PERGAMON

Phytochemistry 52 (1999) 1565–1569

PHYTOCHEMISTRY

Taxane diterpenoids from the seeds of Chinese yew *Taxus chinensis*

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Received 14 September 1998; received in revised form 14 April 1999

Abstract

The taxoid chinentaxunine has been isolated from the seeds of Chinese yew *Taxus chinensis*, and its structure determined on the basis of spectral and chemical methods. In addition, the known taxol C, paclitaxel, 10-deacetyl taxol A, 10-deacetyl-7-*epi*-taxol, 10-deacetyl-10-oxo-7-*epi*-taxol, taxinine M, taxchinin A, 10-deacetyl taxinine B and taxuspine X were also isolated and identified from this source.

Keywords: Taxus chinensis; Taxaceae; Taxane; Diterpenoids; Chinentaxunine

1. Introduction

Paclitaxel, a diterpenoid, has shown promise as an anticancer agent in treatment of human ovarian, breast and lung cancers. However, its development as a drug has been hampered by its limited supply and low water solubility. To solve these problems, the search for practical and renewable source of paclitaxel from various parts of *Taxus* species is under way. Also, it is very important to isolate paclitaxel's precursors for semisynthesis and further biological evaluation. Additionally, the isolation of taxane diterpenoids with a modified or novel skeleton is of significance because very little is known about the biosynthetic pathway of taxoids. More than 250 new taxoids have been isolated in the past decade (Kingston, Molinero & Rimoldi, 1993; Appendino, 1995). In our continuing search for new taxoids (Shen, Chen & Kuo, 1998), we reported previously the isolation, from the seeds of *Taxus chinensis* (Pilg.) Rehd. two new taxoids 2α-acetoxy-2′,7-dideacetoxyaustrospicatine and decinnamoyltaxinine E together with taxachitriene A (Fang, Fang, Liang, Yu & Zheng, 1995; Fang, Fang & Liang, 1996) and related known taxoids (Chen, Chen & Shen, 1999). Further investigation has led to the isolation of nine other taxoids, among which was one new derivative, an 11(1 → 15) *abeo-taxane* named chinentaxunine (**1**), as well as taxuspine X (**4**), taxinine M, taxchinin A, 10-deacetyl-10-oxo-7-*epi*-taxol, taxol C and paclitaxel, 10-deacetyl taxol A, 10-deacetyl-7-*epi*-taxol and 10-deacetyl taxinine B. In this paper, the isolation and the structure elucidation of chinentaxunine from *T. chinensis* are described.

2. Results and discussion

The EtOH extract of the seeds of Chinese yew, *Taxus chinensis*, was partitioned between *n*-hexane and 25% aq. MeOH to give *n*-hexane soluble and 25% aq. MeOH soluble portions. The 25% aq MeOH soluble portion was subjected to sequential Sephadex LH-20 and silica gel chromatographies as well as reversed-phase HPLC to furnish chinentaxunine (**1**, 0.0014%) together with taxuspine X (**4**, 0.0007%) (Shigemori, Wang, Yoshida & Kobayashi, 1997), taxinine M (0.0002%) (Beutler, Chmurny, Look & Witherup, 1991), taxchinin A (0.0003%) (Fuji, Tanaka, Li, Shingu, Sun & Taga, 1993), 10-deacetyl-10- oxo-7-*epi*-

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00310-6

Table 1
^{1}H- and ^{13}C-NMR data for chinentaxunine (**1**)

Atom number	**1**		^{1}H–^{1}H COSY	HMBC
	δ C[b]	δ H[a]		
1	61.7(*s*)			
2	29.2(*t*)	1.38 (*m*)	H-3	C-1, C-8, C-11
		2.10 (*m*)		
3	40.8(*d*)	2.79 (brd, 7.8)	H-2	C-1, 2, 4, 8, 19, 20
4	147.3(*s*)			
5	75.1(*d*)	5.49 (brs)		C-3, C-6, C-20
6	27.7(*t*)	1.70 (*m*)		
		2.04 (*m*)		
7	29.7(*t*)	1.28 (*m*)		
8	41.6(*s*)			
9	80.9(*d*)	5.61 (*d*, 9.9)	H-10	C-7, C-10, C-11, 9-OAc
10	67.6(*d*)	4.52 (*d*, 9.9)	H-9	C-1, C-9, C-11
11	140.7(*s*)			
12	141.9(*s*)			
13	80.2(*d*)	5.43 (*dd*, 6.6, 13.8)	H-14	C-11, C-12
14	44.2(*t*)	1.14 (*m*), 2.39 (*m*)	H-13	C-11, C-12
15	76.8(*s*)			
16	25.8(*q*)	1.38 (*s*)		C-1, C-15, C-17
17	27.9(*q*)	1.16 (*s*)		C-1, C-15, C-16
18	11.2(*q*)	1.92 (*s*)		C-11, C-12
19	17.1(*q*)	0.80 (*s*)		C-8, C-9
20	112.2(*t*)	4.80 (brs)	H-20[b]	C-3, C-5
		5.24 (brs)	H-20[a]	
1′	165.7(*s*)			
2′	118.7(*d*)	6.41 (*d*, 16.0)		
3′	144.4(*d*)	7.66 (*d*, 16.0)		
1″	134.5(*s*)			
2″, 6″	127.9(*d*)	7.49 (*m*)		
3″, 5″	128.9(*d*)	7.38 (*m*)		
4″	130.5(*d*)	7.38 (*m*)		
9-OAc	171.1(*s*)			
	21.2(*q*)	2.13 (*s*)		
13-OAc	171.6(*s*)			
	20.5(*q*)	1.51 (*s*)		

[a] 300 MHz in $CDCl_3$, *J* value in Hz.
[b] 100 MHz in $CDCl_3$.

taxol (0.0003%) (Huang, Kingston, Magri & Samaranayake, 1986), taxol C (0.0002%) (Ma et al., 1994), paclitaxel (0.006%) (Senilh et al., 1984) and 10-deacetyl taxol A (0.0002%) (McLanghlin, Miller, Powell & Smith, 1981; Shen, Tai, Hsieh & Chen, 1996), 10-deacetyl-7-*epi*-taxol (0.0003%) (McLanghlin et al., 1981), and 10-deacetyl taxinine B (0.0004%) (Tong, Fang, Zhou, He, Chen & Fang, 1994). The structures of known compounds were identified on the basis of spectral analysis and comparison with authentic samples. Cinnamoylation of taxachitriene A yielded 5α-cinnamoyl-taxachitriene A (**4**) (Shigemori et al., 1997). The structure of compound **1** was elucidated as follows.

Chinentaxunine (**1**), $[\alpha]_D^{28}$ +43° ($CHCl_3$), had a molecular formula of $C_{33}H_{42}O_8$ as derived from its quasi-molecular ions in the FAB mass spectrum at *m*/*z* 589 (M + Na) and at *m*/*z* 567 (M + H), and DEPT spectra. The UV absorption and IR bands indicated the presence of hydroxyl (3525 cm^{-1}), ester (1737 cm^{-1}), and cinnamoyl (216, 276 nm) groups. The latter was verified by a base peak at *m*/*z* 131 (C_9H_7O) in the EI mass spectrum and the observation of ^{1}H-NMR signals at δ 7.66 (1H, *d*, *J* = 16 Hz), 6.41 (1H, *d*, *J* = 16 Hz), 7.38 (3H, *m*) and 7.49 (2H, *m*). The ^{1}H-NMR spectrum of **1** (Table 1) also showed two acetyl singlets (δ 1.51, 2.13) and four typical methyl singlets (δ 0.80, 1.16, 1.38, 1.92). The signals at δ 4.80 (*brs*) and 5.24 (*brs*) suggested the presence of an exomethylene moiety. The ^{1}H– and ^{13}C-NMR spectral data of **1** were assigned on the basis of 2D-NMR analysis. Detailed analysis of the COSY spectrum of **1** revealed connectivities of H-9/H-10, H-13/H-14, and H-2/H-3. An 11(1 → 15) *abeo-taxane* skeleton bearing a dimethyl carbinol group in C-1 was deduced from the observation of adjacent quaternary sp^3 carbons at δ 61.7 (C-1) and δ

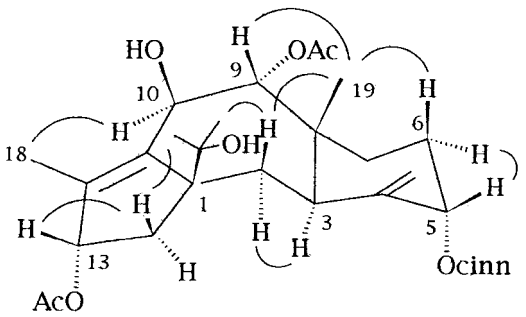
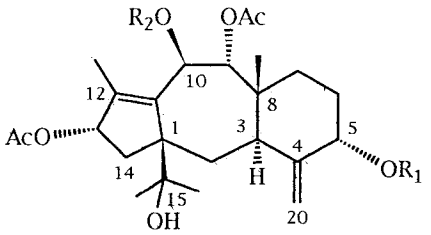
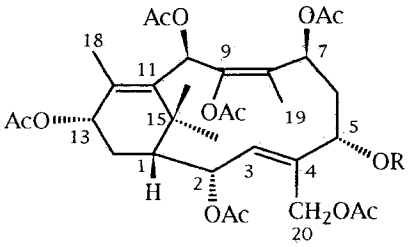


Fig. 1. Selected NOESY correlation of chinentaxunine (**1**).



1 R_1 = COCH=CHC_6H_5 , R_2 = H
2 R_1 = COCH=CHC_6H_5 , R_2 = Ac
3 R_1 = COCH=CHC_6H_5 , R_2 = COC_6H_5



4 R = COCH=CHC_6H_5

76.8 (C-15), and cross-peaks from Me-16 and Me-17 to C-1 and C-15 as well as correlations of geminal dimethyl (16/17) in the HMBC spectrum. Additional HMBC data such as H-2/C-1, C-8, C-11, and H-3/C-1, C-2, C-4, C-8 and H-10/C-1, C-9, C-11 (Table 1) fully supported the structure of **1**, having a rearranged 5/7/6- membered ring system. The location of acetoxyls were assigned at C-9 and C-13 due to HMBC correlation of carbonyl signal (δ 171.1) with H-9 (δ 5.61), and correlation between H-13 (δ 5.43) and C-11 (δ 140.7) and C-12 (δ 141.9). The hydroxyl group was determined at C-10 by strong HMBC correlation of H-10 (δ 4.52) to C-1 (δ 61.7) and C-11. Upon acetylation compound **1** yielded **2**, which showed a single additional acetyl singlet at δ 2.01 in the ^{1}H-NMR spectrum. Also, the chemical shift of H-10 was shifted from 4.52 ppm in **1** to 6.22 ppm in **2**. Benzoylation of **1** gave a monobenzoate (**3**). The cinnamoyl group was placed at C-5 although there was lack of correlation from H-5 (δ 5.49) to the cinnamoyl carbonyl signal (δ 165.7). Relative stereochemistry of **1** was determined from chemical shifts, coupling constants and NOESY data. The NOESY correlations of H-13/H-14β, H- 13/Me-17, H-14β/Me-16, 17 and H10/Me-18 in **1** suggested that H-13 and the dimethyl carbinol group were in β-orientation. Correlation between H-9 and Me-19 agreed with β-configuration of H-9 and Me-19. A coupling constant between H-9 and H-10 of 9.9 Hz indicated that the B ring was in the chair-boat conformation. NOESY correlations from H-5 to H-6α and H-6β to Me-19 were consistent with the proposed relative stereochemistry at C-5. Thus, the relative stereochemistry of **1** is the same as taxchinin A (Hosoyama, Inubushi, Katsui, Shigemori & Kobayashi, 1996), taxuspine A (Kobayashi et al., 1994) and other 11(1 → 15) *abeo-taxanes* (Appendino, 1995; Rao & Juchum, 1998). A possible conformation consistent with the results from NOESY experiments is given in Fig. 1.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR and UV spectra were recorded with a HORIBA FT-720 and a HITACHI U-3210 spectrophotometer, respectively. Mass spectra were measured with a VG Quattro 5022 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded using Varian FT-300 or Bruker AM-400 NMR instruments.

3.2. Plant material

The seeds of *Taxus chinensis* were commercially available (King of Tree) and were identified by one of the authors (Y-C Shen). A voucher specimen of seeds was kept in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

3.3. Extraction and isolation

Dried and ground seeds (3.2 kg) were extracted with EtOH to afford a crude extract, which was defatted with *n*-hexane (600 ml) and 25% aq. MeOH (600 ml) twice to yield a 25% aq. MeOH-soluble residue (32 g). A part of the residue (16 g) was first applied to a Sephadex LH-20 column and eluted with MeOH to

afford three fractions, fr. A (1.17 g), B (12.93 g), C (1.01 g). Fr. B (12 g) was chromatographed on a silica gel column eluted with *n*-hexane-$CHCl_3$-MeOH (30:30:1, 10:10:1, 3:3:1, 1:1:1) to give four taxoid-containing fractions, B-1 (62 mg), B-2 (1.9 g), B-3 (0.57 g), and B-4 (0.9 g). B-2 was rechromatographed by silica gel column and eluted with $CHCl_3$-EtOAc of increasing polarity to give nine fractions, B-2-1 (13 mg), B-2-2 (47 mg), B-2-3 (111 mg), B-2-4 (178 mg), B-2-5 (430 mg), B-2-6 (322 mg), B-2-7 (233 mg), B-2-8 (111 mg), and B-2-9 (335 mg). B-2-6 (322 mg) was further purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, 75% aq MeOH) to give taxuspine X (**4**, 10 mg), decinnamoyltaxinine E (44 mg) and taxezopidine E (91 mg). B-2-9 (200 mg) was purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, CH_3CN: MeOH: H_2O, 2:2:1) to give chinentaxunine (**1**, 20 mg) and taxachitriene A (62 mg). The MeOH-insoluble part of Fr. B-2-7 (27 mg) was purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, 80% aq. MeOH) to give 10-deacetyl taxinine B (7 mg) and taxsuspine X (**4**, 10 mg). Fr. B-3 and Fr. B-4 were combined and rechromatographed together by silica gel column eluted with *n*-hexane-$CHCl_3$-MeOH (5:5:1) to give three taxoid-containing fractions, B-3-1 (95 mg), B-3-2 (62 mg), and B-3-3 (490 mg). B-3-2 (50 mg) was purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, 55% aq CH_3CN) to give 10-deacetyl-10-oxo-7-*epi*-taxol (5 mg). B-3-3 was rechromatographed by silica gel column eluted with *n*-hexane-acetone (4:1, 3:1, 2:1, 1:1) to give paclitaxel (100 mg) and three taxoid-containing fractions, B-3-3-1 (62 mg), B-3-3-2 (104 mg), and B-3-3-3 (70 mg). B-3-3-1 (30 mg) was purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, 50% aq CH_3CN) to give taxol C (4 mg) and taxinine M (3 mg). B-3-3-3 (70 mg) was purified by ODS HPLC (UV: 220 nm, LiChrosorb RP-18 column, 50% aq CH_3CN) to give taxchinin A (5 mg), 10-deacetyl taxol A (3 mg) and 10-deacetyl-7-*epi*-taxol (5 mg).

3.3.1. Chinentaxunine (*1*)

Amorphous solid, $[\alpha]_D^{28}$ +43° ($CHCl_3$, c 0.49), FABMS *m/z*: 589 $[M+Na]^+$, 567 $[M+H]^+$; UV λ_{max} (MeOH) nm: 277, 222; IR ν_{max} (KBr) cm^{-1}: 3525, 2948, 1737, 1238; ^{1}H- and ^{13}C-NMR: Table 1. EIMS *m/z* (rel. int.): 566 (0.1, M^+), 549 (0.2, $[M–OH]^+$), 523 (0.2, $[M–Ac]^+$), 448 (1), 430 (2), 388 (7), 370 (5), 240 (29), 222 (18), 211 (17), 197 (13), 147 (22), 131 (100), 105 (36), 103 (47), 91 (34), 77 (26), 59 (78), 43 (92).

3.3.2. Acetylation of chinentaxunine (*1*)

Acetylation (Ac_2O: Pyr; 1:1; room temperature) of **1** (2 mg) gave a solid (2 mg) after work-up: IR ν_{max} (KBr) cm^{-1}: 3397, 2923, 2856, 1732, 1660, 1628, 1594, 1238, 1025; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.77 (1H, *d*, *J* = 8.4 Hz, H-3), 5.50 (1H, *brs*, H-5), 5.85 (1H, *d*, *J* = 10.2 Hz, H-9), 6.22 (1H, *d*, *J* = 10.2 Hz, H-10), 5.42 (1H, *brt*, *J* = 5.4 Hz, H-13), 2.49 (1H, *dd*, *J* = 13.8, 7.2 Hz, H-14β), 1.32 (3H, *s*, H-16), 1.15 (3H, *s*, H-17), 1.89 (3H, *s*, H-18), 0.82 (3H, *s*, H-19), 4.80 (1H, *brs*, H-20a), 5.26 (1H, *brs*, H-20b), 7.65 (1H, *d*, *J* = 16.2 Hz, H-3′), 7.50 (2H, *m*, H-2″, 6″), 7.40 (3H, *m*, H-3″, 4″, 5″), 6.39 (1H, *d*, *J* = 16.2 Hz, H-2′), 2.04, 2.01, 1.52 (3H × 3, *s*, $OCOCH_3$).

3.3.3. Benzoylation of chinentaxunine (*1*)

A solution of chinentaxunine (**1**, 4 mg) in dry pyridine 0.2 ml was treated with benzoyl chloride (0.3 ml) and stirred for 12 h at room temperature. After work-up as above the residue was purified by a Sephadex LH-20 column using MeOH as eluent to give 10- benzoylchinentaxunine (**3**, 2 mg) as a white powder: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.84 (1H, *d*, *J* = 8.4 Hz, H-3), 5.52 (1H, *brs*, H-5), 6.06 (1H, *d*, *J* = 10.5 Hz, H-9), 6.49 (1H, *d*, *J* = 10.5 Hz, H-10), 5.41 (1H, *brt*, *J* = 7.2 Hz, H-13), 2.52 (1H, *dd*, *J* = 13.8, 7.2 Hz, H-14β), 1.26 (3H, *s*, H-16), 1.16 (3H, *s*, H-17), 1.38 (3H, *s*, H-18), 0.86 (3H, *s*, H-19), 4.86 (1H, *brs*, H-20a), 5.29 (1H, *brs*, H-20b), 6.43 (1H, *d*, *J* = 16.1 Hz, H-2′), 7.68 (1H, *d*, *J* = 16.1 Hz, H-3′), 7.38–7.62 (6H, *m*), 7.89 (2H, *d*, *J* = 8.0 Hz), 8.10 (2H, *d*, *J* = 7.8 Hz) 1.80, 1.53 (3H × 2, *s*, $OCOCH_3$).

3.3.4. Cinnamoylation of taxachitriene A

A solution of taxachitriene A (5.8 mg) in pyridine (0.1 ml) was treated with cinnamoyl chloride (0.3 ml). The reaction mixture was heated in an oil bath (50°C) and stirred for 12 h. After treatment as above, the residue was purified by a Sephadex LH-20 column (MeOH) to yield a product (3.2 mg), which showed identical ^{1}H-NMR, [α], MS data with those of taxuspine X (**4**) Shigemori et al., 1997.

Acknowledgements

The authors thank the National Science Council, Republic of China (NSC 88-2314-B110-006) and the National Institute of Health, Republic of China (DOH 88-HR-809) for financial support.

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