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TAXAYUNTIN E AND F: TWO TAXANES FROM LEAVES AND STEMS OF *TAXUS YUNNANENSIS*

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Key Word Index—*Taxus yunnanensis*; Taxaceae; taxanes; taxayuntin E and taxayuntin F; conformation.

Abstract—Two new 11(15 → 1)-abeotaxanes (taxayuntin E and F) were isolated from the leaves and stems of *Taxus yunnanensis*. The conformational flexibility of the tetracyclic taxane skeleton was observed in the ^{1}H NMR spectrum of taxayuntin F.

INTRODUCTION

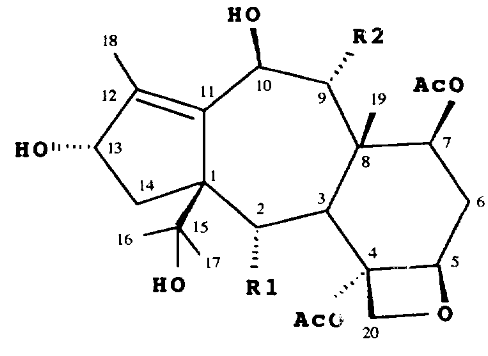
Taxol, originally isolated from the Pacific yew tree in the early 1970s [1], was recently approved by FDA for use against ovarian cancer and has also shown activity against breast, lung, and other cancers. In spite of its encouraging spectrum of activity, progress in developing taxol as a drug has been relatively slow, largely because of the lack of an abundant supply and difficulties in formulation [2]. The promising clinical spectrum of taxol stimulated the search for new related diterpenes having similar activity from other species of the family Taxaceae. *Taxus yunnanensis* Cheng et L. K. Fu is an evergreen tree indigenous to China and from which many taxoids have been already isolated by our research group [3–9]. In continuing investigations on the leaves and stems of *T. yunnanensis*, two new taxoids were obtained and named taxayuntin E (**2**) and taxayuntin F (**3**).

RESULTS AND DISCUSSION

A defatted ethanolic extract from the leaves and stems was extracted with dichloromethane; after removal of the solvent, the residue was chromatographed on silica gel using $CHCl_3$–MeOH (40:1) as eluent. Ten main fractions were collected and the seventh fraction gave the new taxoids **2** and **3**.

Taxayuntin E (**2**), isolated as transparent plates, was determined to have a molecular formula of $C_{33}H_{42}O_{12}$ by analysis of ^{13}C NMR and FAB-MS. Besides the four methyls typical of taxane derivatives, the signals of three acetates and one benzoate were also present in the NMR spectra (Table 1). Two doublets at δ4.31 and δ4.00 (J = 7.5 Hz) were assigned to a C-5, C-20 oxetane moiety. C-1 and C-15 resonated at δ68.84 and 76.71 (DEPT and HETCOR techniques), and thus **2** was a 11(15 → 1)-abeotaxane [6]. By comparison of ^{1}H and ^{13}C NMR spectra with those of taxacustin (**1**) [10], we found that these compounds were very similar except for the presence of one benzoate group in **2** instead of an acetate group in **1**. In order to determine the location of the ester groups, an HMBC experiment was performed. A correlation between the benzoyl carbonyl at δ167.69 and H-2β (δ6.19) located the benzoyl at C-2α. Consequently, the three acetoxyl groups must be at positions C-4α, C-7β and C-9α. The stereochemistry of the molecule was also deduced by comparison with taxacustin.

Taxayuntin F (**3**) showed the ion $[M + K]^+$ in the FAB-MS at m/z 669, as observed with **2**. Interestingly, the ^{1}H NMR spectrum showed marked changes with temperature. At room temperature, the ^{1}H NMR spectrum consisted of very broad lines and spectral analysis was not possible. At elevated temperature (40°), more signals could be resolved, but at low temperature (0°), most signals sharpened, allowing the identification of the spin systems. The ^{1}H NMR signals at δ4.52 and 4.44 (d, J = 7.5 Hz) and the signal at δ75.62 (CH_2) in the ^{13}C NMR spectrum indicated the presence of an oxetane ring in the molecule. Besides the four taxane methyl resonances, the signals of three acetates and of one benzoate were also present in the NMR spectra. Both the ^{1}H- and ^{13}C NMR spectra were very similar to those of **2**. The major difference between the two compounds was in the location of the benzoate group. Indeed, the signal of H-9β in **3** was shifted downfield ($\Delta\ \delta$ + 0.30) and H-2β was upfield ($\Delta\delta$ – 0.08). These changes suggested that **3** has a benzoate group at C-9α and an acetate group at C-2α. A single crystal X-ray diffraction analysis of **3** confirmed the structure and revealed the twist-boat conformation of ring B in the crystalline state (Fig. 1).



		R1	R2
Taxacustin	**1**	OAc	OAc
Taxayuntin E	**2**	OCOPh	OAc
Taxayuntin F	**3**	OAc	OCOPh

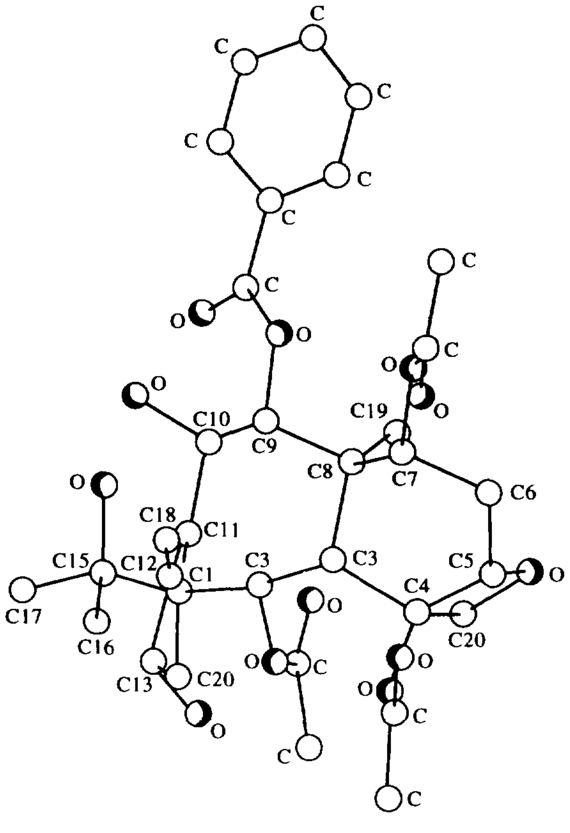


Fig. 1.

The temperature-dependent ^{1}H NMR spectrum of **3** suggested the presence of a slow (on the NMR time scale) conformational equilibrium. A temperature decrease during the NMR experiment (CD_3OD) sharpened most resonances and at 0° two sets of signals were detected. An analysis of the proton–proton coupling constants showed that in the major rotamer ring B is in the twist-boat conformation with H-9β and H-10α pseudoaxial ($J_{9,10}$ = 10.5 Hz), whereas in the minor conformer ring B assumes a twist-chair geometry with these protons pseudoequatorial ($J_{9,10}$ = 7.8 Hz). The flexible behaviour of **3** is not unprecedented, since the conformational equilibrium had already been observed in the NMR spectra of brevifoliol derivatives [11, 12].

EXPERIMENTAL

General. NMR spectra were obtained on a Bruker AM 500 spectrometer in $CDCl_3$ at room temp. ^{1}H Chemical shifts are recorded in ppm from int. TMS and ^{13}C shifts are based on the $CHCl_3$ signal at 77.0 ppm. MS were obtained on a JMS-DX 300 mass spectrometer. UV spectra were recorded on a Shimadzu 240 spectrometer. IR spectra were recorded on Perkin–Elmer 399B spectrometer as KBr pellets. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. Mps were determined with an uncalibrated Boetius thermometer.

Plant material. Taxus yunnanensis Cheng et L. K. Fu was collected in March 1993 in Yunnan province, China and identified by Prof. Chen Yu-heng. A voucher specimen is kept at the Department of Medicinal Plants, Institute of Meteria Medica, Chinese Academy of Medical Sciences.

Extraction and isolation. Dried leaves and stems (25 kg) were extracted with EtOH to afford 1.5 kg of crude extract, which was dissolved with an equivalent amount of H_2O and extracted with petrol, CH_2Cl_2 and EtOAc successively. About 106 g of the CH_2Cl_2 part was eluted with petrol, cyclohexane–Me_2CO (1:1) and Me_2CO by vaccuum chromatography. Concn of the cyclohexane–Me_2CO (1:1) part under red. pres. gave 84 g of residue, which was then subjected to dry-CC on silica gel using MeOH–$CHCl_3$ (1:40) as eluent and 10 frs were collected. The 7th fr. was rechromatographed on silica gel and RP-18 CC to give **2** (20 mg) and **3** (35 mg).

Taxayuntin E (**2**). Plates from MeOH: mp 185–186°, $[\alpha]_D^{12}$ + 8.70 (MeOH; *c* 0.07). UV $\lambda_{\text{Max}}^{\text{MeOH}}$ nm (log ε): 205 (3.94), 227 (3.91). IR $\nu_{\text{Max}}^{\text{KBr}}$ cm^{-1}: 3432, 2983, 2371, 1735, 1719, 1371, 1279, 1237, 1272, 1028, 982, 713. ^{1}H and ^{13}C NMR data see Table 1. FAB-MS, *m/z*: 669, $[M + K]^+$ 284; 653, $[M + Na]^+$ 225.

Taxayuntin F (**3**). Plates from MeOH: mp 185–186°, $[\alpha]_D^{12}$ − 19.0 (MeOH; *c* 0.08). UV $\lambda_{\text{Max}}^{\text{MeOH}}$ nm (log ε): 205 (4.32), 227 (3.95). IR $\nu_{\text{Max}}^{\text{KBr}}$ cm^{-1}: 3526, 3434, 3395, 1737, 1715, 1371, 1259, 1236, 1072, 1027, 984. ^{1}H and ^{13}C NMR data see Table 1. FAB-MS, *m/z*: 669, $[M + K]^+$ 334, 296, 231; 653, $[M + Na]^+$.

X-Ray analysis of **3**. Taxayuntin F crystallized in the orthorhombic system, space group $p2_12_12_1$ with one molecule of composition $C_{33}H_{42}O_{12}$ forming the asymmetric unit. Accurate cell constants of a = 11.913 (3) Å, b = 13.210 (4) Å, c = 20.343 (6) Å, v = 3201.4 Å^3 were determined by a least-squares fit of 15 moderate angle 2θ values. All unique diffraction maxima with $2\theta < 114$ were collected on the NICOLET R3M/E X-ray system using graphite monochromated CuK$_\alpha$ radiation (1.54178 Å) and variable speed ω scans. After correction for Lorentz, polarization and background effects, 2257 of the 2466 unique reflections were judged observed ($|Fo| > 3\sigma|Fo|$). The structure was solved routinely using direct method

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data for **2** and **3** in CD_3OD

C	Type*	**2**	**3**†		**2**	**3**‡
		^{13}C			^{1}H§	
C1	*s*	68.8	68.3			
C2	*d*	70.1	69.9	H-2β	6.19 *d* (7.6)	6.11 *d* (7.45)
C3	*d*	46.5	45.8	H-3α	3.13 *d* (7.6)	3.17 *d* (7.34)
C4	*s*	80.3	80.1			
C5	*d*	86.1	86.3	H-5α	4.91 *br d* (8.4)	5.04 *br d* (7.8)
C6	*t*	35.9	35.9	H-6α	2.35 *m*	2.55 *dt* (15.5, 8.3)
				H-6β	1.77 *m*	1.80 dd (14.0, 9.7)
C7	*d*	72.4	72.4	H-7α	5.42 *br t* (8.7)	5.53 *t* (8.2)
C8	*s*	44.6	44.8			
C9	*d*	80.8	82.1	H-9β	5.86 *d* (10.2)	6.06 *d* (10.2)
C10	*d*	67.6	67.9	H-10α	4.63 *d* (10.3)	4.86 *d* (10.1)
C11	*s*	138.3	138.2			
C12	*s*	148.0	147.8			
C13	*d*	77.7	77.7	H-13β	4.50 *br t* (7.1)	4.56 *t* (7.2)
C14	*t*	40.3	40.1	H-14α	1.86 *m*	1.73 *m*
				H-14β	2.16 *m*	2.35 *dd* (18.7, 7.3)
C15	*s*	76.7	76.7			
C16	*q*	25.3	25.3	16-Me	1.05 *s*	1.20 *s*
C17	*q*	28.1	28.1	17-Me	0.97 *s*	1.08 *s*
C18	*q*	11.3	11.3	18-Me	1.83 *s*	1.81 *s*
C19	*q*	13.1	13.8	19-Me	1.61 *s*	1.74 *s*
C20	*t*	75.6	75.6	H-20α	4.30 *d* (7.5)	4.52 *d* (7.5)
				H-20β	4.50 *d* (7.6)	4.44 *d* (7.5)
COMe	*s*	172.7	172.2			
	q	21.4	21.8	COMe	2.05 *s*	1.94 *s*
COMe	*s*	172.1	172.2			
	q	21.8	22.1	COMe	2.09 *s*	2.08 *s*
COMe	*s*	171.9	172.0			
	q	22.3	22.3	COMe	2.16 *s*	2.20 *s*
COph	*s*	167.7	169.1			
1′	*d*	131.5	132.3			
2′	*d*	130.8	130.8		8.00 *d* (7.5)	8.01 *d* (7.5)
3′	*d*	129.8	129.5		7.47 *m*	7.53 *m*
4′	*d*	134.8	134.1		7.58 *m*	7.65 *m*
5′	*d*	129.7	129.5			
6′	*d*	134.4	131.4			

*s = C, d = CH, t = CH_2, q = Me. Assignments made by the DEPT technique.

†Assignment was based on analogy to **2**.

‡Recorded at 0°.

§Multiplicity and coupling constant(s) in Hz in parentheses.

SAPI. Hydrogen atoms were located in difference electron density synthesis after least-squares refinement of the non-hydrogen atoms. The residual factors were $R = 0.0712$ for 2257 observed reflections.

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