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TAXOIDS FROM THE BARKS OF *TAXUS WALLICHIANA*

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Key Word Index—*Taxus wallichiana*; Taxaceae; taxoid; diterpenoids; bark.

Abstract—Two new taxane diterpenoids, 1-hydroxy-2-deacetoxytaxinine J and 7,2′-bisdeacetoxyaustrospicatine, were isolated from the barks of *Taxus wallichiana* together with 13 known compounds, including taxol. Their structures were elucidated and identified by spectroscopic methods. The structure of 7,2′-bisdeacetoxyaustrospicatine was further confirmed by X-ray crystallographic analysis.

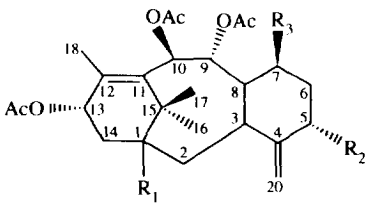
INTRODUCTION

Taxol, a diterpenoid first isolated by Wani *et al.* [1] from *Taxus brevifolia* Nutt. was recently developed as a new and exciting anti-tumor agent for the treatment of ovarian and breast cancer. Its novel activity, along with the resource scarcity, has promoted chemical researches on *Taxus* species all over the world. In our continuous work on the chemical constituents of *Taxus* species indigenous to China, *T. wallichiana* Zucc. has been studied. There have been several reports on the chemical constituents of this plant [2–11], including taxoids, diflavonoids and lignans. The present paper reports the isolation and structure determination of two new and 11 known taxoids and two common steroids, sitosterol and daucosterol.

RESULTS AND DISCUSSION

Besides the two new taxoids **1** and **2** described below, 13 known compounds, in which 11 are also taxoids, were isolated from ethanol extracts of barks of *T. wallichiana* Zucc. collected in Tibet, China. These known compounds are taxol, cephalomannine, 10-deacetylbaccatin III, 7-xylosyltaxol, 10-deacetyl-7-xylosyltaxol [12], 10-deacetyl-7-xylosyltaxol C [12], 2-deacetoxytaxinine J [13], 2-deacetoxy-5-decinnamyltaxinine J [14], 2′-deacetoxyaustrospicatine (**3**) [15], taxayuntin [16], taxacustin, sitosterol and daucosterol. Their structures were determined by comparing the physical and spectral data with those for authentic material and/or with the data in the literature.

Compound **1** was determined to have a molecular formula of $C_{37}H_{46}O_{11}$ by analysis of the ^{13}C NMR and FAB-mass spectral data. The ^1H NMR signals at δ5.09 (1H, *br s*) and 5.43 (1H, *br s*) and those in the ^{13}C NMR at δ145.6 (*tert*-C) and 116.1 (CH_2) suggest the presence of an exomethylene moiety. The ^1H NMR singlets at 1.18, 1.63, 2.33 and 0.93 (each 3H) are characteristic of the four methyl groups on the taxane skeleton. Four-*O*-acetyl groups were revealed by ^1H NMR signals at δ1.73, 2.00, 2.05 and 2.07, and ^{13}C NMR signals at δ169.1, 169.8, 170.2 and 170.5. Furthermore, the correlations in the HMBC spectrum indicate that the four *O*-acetyl groups are connected at 7, 9, 10 and 13 positions (Table 1). The presence of a cinnamate was verified by the observation of ^1H NMR signals at δ7.77 *d* (1H, J = 16.0 Hz), 6.55 *d* (1H, J = 16.0 Hz), 7.49 *m* and 7.40 *m*, and ^{13}C NMR signals at δ166.0, 118.3, 130.1, 130.7, 134.1 138.1 and 145.9, and its location was determined to be at the C-5 position by comparing the ^1H NMR data with that for its congener, 2-deacetoxy taxinine J. Therefore, **1** is elucidated as 1β-hydroxy-7β,9α,10β,13α-tetraacetoxy-5α-cinnamyloxy-taxa-4(20),11-diene, named 1-hydroxy-2-deacetoxytaxinine J.



	R_1	R_2	R_3
1	OH	O_{cinn}	OAc
2	H	$OCOCH_2CH(NMe_2)Ph$	H
3	H	$OCOCH_2CH(NMe_2)Ph$	OAc

Compound **2** is obtained as colourless needles. Its FAB mass spectrum showed peaks at *m/z* 638 and 676, corres-

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Table 1. ^1H and ^{13}C NMR spectral data for compound **1** (δ, $CDCl_3$)

Position	C	HMBC	H	H–H COSY
1	76.2	Me-16, 17; H-3, 14		
2	37.0	H-3, 14	2.00 *m*	H-3
3	41.2	Me-19; H-20	2.97 *d* (5.5)	H-2
4	145.6	H-6		
5	74.5	H-20	5.60 *t* (2.8)	H-6
6	34.6		1.97 *m*	H-5, 7
7	69.8	Me-19; H-9	5.67 *dd* (11.4; 5.0)	H-6
8	46.6	Me-19; H-3, 9		
9	76.0	Me-19; H-10	5.95 *d* (11.2)	H-10
10	71.3	H-9	6.31 *d* (11.2)	H-9
11	134.8	Me-16, 17, 18; H-10		
12	139.2	Me-18; H-10, 14		
13	71.0	Me-18; H-14	5.96 *br s*	H-14
14	41.6		2.58 *dd* (14.8; 9.9)	H-13
			1.53 *dd* (14.8; 6.6)	
15	43.7	Me-16, 17; H-2, 10		
16	27.3	Me-17	1.18 *s*	
17	21.4	Me-16	1.63 *s*	
18	15.3		2.33 *s*	
19	13.2	H-7, 9	0.93 *s*	
20	116.1		5.09 *s*	
			5.43 *s*	
4 × OAc				
C=O	169.1	H-10		
	169.8	H-7		
	170.2	H-13		
	170.5	H-9		
– Me	20.8		1.73 *s*	
	20.9		2.00 *s*	
	21.0		2.05 *s*	
	21.8		2.07 *s*	
– *O* cinn				
C=O	166.0			
CH=CH	118.3		7.77 *d* (16.0)	
	145.9		6.55 *d* (16.0)	
Ph	138.1			
	130.1		7.49 *m*	
	130.7		7.40 *m*	
	134.1			

ponding respectively to $[MH^+]$ and $[MK^+]$. The EI-MS of compound **2** gave a molecular ion peak at *m/z* 637. In the ^1H NMR spectrum, four typical methyls of taxane derivatives were present at δ0.66, 1.09, 1.59 and 2.12 ppm, and two doublets at 5.15 (1.3 Hz), 4.81 ppm (1.3 Hz) showed the presence of a normal C-4 exomethylene group. Three acetates and one 3′-dimethylamino-3′-phenylpropionyl group were revealed in the ^1H NMR spectrum as well, and the latter was confirmed by the fragment ion of *m/z* 134 (Me_2N^+ =CHPh) in EI-MS. The ^1H NMR spectrum of **2** was very similar to that of 2′-deacetoxyaustrospicatine (**3**) (see Table 2), the main difference being the number of the acetates (three instead of four) and the signal of C7-H (δ1.5 *br t*; 2.01 *m* in **2** and δ5.41 *dd* in **3**). From this comparison and the ^1H–^1H COSY spectrum, the molecular formula can be established as $C_{37}H_{51}NO_8$ and its structure is deduced as 9α,10β,13α-triacetoxy-5α-(3′-dimethylamino-3′-phenyl)-propionyloxy-taxa-4(20),11-diene, named 7,2′-bisdeacetoxyaustrospicatine. This structure was further confirmed by X-ray diffraction analysis (Fig. 1), and was reported in preliminary form earlier [17].

EXPERIMENTAL

General. Mps: uncorr. NMR: 500 MHz, with TMS as int. standard.

Plant material. Taxus wallichiana Zucc. barks were collected in Jilong, Tibet, P. R. China, in November, 1992, and identified by Prof. Y. H. Chen of our Institute where a voucher specimen has been deposited.

Table 2. ^{1}H NMR spectral data for compounds **2** and **3** (δ, $CDCl_3$)

Position	2	3
1	1.82 *m*	1.84 *br d* (5.3)
2	1.74 *ddd* (15.8; 8.5; 2.2)	1.83 *dd* (14.1; 6.5)
2′	1.65 *dd* (14.7; 5.0)	1.70 *dd* (15.4; 5.9)
3	2.84 *br d* (5.5)	2.80 *br d* (5.5)
5	5.27 *t* (2.8)	5.37 *dd* (3.9; 2.3)
6	1.43 *m*	1.62 *m*
6′	1.09 *m*	1.27 *m*
7	2.01 *m*	5.41 *dd* (11.6; 5.0)
7′	1.53 *br t* (5.8)	
9	5.82 *d* (10.6)	5.87 *d* (11.0)
10	6.11 *d* (10.6)	6.29 *d* (11.0)
13	5.81 *t* (7.2)	5.88 *t* (5.6)
14	1.07 *dd* (14.6; 7.8)	0.98 *dd* (15.5; 7.4)
14′	2.67 *ddd* (14.6; 9.8; 4.8)	2.63 *ddd* (14.5; 9.8; 7.4)
16	1.09 *s*	1.10 *s*
17	1.59 *s*	1.59 *s*
18	2.12 *s*	2.22 *s*
19	0.66 *s*	0.78 *s*
20	5.15 *d* (1.3)	5.25 *d* (1.0)
20′	4.81 *d* (1.3)	4.93 *d* (1.0)
OAc	2.05 *s*, 2.01 *s*	2.05 *s*, 2.01 *s*, 1.97 *s*
CO | CH_2	3.00 (*br s*), 2.80 (*br s*)	2.97 (*br s*), 2.88 *m*
| CH	3.80 (*br s*)	3.92 (*br s*)
| |—(NMe_2)	2.21 *s*	2.16 *s*
Ph	7.39 *m*, 7.28 *m*	7.30 *t* (7.6), 7.23 *d* (7.6)

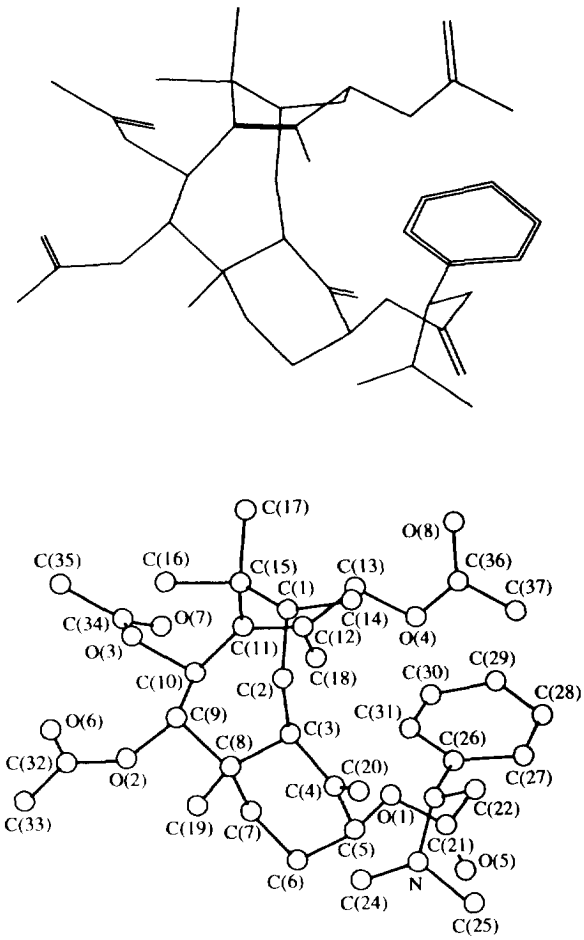

Fig. 1.

Extraction and isolation. Dried and powdered barks (4.4 kg) were extracted with 95% EtOH. The crude extract was concd *in vacuo*, diluted with dist. H_2O and partitioned against petrol followed by CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 extracts were concd, yielding a dark residue (38.8 g). CC of this residue on silica gel (100–200 mesh) eluted with CH_2Cl_2 and CH_2Cl_2–MeOH gradient provided 67 frs. Isolation and purification by repeated chromatography, including CC, prep. TLC and recrystallization furnished 15 pure compounds. β-Sitosterol (110 mg) was obtained from frs 38–41. Compounds **1** (7 mg), **2** (5 mg), **3** (237 mg) and taxol (64 mg), cephalomannine (43 mg), 2-deacetoxy-5-decinnamoyltaxinine J (48 mg) and taxayuntin (22 mg) were isolated from frs 42–48. Compound taxacustin (21 mg) was obtained from frs 49 and 50. Compounds 10-deacetylbaccatin III (78 mg) and 7-xylosyltaxol (34 mg) were isolated from frs 55–58. Frs 66 and 67 afforded 10-deacetyl-7-xylosyltaxol (35 mg), 10-deacetyl-7-xylosyltaxol C (18 mg) and daucosterol (120 mg).

1-Hydroxy-2-deacetoxytaxinine J (**1**). Powder, mp 112–114°, $[\alpha]_D^{23}$ + 64.0° (*c* 0.58, $CHCl_3$). FAB-MS, *m/z*: 689 $[MNa]^+$, 705 $[MK]^+$, 536 [MH – PhCHCHCO]. EI-MS, *m/z* (rel. int.): 606 $[M - HOAc]^+$ (0.8), 547 (4), 504 (7), 486 (8), 458 (9), 446 (10), 358 (7), 296 (79), 220 (57), 209 (27), 195 (100), 178 (27), 161 (45), 150 (39), 131 (43), 121 (43), 106 (26).

7,2′-Bisdeacetoxyaustrospicatine (**2**). Needles, mp 199–200° (Me_2CO), $[\alpha]_D^{15}$ + 112.9° (*c* 0.95, $CHCl_3$). FAB-MS, *m/z*: 638 $[MH]^+$, 676 $[MK]^+$, 194 $[Me_2NPhCHCH_2COOH]$. EI-MS, *m/z* (rel. int.): 637 $[M]^+$ (0.2), 578 (0.1), 518 (0.3), 444 (0.9), 402 (1.5), 282 (16), 249 (22), 192 (22), 163 (21), 147 (41), 134 (100), 119 (30), 60 (21), 43 (56).

Compound **2** crystallized in the orthorhombic space group $P2_12_12_1$ with one molecule of composition $C_{37}H_{51}NO_8$ forming the asymmetric unit. Accurate cell constants of a = 8.234 (2) Å, b = 17.191 (3) Å, c = 24.889 (5) Å, v = 3523.05 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 using graphite monochromated Cu K_α radiation (1.54178 Å) and variable speed ω scans. After correction for Lorentz, polarization and background effects, 871 of the 2089 (42%) unique reflections were judged observed ($|F_0| > 3\sigma(F_0)$). The structure was solved by SAPI programs. H atoms were located in a difference electron density synthesis after full least-squares refinement of the non-H atoms. The final conventional crystallographic residual is R = 0.076 for 871 observed reflections. Crys-

tallographic data have been deposited with The Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K., and are available from them. The final X-ray model is shown in Fig. 1.

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