A New Taxoid, 19-Acetoxytaxagifine, from *Taxus chinensis*

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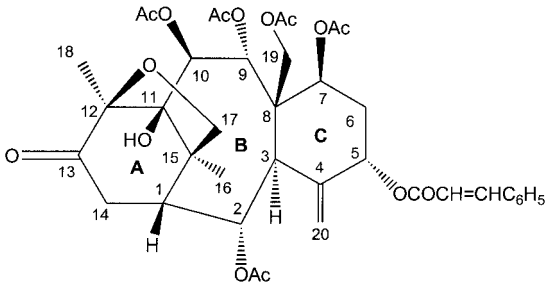
A new taxane diterpene, 19-acetoxytaxagifine (**1**), was isolated from an ethanol extract of the aerial parts of *Taxus chinensis*. Its structure was determined on the basis of spectral evidence.

Paclitaxel (Taxol), which was isolated from *Taxus brevifolia* by Wani et al. in 1971, has a unique mode of action and is used as an antitumor agent for treating breast and ovarian cancer.[1] Early supply problems prompted extensive investigation of the constituents of various *Taxus* species, and many taxane diterpenoids have been isolated.[2−10] Several of these reports concentrated on the taxoid constituents of *Taxus chinensis* (Pilg.) Rehd (taxaceae), especially on novel tricyclo 5/7/6-membered ring compounds.[3−6] In our continuing studies of new antitumor agents from higher plants, we have also investigated the taxane diterpene constituents of *T. chinensis* var. *mairei*. We previously reported the isolation of taxuchin A, a new 11(15→1)*abeo*-taxine type diterpene, from *Taxus chinensis*.[11] In this paper, we report the isolation of a new taxane diterpene, along with the known 2-deacetoxytaxinine J.[12] Structural elucidation established the new taxoid as structure **1**, named as 19-acetoxytaxagifine.



1: 19-Acetoxytaxigifine

Compound **1** was obtained as a colorless amorphous powder. Its IR spectrum showed the presence of hydroxyl (3420 cm^{-1}), ester (1740 cm^{-1}), carbonyl (1720 cm^{-1}), and α,β-unsaturated carbonyl (1665 cm^{-1}) groups. Its UV spectrum showed an absorption maximum at 274 nm due to a conjugated aromatic ring. Its molecular formula was established as $C_{39}H_{46}O_{15}$ from its HREIMS (*m*/*z* 754.2824), ^1H and ^{13}C NMR and DEPT spectra. Since its ^1H and ^{13}C NMR spectra did not coincide with those of any known taxoid compound, this compound was assumed to be a new taxane diterpenoid.

Assignment of H-5, H-7, H-9, H-10, and H-20′ was difficult due to the overlapping ^1H NMR signals found when measured in $CDCl_3$ (Table 1); thus, the structure of **1** was elucidated by analysis of 1D and 2D NMR spectra ($^1H-^1H$ COSY, $^1H-^{13}C$ COSY, HMBC, and NOESY) measured in C_5D_5N. The ^1H NMR spectrum of **1** disclosed the presence of two quaternary methyl groups [δ 1.99 (H-16) and 1.66 (H-18)], five acetyl methyl groups [δ 1.96 (2-OAc), 1.96 (7-OAc), 2.36 (9-OAc), 2.06 (10-OAc), and 2.19 (19-OAc)], two methylene groups connected to an oxygen atom [δ 3.94 (H-17), 4.40 (H-17′), 4.59 (H-19), and 4.70 (H-19′)], and a cinnamoyl group [δ 7.04 (H-2′), 8.33 (H-3′), 8.03 (H-5′), 7.38 (H-6′), and 7.33 (H-7′)]. The ^{13}C NMR and DEPT spectra of **1** indicated the presence of a ketone carbonyl [δ 204.9 (C-13)], six ester carbonyls [δ 169.9 (OAc-2), 168.8 (OAc-7), 169.7 (OAc-9), 170.5 (OAc-10), 170.5 (OAc-19), and 165.6 (C-1′)], four aromatic ring carbons [δ 136.0 (C-4′), 129.3 (C-5′), 129.2 (C-6′), and 130.6 (C-7′)], four olefinic carbons [δ 148.8 (C-4), 115.0 (C-20), 118.8 (C-2′), and 146.0 (C-3′)], two methylene carbons connected to an oxygen atom [δ 82.0 (C-17) and 61.8 (C-19)], and two methylene carbons [δ 36.6 (C-6) and 34.5 (C-14)].

The ^1H- and ^{13}C NMR spectra of **1** were quite similar to those of taxagifine,[7] taxacin (19-benzoxytaxagifine),[8] and 19-debenzoyl-19-acetyltaxinine M,[9] suggesting that all four compounds have the same basic skeleton. However, comparison of spectral data revealed the following differences. The ^1H NMR spectra of **1** lacked one methyl group found in taxagifine[7] but contained additional methylene and acetyl methyl groups. Furthermore, the H-2 and H-7 signals in **1** (δ 6.18 and 5.54) appeared at lower field by approximately 0.76 and 0.62 ppm, respectively. The ^1H NMR signals due to a benzoate group in taxacin were absent in **1**, and both H-19 (0.11 ppm, δ 4.34) and H-19′ (1.08 ppm, δ 4.44) were shifted upfield in the latter compound. Compound **1** showed the presence of signals due to a cinnamoyl group that were not found in 19-benzoyl-19-acetyltaxinine M. In addition, the H-5, H-6α, and H-6β signals (δ 5.37, 2.37, and 2.20) in **1** were also shifted to lower field by 0.99, 0.21, and 0.58 ppm. The data suggest that these four compounds vary only in the substituents at C-5 and C-19. At position 5, compound **1**, taxagifine, and taxacin have a cinnamoyl ester, while 19-debenzoyl-19-acetyltaxinine both have an unesterified hydroxyl group. At position 19, taxagifine contains an unsubstituted methyl group, taxacin contains a benzoxymethylene group, and both **1** and 19-debenzoyl-19-acetyltaxinine M contain an acetoxymethylene group.

The positions of the cinnamoyl and acetate esters in **1** were confirmed by long-range correlations with the respective ring protons in the HMBC spectrum (C-1′ at δ 165.6, OAc-2 at δ 169.9, OAc-7 at δ 168.8, OAc-9 at δ 169.7, OAc-10 at δ 170.5, and OAc-19 at δ 170.5) showed long-range

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Table 1. ^1H, ^{13}C, and HMBC NMR Spectral Data of Compound **1**[a]

	in $CDCl_3$		in C_5D_5N		
position	^1H	^{13}C	^1H	^{13}C	HMBC correlations
1	2.53 (m)	48.7	2.92 (dd, 2.8, 12.4)	48.6	H-3, H-14α, H-17, H-17′
2	6.18 (m)	70.5	6.69 (dd, 2.8, 10.8)	70.6	H-1, H-3, H-14α, H-14*β*
3	3.47 (d, 9.6)	39.9	3.99 (d, 10.8)	41.4	H-19, H-19′
4	–	140.8	–	148.8	H-3
5	5.37 (m)	73.8	5.76 (d, 4.6)	75.0	–
6α	2.37 (m)	37.1	2.48 (m)	36.6	H-7
6*β*	2.20 (m)	–	1.84 (m)	–	–
7	5.54 (m)	69.2	5.98 (dd, 6.4, 10.8)	69.3	H-3, H-5, H-9, H-19, H-19′
8	–	49.1	–	49.8	H-3. H-7, H-9, H-10, H-19, H-19′
9	5.54 (m)	68.4	6.35 (d, 2.0)	69.3	H-3, H-7, H-10, H-19
10	5.54 (m)	65.3	5.90 (d, 2.0)	65.5	H-9
11	–	82.3	–	82.2	H-1, H-17
12	–	91.7	–	92.2	H-17, H-18
13	–	204.4	–	204.9	H-14α, H-14*β*, H-18
14α	2.65 (d, 19.2)	32.3	3.30 (d, 19.6)	34.5	H-2
14*β*	2.98 (dd, 12.0, 19.2)	–	3.35 (dd, 12.4, 19.6)	–	–
15	–	49.7	–	50.1	H-1, H-14α, H-14*β*, H-16, H-17, H-17′
16	1.21 (s)	15.1	1.99 (s)	15.6	–
17	3.69 (d, 8.0)	80.4	3.94 (d, 8.0)	82.0	–
17′	4.20 (d, 8.0)	–	4.40 (d, 8.0)	–	–
18	1.25 (s)	12.2	1.66 (s)	14.0	–
19	4.34 (d, 12.4)	61.6	4.59 (d, 12.0)	61.8	H-3, H-7
19′	4.44 (d, 12.4)	–	4.70 (d, 12.0)	–	–
20	4.59 (s)	115.4	4.88 (s)	115.0	H-3, H-5
20′	5.54 (m)	–	5.51 (s)	–	–
OAc-2	1.97[b](s)	20.9[b]	1.96 (s)	21.1	–
OAc-7	1.97[b](s)	20.6[b]	1.96 (s)	20.8	–
OAc-9	2.20[b](s)	21.3[b]	2.36 (s)	21.1	–
OAc-10	2.12[b](s)	20.9[b]	2.06 (s)	20.9	–
OAc-19	2.15[b](s)	21.3[b]	2.19 (s)	20.9	–
2-CO	–	168.9[b]	–	169.9	H-2
7-CO	–	167.0[b]	–	168.8	H-7
9-CO	–	169.9[b]	–	169.7	H-9
10-CO	–	170.8[b]	–	170.5	H-10
19-CO	–	172.2[b]	–	170.5	H-19, H-19′
1′	–	166.0	–	165.6	H-5
2′	6.80 (d, 16.0)	117.7	7.04 (d, 16.4)	118.8	H-3′
3′	7.92 (d, 16.0)	146.3	8.33 (d, 16.4)	146.0	H-2′
i-phenyl	–	134.7	–	136.0	H-3′
o-phenyl	7.32–7.81 (m)[c]	128.8[b]	8.03 (d, 7.2)	129.3[b]	–
m-phenyl		127.9[b]	7.38 (dd, 7.2, 7.2)	129.2[b]	–
p-phenyl		130.2	7.33 (d, 7.2)	130.6	–

[a] Values are in ppm. The multiplicities (*s* = singlet, d = doublet, m = multiplet) and coupling constants (*J* in Hz) are in parentheses; ^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz. [b] These values may be interchanged. [c] Individual phenyl peaks were not assignable.

correlations with the respective ring protons (H-5 at δ 5.76, H-2 at δ 6.69, H-7 at δ 5.98, H-9 at δ 6.35, H-10 at δ 5.90, and both H-19 at δ 4.59 and H-19′ at δ 4.70) (Table 1). The relative stereochemistry of **1** was confirmed by NOE correlations. From these data, the structure of **1** was definitely determined as 19-acetoxytagifinine.

The known 2-deacetoxytaxinine J was identified by comparing its physical and spectroscopic data with literature values.[12]

Experimental Section

General Experimental Procedures. Melting points were determined on an MRK air-bath type melting point apparatus and are uncorrected. Specific rotations were obtained on a JASCO DIP-370 digital polarimeter (L = 0.5 dm). IR and UV spectra were recorded on JASCO IR-810 and Hitachi 320-S spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were determined on a JEOL JNM-A400 instruments in $CDCl_3$ and C_5D_5N using TMS as an internal standard. Mass spectra were recorded on a Hitachi M-80 instrument. Si gel (Merck, type 60, 70–320 mesh) was used for column chromatography. Precoated Si gel plates (Merck 60 F_{254}) of 0.25 mm thickness were used for analytical TLC, and plates of 1 mm and 2 mm thickness were used for preparative TLC. Components were detected on TLC plates using a UV lamp (254 and 365 nm). Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector at 254 nm and a reversed-phase column (TSK-gel ODS-80Ts) using a solvent mixture of MeOH–H_2O. Preparative HPLC was carried out on Tosoh or Gilson liquid chromatographs equipped with a reversed-phase column (Lichrosorb RP-18) at 254 nm using the same solvents as employed for analytical HPLC.

Plant Material. The plant bark, twigs, and leaves of *T. chinensis* were collected in August 1993, inYunnan Province, People's Republic of China. The voucher specimen is deposited at Shanghai Medical University, Shanghai, People's Republic of China.

Extraction and Isolation. The plant bark, twigs, and leaves of *T. chinensis* (air-dried material, 6.2 kg) were extracted with EtOH, and the EtOH extract (507 g) was obtained by evaporation of the solvent. The extract was diluted with EtOH and H_2O (3:1) and then extracted with *n*-hexane. The EtOH–H_2O layer then was extracted with CH_2Cl_2 to give a CH_2Cl_2 extract (120 g). Silica gel column chromatography of this extract eluting with benzene–EtOAc–*n*-hexane (14:5:6) (29 L) gave 11 fractions. Each fraction was checked by analytical TLC and HPLC.

Fraction 11 (1.14 g) was suspended in MeOH and the insoluble material (34.8 mg), was filtered. The remaining material (1.09 g) was subjected to preparative HPLC (MeOH–H_2O, 7:3) to give 16 subfractions of which fractions 11-1 to

11-5 and fractions 11-11 to 11-13 were separately pooled, according to their HPLC analysis. Fraction 11-9 (16.9 mg) was further subjected to repeated preparative HPLC (MeOH−H_2O, 7:3) to afford the new taxane diterpene **1** (5.6 mg, 0.000090%) as a colorless amorphous powder. Fraction 11-16 (445 mg) was purified by preparative HPLC (MeOH−H_2O, 85:15) to yield fractions 11-16-1 (20.6 mg) and 11-16-0 (217 mg). Purification of fraction 11-16-0 by recycle HPLC (MeOH−H_2O, 75:25) afforded the known 2-deacetoxytaxinine J (4.0 mg, 0.000065%).

19-Acetoxytaxagifine (1): Colorless amorphous powder: mp 106−108 °C; $[\alpha]^{27}_D$ −2.4° (*c* 0.33, MeOH); UV (EtOH) λ_{max} (log ϵ) 274 (4.03) nm; IR (KBr) ν_{max} 3420 (OH), 1740 (ester C═O), 1665 (α,β-unsaturated C═O) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 754 (6), 606 (26.2), 564 (15), and 131(100); HREIMS *m*/*z* 754.2824 (calcd for $C_{39}H_{46}O_{15}$, 754.2833).

2-Deacetoxytaxinine J: Colorless amorphous powder: mp 169−171 °C (lit. mp 171−172 °C);[12] and spectral data (UV, IR, ^1H NMR, ^{13}C NMR, EIMS) comparable to literature values.[12]

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