New Taxoids from the Seeds of *Taxus chinensis*

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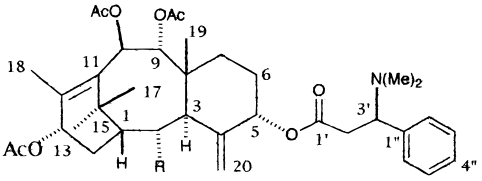
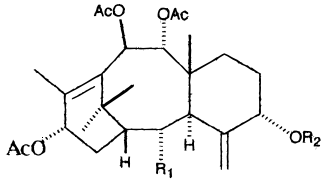
Two new taxoids, 2α-acetoxy-2′,7-dideacetoxyaustrospicatine (**1**) and decinnamoyltaxinine E (**2**), have been isolated from the extracts of the seeds of the Chinese yew *Taxus chinensis* (Pilg.) Rehd. in addition to taxuspine Z (**3**), taxin B (**4**), taxezopidine G (**5**), 7,2′-bisdeacetoxyaustropicatine (**6**), 5α-cinnamoyl-9α,10*β*,13α-triacetoxytaxa-4(20),11-diene (**7**), taxachitriene A, 20-deacetyltaxachitriene A, 13-*O*-acetylbrevifoliol, taxinines A and J, and taxacin. The structures of these compounds were elucidated on the basis of spectral analysis and chemical derivatizations.

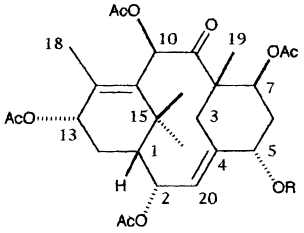
The clinical application of paclitaxel against breast and ovarian cancers has spurred a worldwide search for a new source of this drug. Chemical modifications of paclitaxel for better activity and determination of structure−activity relationships (SAR) have been extensively investigated during the past decade.[1−3] Although many taxoids have been isolated to date, new taxoids continue to be discovered, and some of these could become precursors of paclitaxel and its analogues.[4,5] Previously, we isolated new taxumairols A−F and K from the roots of *Taxus mairei* (Lemee & Levl.) S. Y. Hu (Taxaceae).[6−9] In our continuing search for biorenewable sources of taxoids and for the study of their SAR, a phytochemical study of the seeds of *T. chinenesis* (Pilg.) Rehd. was carried out. As far as we know, very few studies on the constituents of the seeds were reported.[10,11] The seeds of the Chinese yew *Taxus chinensis* are used in Chinese folk medicine as an anthelmintic. We report herein the isolation and structural determination of two new taxoids together with 11 known compounds from the seeds of *T. chinensis*.



1 R = OAc
3 R = OH
6 R = H



2 R_1 = OAc, R_2 = H
5 R_1 = OH, R_2 = COCH=CHC$_6$H$_5$
7 R_1 = H, R_2 = COCH=CHC$_6$H$_5$
8 R_1 = OAc, R_2 = Ac



4 R = H

The EtOH extract of the seeds of *T. chinensis* was partitioned between *n*-hexane and 25% aqueous MeOH to give *n*-hexane-soluble and 25% aqueous MeOH-soluble portions (32 g). Extensive chromatography of the 25% aqueous MeOH-soluble portion (16 g) by a combination of Sephadex LH-20, silica gel, and reversed-phase C_{18} chromatography gave 2α-acetoxy-2′,7-dideacetoxyaustrospicatine (**1**, 0.0013%) and decinnamoyltaxinine E (**2**, 0.0028%), together with the known taxane diterpenes, taxuspine Z (**3**, 0.0014%),[12] taxin B (**4**, 0.00063%),[13] taxezopidine G (**5**, 0.0056%),[14] 7,2′-bisdeacetoxyaustropicatine (**6**, 0.00063%),[15] 5α-cinnamoyl-9α,10*β*,13α-triacetoxytaxa-4(20),11-diene (**7**, 0.0053%),[16] taxachitriene A (0.0039%),[17] 20-deacetyltaxachitriene A (0.00044%),[17,18] 13-*O*-acetylbrevifoliol (0.00038%),[10,19] taxinines A (0.0026%),[20] J (0.01%),[21] and taxacin (0.0007%).[22,23] The structures of the known taxoids were confirmed by comparison of their spectroscopic data (FABMS, ^{1}H and ^{13}C NMR) with literature data.

Compound **1**, an isomer of 2′-deacetoxyaustrospicatine,[5] was isolated as a white solid, $[\alpha]_D$ +33.7°, and a composition of $C_{39}H_{53}O_{10}N$ for it was deduced by a combination of low-resolution EIMS and DEPT spectroscopy. Its UV and IR spectra were similar to those of **3**. The ^{1}H and ^{13}C NMR spectra (Table 1) of **1**, **3**, and **6** were similar, suggesting compound **1** belonged to the austrospicatine class of taxoids.[15,24−27] The primary difference between these compounds was in the number of and placement of hydroxy and acetoxy groups. The presence of an exomethylene (*δ* 5.22 brs, 4.78 brs), four methyl (*δ* 0.80 s, 1.14 s, 1.73 s, 2.14 s), and the 3′-(dimethylamino)-3′-phenylpropionyl (*δ* 2.84, dd, 9.3, 13.5 Hz; *δ* 3.03, dd, 6.3, 13.5 Hz; *δ* 3.73, dd, 6.3, 9.3 Hz; *δ* 2.19 s, *δ* 7.30 m) moieties in the ^{1}H NMR spectrum suggested that **1** was an analogue of **3**. The Winterstein acid side chain was further confirmed by the fragment ions in the EIMS at *m/z* 134 (PhCH=NMe$_2$) and *m/z* 192 ($C_{11}H_{14}NO_2$). A comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those of **3** revealed that compound **1** had four acetyl (*δ* 2.00, 2.01, 2.04 and *δ* 2.09) groups. In addition, the signal of methine H-2 (*δ* 4.16) in **3** was downshifted to *δ* 5.42 in **1**, suggesting that the additional acetate was at C-2. This was verified by a COSY spectrum of **1**, which clearly indicated the correlations between C-2 methine proton (*δ* 5.42, brd, J = 6.3 Hz) and H-3 at *δ* 3.09. The structure and stereochemistry of **1**, including the stereochemistry of the side chain, were confirmed by acetylation of the known taxuspine Z (**3**) to give a product identical with **1**.[12]

Decinnamoyltaxinine E (**2**) had the composition $C_{28}H_{40}O_9$, as derived from a molecular ion peak at *m/z* 520.2672 in its HREIMS spectrum. Its IR absorption indicated the presence of hydroxyl (3432 cm^{-1}) and acetyl (1739 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** showed the characteristic signals for a taxa-(20),11-diene with signals

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Table 1. ^{1}H and ^{13}C NMR Data for 2α-Acetoxy-2′,7-dideacetoxyaustrospicatine (**1**)

no.	δ_C[b]	δ_H[a]	COSY
1	48.4 (d)	1.90 (m)	H-2, H-14β
2	71.9 (d)	5.42 (brd, 6.3)	H-1, H-3, OH
3	44.0 (d)	3.09 (brd, 6.3)	H-2
4	141.9 (s)		
5	77.9 (d)	5.28 (brs)	H-6
6	28.1 (t)	1.06 (m), 1.40 (m)	H-5, H-7
7	27.0 (t)	1.50 (m)	H-6
8	44.0 (s)		
9	77.4 (d)	5.88 (d, 10.8)	H-10
10	72.2 (d)	5.98 (d, 10.8)	H-9
11	133.1 (s)		
12	137.2 (s)		
13	70.0 (d)	5.88 (dd, 8.1, 8.7)	H-14, OAc
14	28.2 (t)	1.40 (m)	H-13, 14β
		2.54 (ddd, 4.8, 9.3, 15.0)	H-1, 13, 14α
15	37.4 (s)		
16	31.3 (q)	1.14 (s)	
17	26.8 (q)	1.73 (s)	
18	15.3 (q)	2.14 (s)	H-13
19	17.8 (q)	0.80 (s)	
20	118.4 (t)	5.22 (brs)	
		4.78 (brs)	
Ac	170.6 (s)		
	170.1 (s)		
	170.0 (s)		
	169.5 (s)		
	31.3 (q)	2.09 (s)	
	21.0 (q)	2.04 (s)	
	20.8 (q)	2.01 (s)	
	20.7 (q)	2.00 (s)	
1′	171.0 (s)		
2′	40.2 (t)	3.03 (dd, 13.5, 6.3, a)	H-2′b, 3′
		2.84 (dd, 13.5, 9.3, b)	H-2′a, 3′
3′	67.9 (d)	3.73 (dd, 6.3, 9.3)	H-2′
1″	139.0 (s)		
2″	128.4 (d)	7.30 (m)	
3″	128.3 (d)	7.30 (m)	
4″	127.7 (d)	7.30 (m)	
5″	128.3 (d)	7.30 (m)	
6″	128.4 (d)	7.30 (m)	
$N(Me)_2$	42.8 (q)	2.19 (s)	

[a] 300 MHz in $CDCl_3$, *J* values in Hz. [b] 75.4 MHz in $CDCl_3$.

for the exocyclic methylene at δ 4.84 (brs) and δ 5.24 (brs) and carbon signals at δ 115.2 (t) and δ 147.0 (s). In addition, the presence of four methyl singlets (δ 0.86, 1.03, 1.71, and 2.15) and four acetyl groups was verified by the observation of ^{1}H and ^{13}C NMR spectral data (Table 2). The proton connectivities were established by the COSY spectrum. A methine proton at δ 5.46 was assigned to H-2 because it correlated with H-3 at δ 3.56 and H-1 at δ 1.83. A doublet of doublets at δ 5.75 (J = 4.8, 10.5 Hz), assigned to H-13, was coupled to H-14 at δ 2.64 and δ 1.52. The remaining signal at δ 4.24 (brs) was assigned to the C-5 proton due to its correlation with the methylene protons (H-6) at δ 1.60 and 1.80. Acetylation of **2** yielded a monoacetate, which was identical with the known 2α,5α,9α,10β,13α-pentaacetoxy-(4)20,11-taxadiene (**8**).[28] This reaction established the structure and stereochemistry of **2**.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR and UV spectra were recorded on HORIBA FT-720 and HITACHI V-3210 spectrophotometers, respectively. EI, FAB, and HREI mass spectra was measured with VG Quattro5022 and JEOL JMS-HX 110 mass spectrometers. ^{1}H and ^{13}C NMR and COSY spectra were recorded on a Varian FT-300 or a Bruker AMX 400 NMR spectrometer.

Table 2. ^{1}H and ^{13}C NMR Data for Decinnamoyltaxinine E (**2**)

no.	δ_C[b]	δ_H[a]	COSY
1	47.7 (d)	1.83 (m)	H-2, H-14β
2	72.7 (d)	5.46 (dd, 6.0, 7.5)	H-1, H-3
3	41.5 (d)	3.56 (d, 6.0)	H-2, H-20
4	147.0 (s)		
5	69.7 (d)	4.24 (brs)	H-6a, H-6b
6	30.2 (t)	1.60 (m), 1.80 (m)	H-5
7	26.4 (t)		
8	44.7 (s)		
9	76.4 (d)	6.07 (d, 10.5)	H-10
10	71.5 (d)	5.80 (d, 10.5)	H-9
11	134.2 (s)		
12	137.9 (s)		
13	77.2 (d)	5.75 (dd, 4.8, 10.5)	H-14, Me-18
14	28.6 (t)	1.52 (dd-like, 5.0, 15.6)	H-13, 14β
		2.64 (ddd, 7.8, 10.5, 15.6)	H-1, 13, 14α
15	37.1 (s)		
16	32.2 (q)	1.03 (s)	
17	25.8 (q)	0.86 (s)	
18	15.8 (q)	2.15 (s)	
19	17.3 (q)	1.71 (s)	
20	115.2 (t)	5.24 (brs)	
		4.84 (brs)	
Ac	170.6 (s)		
	170.1 (s)		
	170.0 (s)		
	169.4 (s)		
	20.7 (q)	2.01 (s)	
	21.0 (q) × 2	2.05 (s) × 2	
	21.4 (q)	2.11 (s)	

[a] 300 MHz in $CDCl_3$, *J* values in Hz. [b] 75.4 MHz in $CDCl_3$.

Plant Material. The seeds of *T. chinensis* were commercially available and were identified by one of the authors (Y.-C.S.). A voucher specimen of seeds was deposited in the Institute of Marine Resources, National Sun Yat-sen University.

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% aqueous MeOH (600 mL) two times to yield a 25% aqueous MeOH-soluble residue (32 g). A part of the residue (16 g) was applied to a Sephadex LH-20 column and eluted with MeOH to afford three fractions, fractions A (1.17 g), B (12.93 g), and C (1.01 g). Fraction A (1.15 g) was chromatographed on a Si gel column eluted with *n*-hexane−$CHCl_3$−MeOH (20:20:1, 10:10:1, 5:5:1, 1:1:1) to give three taxoid-containing fractions, fractions A-1 (173 mg), A-2 (205 mg), and A-3 (187 mg). Fraction A-1 (173 mg) was rechromatographed on a Si gel column ($CHCl_3$−EtOAc 10:1, 5:1) and by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 75% aqueous MeOH) to give a new taxoid, 2α-acetoxy-2′,7-dideacetoxyaustrospicatine (**1**, 23 mg) and known 7,2′-disdeacetoxyaustropicatine (**6**, 10 mg). Fraction A-2 (205 mg) was further purified on a Si gel column [$CHCl_3$−MeOH−H_2O 100:3:1 (lower)] and by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 70% aqueous MeOH) to give taxuspine Z (**3**, 21 mg) and taxachitriene A (62 mg). 20-Deacetyltaxachitriene A (7 mg) and 13-*O*-acetylbrevifoliol (6 mg) were isolated from fraction A-3 on a Si gel column [$CHCl_3$−MeOH−H_2O 100:3:1(lower)] and by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 70% aqueous MeOH).

Fraction B (12 g) was chromatographed on a Si 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 (1877 mg), B-3 (570 mg), and B-4 (934 mg). Fraction B-2 was rechromatographed on a Si gel column 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). Fraction B-2-2 was purified by HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 90% aqueous MeOH) to give 5α-cinnamoyl-9α,10β,13α-triacetoxytaxa-4(20),11-diene (**7**, 35 mg). Fraction B-2-4 (173 mg) was recrystallized with

MeOH to give taxinine J (120 mg). Fraction B-2-6 (322 mg) was further purified by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 75% aqueous MeOH) to give two taxoids, decinnamoyltaxinine E (**2**, 44 mg) and taxezopidine G (**5**, 91 mg). Fraction B-2-7 (233 mg) was dissolved in MeOH to give an amorphous solid (27 mg). The solid was further purified by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-C_{18} column, 80% aqueous MeOH) to yield taxin B (**4**, 10 mg). Fraction B-2-8 (111 mg) was rechromatographed on a Si gel column [$CHCl_3$−MeOH−H_2O 100:3:1 (lower)] to give fractions I (36 mg) and II (70 mg). Fraction II was further purified by reversed-phase HPLC (UV: 220 nm, LiChrosorb RP-18 column, 80% aqueous MeOH) to afford taxinine A (42 mg). Taxacin (14 mg) was obtained from recrystallization of the residue fraction I in MeOH.

2α-Acetoxy-2′,7-dideacetoxyaustrospicatine (1): white powder; $[\alpha]^{28}_D$ +33.7 (*c* 3.03, $CHCl_3$); UV (MeOH) λ_{max} 276, 216 nm; IR (KBr) ν_{max} 3525, 2948, 1737, 1238 cm^{-1}; HREI-MS *m*/*z* 695.3670, calcd for $C_{39}H_{53}O_{10}N$ 695.3670; EI-MS *m*/*z* 695 $[M]^+$ (0.1), 680 (0.2), 636 (0.3), 594 (0.1), 576 (0.1), 400 (0.2), 340 (0.4), 281 (0.8), 265 (0.8), 247 (1.3), 221 (1), 209 (2), 192 (0.3), 134 (100), 43 (83); ^1H and ^{13}C NMR, see Table 1.

Acetylation of Taxuspine Z (3). Acetylation (Ac_2O−pyridine 2:1; room temperature) of **3** (9 mg) gave, after workup, a solid (5 mg) that showed spectral data (^1H NMR, FABMS, [α], HPLC) identical with those of **1**.

Decinnamoyltaxinine E (2): white powder; $[\alpha]^{28}_D$ +33.8 (*c* 1.61, $CHCl_3$); UV (MeOH) λ_{max} 216 nm; IR (KBr) ν_{max} 3432, 1739, 1681, 1648, 1238 cm^{-1}; HREI-MS *m*/*z* 520.2672, calcd for $C_{28}H_{40}O_9$, 520.2671; EI-MS *m*/*z* 520 $[M]^+$(0.2), 502 (0.1), 461 (0.5), 443 (0.2), 401 (0.4), 400 (0.7), 358 (0.6), 341 (0.9), 340 (1.6), 298 (2.4), 280 (4), 265 (4), 247 (5), 237 (5), 209 (4), 195 (4), 149 (9), 145 (9), 135 (14), 121 (15), 105 (16), 91 (15), 79 (9), 55 (18), 43 (100); ^1H and ^{13}C NMR, see Table 2.

Acetylation of Decinnamoyltaxinine E (2). Acetylation (Ac_2O−pyridine 2:1; room temperature) of **2** (10 mg) gave a solid (8 mg) identical with 2α,5α,9α,10*β*,13α-pentaacetoxy-20(4),11-taxadiene[28] (^1H NMR, FABMS, EIMS, and [α]).

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