+$ (38), 236 $[M-5\times HOAc-Me_2CO-H_2O]^+$ (74), 43 $[MeCO]^+$ (100); ^1H and ^{13}C NMR: Table 1.

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