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2-DEACETOXYTAXININE B: A TAXANE DITERPENOID FROM *TAXUS CHINENSIS*

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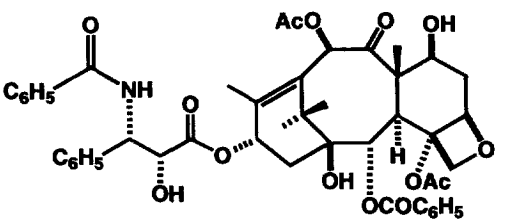
Key Word Index—*Taxus chinensis*; Taxaceae; taxane diterpenoids; 2-deacetoxytaxinine B; brevitaxin.

Abstract—2-Deacetoxytaxinine B, a taxane diterpenoid, was isolated from the stem bark of *Taxus chinensis* along with six known taxane diterpenoids and the diterpenolignan, brevitaxin. The structure of 2-deacetoxytaxinine was determined with the aid of spectroscopic techniques, including 2D NMR spectroscopy.

INTRODUCTION

The emergence of taxol (**1**) as one of the most promising anticancer natural products has stimulated worldwide interest in phytochemical studies of *Taxus* species with a view to identifying alternate sources of taxol and precursors for the semisythesis of its analogues for biological evaluation. The vigorous activity in this field is apparent from the appearance of reports describing over 100 new taxane diterpenoids (taxoids [1]) in barely two and a half years [2]. Currently over 200 natural taxoids are known [1, 2]. In the course of our continuing phytochemical studies of Chinese *Taxus* species we have investigated the stem bark of *Taxus chinensis* (Pilgre) Rehd. (Taxaceae) and in this paper we report the isolation of a new taxoid, 2-deacetoxytaxinine B (**2**), along with taxol (**1**), 10-deacetyltaxol, 1-deoxybaccatin VI, 2-deacetoxytaxinine J, taxinine J, 2-deacetyl-5-decinnamoyltaxinine J, and the diterpenolignan, brevitaxin (**3**). Previous studies on *T. chinensis* have resulted in the isolation of a variety of taxane and A-*nor*-taxane diterpenoids [3–8]. This is the first report of the occurrence of a diterpenolignan in *T. chinensis*. Brevitaxin (**3**) has previously been isolated only from *T. brevifolia* [9].



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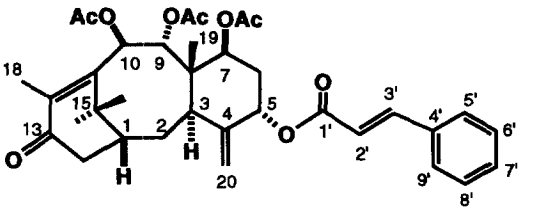

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RESULTS AND DISCUSSION

An ethanolic extract of the stem bark of *T. chinensis* was partitioned between dichloromethane and water and the dichloromethane fraction was adsorbed on to silica gel and extracted sequentially and exhaustively with hot petrol, dichloromethane, ethyl acetate and methanol. Of these, the dichloromethane extract was chromatographed twice on silica gel furnishing the new taxoid **2**, the diterpenolignan **3**, and several known taxane diterpenoids. All known compounds were identified by comparison of their spectral data with those reported in the literature. The structure elucidation of the new taxoid is presented here.

The new taxoid was determined to have the molecular formula $C_{35}H_{42}O_9$ based on its HRFAB mass

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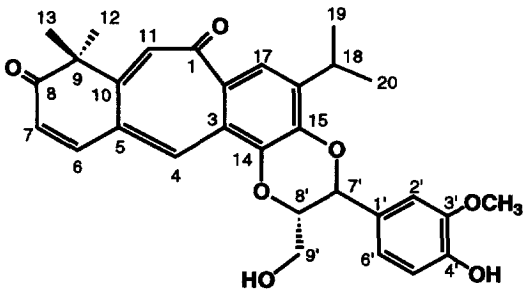

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spectrum and the analysis of ^1H and ^{13}C NMR data. The four methyl groups, characteristic of the taxane skeleton [1], appeared at δ_H 2.37, 1.61, 1.11, and 0.86 (each 3H, *s*), and at δ_C 37.1, 25.6, 14.1 and 12.8 in its NMR spectra. The presence of an *O*-cinnamoyl group was verified by observation of the ^1H NMR signals at δ 7.64 (1H, *d*, J = 16.0 Hz), 6.326 (1H, *d*, J = 16.0 Hz), 7.71 (2H, *dd*, J = 8.2, 1.9 Hz), and 7.39 (*m*, 3H) and the ^{13}C NMR signals at δ 145.9, 134.5, 130.4, 128.9, 128.5, and 117.5 ppm due to aromatic and olefinic moieties. The NMR signals at δ_H 4.93 (1H, *d*) and 5.29 (1H, *br s*), δ_C 146.9 (*s*) and 114.6 (*t*) suggested an exocyclic double bond and this was located at biogenetically favourable C-4(20) of the taxane skeleton. In the HMBC spectrum of **2** one of the olefinic signals at δ_H 4.93 (20-H) showed correlations to signals at δ_C 36.9 and 74.4. The latter had a cross-peak to the signal at δ_H 5.43 in its HMQC spectrum suggesting this to be due to 5-H. Weak HMBC correlations were seen between this signal and the signals at δ_C 69.8 and 166.1 (ester carbonyl). Cross-peaks were also observed between the latter signal and the signals due to olefinic protons of the cinnamoyl moiety in the HMBC spectrum suggesting the attachment of the cinnamoyloxy function to C-5. This was further confirmed by comparison of the ^1H NMR spectral data of **2** (Table 1) with those of taxinine B [6] and 10-deacetyltaxinine B [7].

The presence of 3 acetoxy groups in **2** were revealed by sharp 3H singlets at δ_H 2.01, 2.04, and 2.05 and at δ_C 169.1 (*s*), 169.8 (*s*) and 170.1 (*s*). The ^1H signals at δ 5.52 (1H, *dd*), 5.90 (1H, *d*), and 6.29 (1H, *d*) attached to carbons bearing these acetoxy groups were assigned, respectively, to 7α-H, 9β-H and 10α-H with the aid of its HMBC spectrum (Table 1). The signal due to 5-H at δ 5.43 (see above) showed an HMBC correlation with one of the acetoxylated carbons at δ_C 69.8 and this was assigned to C-7. This signal also showed an HMQC correlation with δ_C at 5.52 which appeared as a *dd* (J = 11.5 and 5.2 Hz) characteristic of 7α-H of taxoids [1]. The ^1H NMR signal at δ 5.90 (*d*, J = 10.8 Hz) showed cross-peaks with ^{13}C signals at δ 69.8 (C-7), 72.9 and 170.1 ppm. Therefore the signal at δ_H 5.90 was assigned to 9-H and the one at δ_C 72.9 to C-10. The allylic proton at C-10 was located at δ 6.29 with the help of ^1H-^1H DQCOSY and HMQC correlations. This proton showed a number of cross-peaks in the HMBC spectrum especially to C-9 (δ_C 75.7; assigned using the HMQC correlation with the signal at δ_H 5.90), C-11 (δ_C 152.2) and C-12 (δ_C 138.2). Chemical shifts of C-11 and C-12 suggested the presence of a carbonyl groups at C-13. A weak four-bond HMBC correlation was observed between 10-H (δ 6.29) and C-1 (δ 40.9). The ^{13}C signal at δ 40.9 (C-1) showed a cross-peak with signal at δ_H 2.21 (*m*) in its HMQC spectrum and the latter signal showed coupling with the signal at δ_C 2.92 (*dd*, J = 19.8 and 7.3 Hz) in its ^1H-^1H DQCOSY spectrum, which in turn was coupled to the signal at δ_H 1.94 (*br d*, J = 19.8 Hz). The presence of a carbonyl group at C-13 was further confirmed by the observation of an HMBC correlation between the signals at δ_H 1.94 (14-H) and δ_C 200.0. Based on above evidence the structure of compound **2** was elucidated as 7β,9α,10β-triacetoxy-5α-cinnamoyloxytaxa-4(20),11-dien-13-one or 2-deacetoxytaxinine B. The complete assignment of ^1H and ^{13}C NMR signals were made by the application of ^1H-^1H DQCOSY, HMQC, and HMBC techniques and comparing with data reported for related taxoids [6, 7]. These assignments are given in Table 1.

Compound **3**, obtained as a yellow solid had in its EI-mass spectrum a $[M]^+$ at *m/z* 502. Based on its mass spectrum, ^1H and ^{13}C NMR spectroscopic data, the molecular formula was determined to be $C_{30}H_{30}O_7$. Its ^1H and ^{13}C NMR spectral data recorded in $CDCl_3$ showed a close resemblance to those reported for brevitaxin [9]. However, a direct comparison was not possible as these data for brevitaxin have been reported in DMSO-d_6. Thus in order to confirm its identity the NMR spectroscopic data for compound **3** was obtained in DMSO-d_6 and were found to be identical with those reported for brevitaxin [9]. Our ^1H and ^{13}C NMR data for **3** in $CDCl_3$ are listed in Table 2, the assignments of which have been made with the aid of DEPT and 2D NMR (DQCOSY, HMQC, and HMBC) techniques.

EXPERIMENTAL

General. Mps are uncorr. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Nicolet-170 SX spectrometer as KBr pellets and UV spectra on a Perkin–Elmer Lambda 2 UV/VIS spectrometer in MeOH. MS spectra were obtained on a MAT 711 mass spectrometer using EI or FAB modes. NMR spectra were recorded using a Varian Unity 400 spectrometer in $CDCl_3$ or DMSO-d_6 at ambient temp.; solvent resonances were used as references.

Plant material. The stem bark of *Taxus chinensis* was collected in June 1995 by Prof. Chen Sai-Zhi from Hupei Province, Peoples Republic of China, and a voucher specimen has been deposited in the Department of Phytochemistry, China Pharmaceutical University.

Extraction and isolation. Air-dried and powdered

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **2** in $CDCl_3$

Position	^{1}H	^{1}H-^{1}H DQCOSY	HMBC	^{13}C*
1	2.21 *m*	H-14, H-2		40.9 (*d*)
2	1.88 *m*	H-1		25.7 (*t*)
	2.04 *m*†	H-3		
3	3.04 *d* (4.9)	H-2		36.9 (*d*)
4				146.9 (*s*)
5	5.43 *t* (3.2, 2.4)	H-6	69.8, 166.1	74.4 (*d*)
6	1.77 *m*	H-5, H-7		34.0 (*t*)
	2.04 *m*†			
7	5.52 *dd* (11.5, 5.2)	H-6	169.8	69.8 (*d*)
8				46.6 (*s*)
9	5.90 *d* (10.8)	H-10	46.6, 69.8, 72.9, 170.1	75.7 (*d*)
10	6.29 *d* (10.8)	H-9	40.9, 75.7, 138.2, 152.2, 169.1	72.9 (*d*)
11				152.2 (*s*)
12				138.2 (*s*)
13				200.0 (*s*)
14	1.94 *br d* (19.8)		25.6, 25.7, 200.0	39.5 (*t*)
	2.92 *dd* (19.8, 7.3)	H-1		
15				39.8 (*s*)
16	1.61 *s*			25.6 (*q*)
17	1.11 *s*			37.1 (*q*)
18	2.37 *s*			14.1 (*q*)
19	0.86 *s*			12.8 (*q*)
20	4.93 *d* (1.4)		36.9, 74.4	114.6 (*t*)
	5.29 *br s*			
7-$\underline{C}OCH_3$				169.8 (*s*)
7-CO$\underline{CH_3}$	2.01‡			20.8 (*q*)‡
9-$\underline{C}OCH_3$				170.1 (*s*)
9-CO$\underline{CH_3}$	2.04‡			20.9 (*q*)‡
10-$\underline{C}OCH_3$				169.1 (*s*)
10CO$\underline{CH_3}$	2.05‡			21.4 (*q*)‡
Cinn.				
1′				166.1 (*s*)
2′	6.36 *d* (16.0)	H-3′	134.5, 166.1	145.9 (*d*)
3′	7.61 *d* (16.0)	H-2′	166.1	117.5 (*d*)
4′				134.5 (*s*)
5′, 9′	7.71 *dd* (8.2, 1.9)		128.5, 130.4	128.5 (*d*)
6′, 8′	7.39 *m*		128.9	128.9 (*d*)
7′	7.39 *m*		128.5	130.4 (*d*)

* Type of carbon by DEPT experiment; *s* = C, *d* = CH, *t* = CH_2, *q* = CH_3.
† Overlapping multiplets.
‡ Those in the same column may be interchanged.

stem bark (12.8 kg) of *T. chinensis* was extracted with EtOH. The EtOH extract was evapd *in vacuo* and the resulting residue was suspended in H_2O and extracted with CH_2Cl_2 yielding on evapn a brown residue (287 g). This was then adsorbed on to silica gel (300 g; 200–400 mesh) and extracted sequentially and exhaustively with petrol, CH_2Cl_2, EtOAc, and MeOH in a Soxhlet extractor. The CH_2Cl_2 extract (146.1 g) thus obtained was subjected to repeated CC on silica gel eluting with various mixts of petrol–CH_2Cl_2 of increasing polarity to afford 2-deacetoxytaxinine B (**2**) (25 mg, 0.0002%), brevitaxin (**3**) (12 mg, 0.00009%) [9], taxol (**1**) (120 mg, 0.0009%) [1], 10-deacetyltaxol (12 mg, 0.00009%) [10], 1-deoxybaccatin VI (30 mg, 0.0002%) [11], 2-deacetyltaxinine J (400 mg, 0.003%) [12], taxinine J (8 mg, 0.00006%) [13], and 2-deacetyl-5-decinnamoyltaxinine J (24 mg, 0.0002%) [14].

2-Deoxytaxinine B (**2**). Colourless powder, nmp 284–286° (MeOH); $[\alpha]_D^{20}$ 137.1° (*c*, 0.03, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1740, 1710, 1695, 1670, 1640, 1250, and 1230; ^{1}H and ^{13}C NMR, see Table 1; HREIMS: *m/z* (rel. int.) $[MH]^+$ 607.2916 (65%) [$C_{35}H_{43}O_9$ requires 607.2907], 547 (25) $[MH\text{-}HOAc]^+$, 459 (48) $[MH\text{-}PhCH{=}CHCO_2H]^+$, 399 (9) $[MH\text{-}PhCH{=}CHCO_2H\text{-}HOAc]^+$, and 131 (100) $[PhCH{=}CHCO]^+$.

Brevitaxin (**3**). Pale yellow solid, mp 252–254°; lit. [9] 280°; $[\alpha]_D^{20}$ 0° (*c*, 1.0, CH_2Cl_2); IR ν_{max}^{KBr} cm^{-1}: 1670, 1620, 1570, 1520, and 1470; UV λ_{max}^{MeOH} nm 317 (log ε 19,400) 380 sh; ^{1}H and ^{13}C NMR, see Table 2; EIMS: (rel. int.) 502 (<0.1) $[M]^+$, 342 (3), 300 (57), 254 (30), 253 (58), 131 (84), 106 (77), 105 (69), 91 (44), and 43 (100).

Acknowledgements—The authors thank Prof. Chen

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **3**

Position	^{1}H*	^{1}†	HMBC*	^{13}C*,‡
1				188.0 (*s*)
2				133.7 (*s*)
3				144.2 (*s*)
4	7.95 *s*	8.17 *s*	133.7, 140.6, 147.8, 150.6	132.8 (*d*)
5				130.7 (*s*)
6	7.26 *d* (9.8)	7.63 (10.00)	132.8, 150.6, 200.9	147.8 (*d*)
7	6.08 (9.9)	6.13 *d* (9.4)	130.7	123.9 (*d*)
8				200.9 (*s*)
9				50.5 (*s*)
10				150.6 (*s*)
11	6.94 *s*	6.82 *s*	50.5, 130.7, 133.7	131.7 (*d*)
12	1.46 *s*	1.38 *s*	150.6, 200.9	28.0 (*q*)
13	1.42 *s*	1.34 *s*	150.6, 200.9	25.5 (*q*)
14				140.6 (*s*)
15				142.2 (*s*)
16				122.5 (*s*)
17	7.79 *s*	7.60 *s*	122.5, 144.2, 188.0	120.0 (*d*)
18	3.35 *m*	3.26 *m*		27.7 (*d*)
19	1.28 *d* (7.2)	1.24 *d* (3.7)	142.2	22.0 (*q*)
20	1.26 *d* (7.7)	1.23 *d* (3.2)	142.2	22.3 (*q*)
1′				127.4 (*s*)
2′	6.99 *br s*	7.08 *d* (1.7)		109.4 (*d*)
3′				146.9 (*s*)
4′				146.5 (*s*)
5′	6.95 *s*	6.83 *d* (8.3)	120.6, 127.4, 146.5	114.8 (*d*)
6′	6.99 *br s*	6.91 *dd* (8.3, 1.7)		120.6 (*d*)
7′	5.11 *d* (8.2)	5.08 *d* (8.0)	109.4, 120.6, 127.4	76.0 (*d*)
8′	4.07 *m*	4.32 *m*		78.7 (*d*)
9′	3.68 *m*	3.51 *m*		61.5 (*t*)
	4.04 *m*	3.62 *m*		
OMe	3.92 *s*	3.76 *s*	146.9	56.0 (*q*)
4′-OH	5.72 *s*	9.25 *s*	114.8, 146.5	
9′-OH	1.99 *br s*	5.19 *t* (5.8)		

* Measured in $CDCl_3$.
† Measured in DMSO-d_6.
‡ Type of carbon by DEPT experiment; s = C, d = CH, t = CH_2, q = CH_3.

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