Notes

Dantaxusins A and B, Two New Taxoids from *Taxus yunnanensis*

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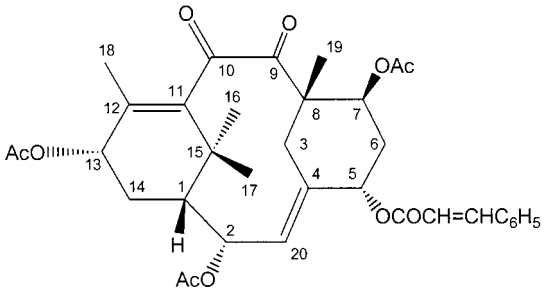
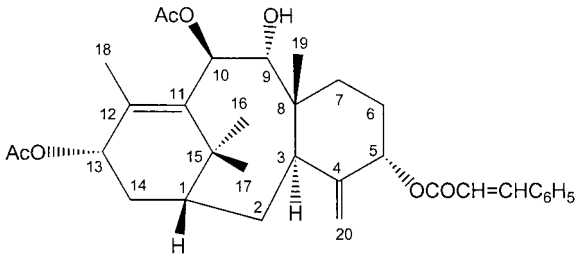
Two new taxane diterpenes, dantaxusin A [5α-cinnamoyloxy-2α,7*β*,13α-triacetoxy-2(3→20)*abeo*-taxa-4(20),11-diene-9,10-dione (**1**)] and dantaxusin B [5α-cinnamoyloxy-9α-hydroxy-10*β*,13α-diacetoxytaxa-4(20),11-diene (**2**)], were isolated from an ethanol extract of the aerial parts of *Taxus yunnanensis* along with taxuspine B, 2-deacetoxytaxinine J, taxuyunnanine C, taxinine B, taxuspine C, and taxinine NN-4. The structures of **1** and **2** were established on the basis of 1D and 2D NMR and HRMS spectroscopic methods.

Paclitaxel (Taxol), which was isolated from *Taxus brevifolia* (Taxaceae) by Wani et al. in 1971, acts as a mitotic inhibitor by promoting microtubule assembly and is used as an antitumor agent for treating breast and ovarian cancer.[1,2] Early supply problems promoted extensive investigation of the constituents of various *Taxus* species, and many taxane diterpenoids have been isolated.[3,4] Several of these reports have concentrated on the taxoid constituents of *Taxus yunnanensis* Cheng et L.K. Fu[6] and *T. chinensis* (Pilgre) Rehd. var. *maireii*.[5−11] In our continuing studies of new antitumor agents from higher plants, we have investigated the taxane diterpene constituents of these two species. Previously, we reported the isolation of taxuchin A, a 11(15→1)*abeo*-taxane type diterpene, and 19-acetoxytaxagifine, and the evaluation of seven isolated taxane diterpenes for cytotoxicity against nine human cell lines, including a *β*-tubulin-mutant resistant to paclitaxel.[9−11] As part of our continuing studies on the constituents of *Taxus* species, we report here the isolation and structural elucidation of two new taxane diterpenes from *T. yunnanensis*. Spectroscopic characterization established the two new taxoids as structures **1** and **2**.

The air-dried aerial parts of *T. yunnanensis* were extracted with EtOH to afford a crude extract. After evaporation of the solvent, the crude extract was dissolved in aqueous EtOH and re-extracted with *n*-hexane, CH_2Cl_2, and then *n*-BuOH. The CH_2Cl_2 extract was further fractionated using a combination of Si gel chromatography and preparative HPLC to give two new taxoids, **1** and **2**, and six known taxoids. The known taxoids, taxuspine B, 2-deacetoxytaxinine J, taxuyunnanine C, taxinine B, taxuspine C, and taxinine NN-4, were identified by comparison of their physical and NMR spectral data with those reported in the literature.[12−15]



1



2

Compound **1** was obtained as a colorless amorphous powder. Its IR spectrum showed the presence of ester (1740 cm^{-1}), carbonyl (1720 cm^{-1}), and α,*β*-unsaturated carbonyl (1665 cm^{-1}) groups. Its molecular formula was established as $C_{35}H_{40}O_{10}$ from HRMS (*m*/*z* 620.2602) and ^1H, ^{13}C, and DEPT NMR spectra. The UV spectrum of **1** showed an absorption maximum at 274 nm due to a conjugated aromatic ring.

The ^1H NMR spectrum of **1** showed four quaternary methyl groups [*δ* 1.12 (H-16), 1.75 (H-18), 1.55 (H-17), and 1.25 (H-19)], three acetyl methyl groups [*δ* 2.02 (OAc-13), 2.14 (OAc-7), and 2.15 (OAc-2)], an olefinic proton [*δ* 5.28 (H-20)], and a cinnamoyl group [*δ* 6.45 (H-2′), 7.75 (H-3′), 7.40 (H-6′, H-7′), 7.50 (H-5′)] and four methine groups connected to ester oxygen atoms [*δ* 5.85 (H-2), 5.55 (H-5), 5.75 (H-7), 5.40 (H-13)]. The relationships between the proton signals in **1** were established by the $^1H-^1H$ COSY

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Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) Spectral Data of **1** and **2**

	1				**2**			
position	$^{13}C^a$	$^1H^b$	COSY	HMBC	$^{13}C^a$	$^1H^b$	COSY	HMBC
1	40.3	1.80 (m)	H-2, 14α	H-2, 14, 20, Me-16,17	40.3	1.80 (m)	H-2, 14-α,*β*	H-2, 3, 13, 14, Me-16, 17
2α					28.2	1.65−1.75 (m)	H-1, 3	H-1, 3, 14
2*β*	70.7	5.85 (d, 8.8)	H-1, 20	H-1, 14, 20		1.65−1.75 (m)		
3a	32.4	2.54 (d, 16)	H-3b	H-5, 7, Me-19	37.9	3.08 (br s)	H-2	H-1, 2, 5, 7, 9, Me-19
3b		2.81 (d, 16)	H-3a					
4	135.4			H-2, 3, 5, 6	149.2			H-2, 3, 5, 6, 20
5	68.6	5.55 (dd, 5.2)	H-6α,*β*	H-3, 6, 7, 20	76.7	5.54 (br s)	H-6*β*	H-3, 6, 7, 20
6α	34.4	1.65 (m)	H-5, 6*β*, 7	H-5, 7	28.0	1.80 (m)	H-6*β*, 7	H-5, 7
6*β*		2.33 (m)	H-5, 6α, 7			1.88 (m)	H-5, 6α	
7α	71.5	5.75 (br s)	H-6α, 6*β*	H-3, 5, 6, Me-19	26.1	1.91 (m)	H-6α	H-3, 5, 6, 9, Me-19
7*β*						1.65−1.75 (m)		
8	53.0			H-3, 6, 7, Me-19	43.3			H-2, 3, 6, 7, 9, 10, Me-19
9	209.9			H-3, 7, Me-19	77.2	4.28 (dd, 10)	H-10	H-3, 7, 10, Me-19
10	199.6				76.2	5.92 (d, 10)	H-9	H-9
11	136.4			H-1, Me-16, 17, 18	135.7			H-1, 9, 10, 13, Me-18
12	146.4			H-13, 14, Me-18	136.4			H-10, 14, Me-18
13	67.7	5.40 (d, 9.6)	H-14*β*	H-1, 14, Me-18	70.7	5.75 (t, 9.2, 8.4)	H-14α,*β*	H-1, 14, Me-18
14α	27.0	1.9 (ddd, 2.4, 5.2, 9.6)	H-1, 14*β*	H-1, 2, 13	32.4	1.00−1.08 (m)	H-1, 13, 14*β*	H-1, 2, 13
14*β*		2.72 (ddd, 2.4, 5.2, 9.6)	H-13, 14α			2.74 (m)	H-1, 13, 14α	
15	36.7			H-1, 2, 14, Me-16, 17	39.3			H-1, 2, 10, 14, Me-16, 17
16	31.2	1.12 (s)		H-1, Me-17	31.2	1.07 (s)		H-1, Me-17
17	25.7	1.55 (s)		H-1, Me-16	27.3	1.49 (s)		H-1, Me-16
18	18.3	1.75 (s)		H-13	15.3	2.24 (s)		H-13
19	23.9	1.25 (s)		H-3, 7	18.2	0.96 (s)		H-3, 7, 9
20	121.1	5.28 (d, 8.8)	H-2	H-1, 2, 3, 5	113.8	5.29 (s)		H-3, 5
20*β*						4.88 (s)		
1′	165.7			H-2′, 3′	166.3			H-5, 2′, 3′
2′	117.2	6.45 (d, 16)	H-3′	H-3′	118.9	6.58 (d, 16)	H-3′	H-3′
3′	146.0	7.75 (d, 16)	H-2′	H-2′, 5′	145.0	7.75 (d, 16)	H-2′	H-2′, 5′
4′	134.0			H-2′, 3′, 5′, 6′	134.4			H-2′, 3′, 5′, 6′
5′	128.0	7.50 (m)	H-6′	H-3′, 6′, 7′	129.0	7.50 (m)	H-6′	H-3′, 6′, 7′
6′	129.1	7.40 (m)	H-5′, 7′	H-5′, 7′	127.9	7.40 (m)	H-5′, 7′	H-5′, 7′
7′	130.8	7.40 (m)	H-6′	H-6′	130.5	7.40 (m)	H-6′	H-6′
OAc Groups								
2	169.8			H-2				
	21.0	2.15 (s)						
7	168.5			H-7				
	21.0	2.14 (s)						
10					170.5			H-10
					21.4	2.12 (s)		
13	170.2			H-13	170.8			H-13
	21.4	2.02 (s)			21.0	1.72 (s)		

[a] Assignments made using the HSQC and HMBC techniques. [b] Multiplicities and coupling constants in Hz in parentheses.

technique. The ^{13}C and DEPT NMR spectral data indicated the presence of two ketone carbonyls [δ 209.9 (C-9), 199.6 (C-10)], three ester carbonyls [δ 169.8 (OAc-2), 168.5 (OAc-7), 170.2 (OAc-13)], six aromatic ring carbons [δ 134.0, 128.0, 129.1, 130.8], six olefinic carbons [δ 135.4 (C-4), 136.4 (C-11), 146.4 (C-12), 121.1 (C-20), 117.2 (C-2′), 146.0 (C-3′)], three methylene carbons [δ 32.4 (C-3), 34.4 (C-6), 27.0 (C-14)], and four methine carbons connected to oxygen atoms [δ 70.7 (C-2), 68.6 (C-5), 71.5 (C-7), 67.7 (C-13)].

The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of taxuspine B (**2**),[12] suggesting that **1** has the same 4(20→3)*abeo*-taxane skeleton. However, comparison of their spectral data revealed the following differences. The ^{1}H NMR spectrum of **1** lacked one methine proton attached to the hydroxyl-bearing carbon found in taxuspine B. Furthermore, the Me-17 and H-3a signals in **1** (δ 1.55 and 2.54) were shifted to lower field by approximately 0.35 and 0.54 ppm, respectively, and the Me-19 signal (δ 1.25) appeared at higher field by 0.10 ppm. The ^{13}C NMR spectrum of **1** showed the presence of an additional signal due to a carbonyl at δ 199.6 (C-10) rather than the C-10 methine carbon connected to a hydroxyl group, as was found in taxuspine B. These findings indicated that **1** has a ketone group at C-10.[16]

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 (Table 1). The ester carbonyl carbon signals (C-1′ at δ 165.7, OAc-2 at δ 169.8, OAc-7 at δ 168.5, OAc-13 at δ 170.2) showed long-range correlations with the respective ring protons (H-2 at δ 5.85, H-5 at δ 5.55, H-7 at δ 5.75, and H-13 at δ 5.40). Other correlations were also in agreement with the structure proposed. The relative stereochemistry of **1** was confirmed by NOE correlations, as shown by arrows in Figure 1. From these data, the structure of **1** was established as 5α-cinnamoyloxy-2α,7*β*,13α-triacetoxy-2(3→20)-*abeo*-taxa-4(20),11-diene-9,10-dione and given the trivial name dantaxusin A.

Compound **2** was obtained as a colorless amorphous powder. Its IR spectrum showed the presence of hydroxyl

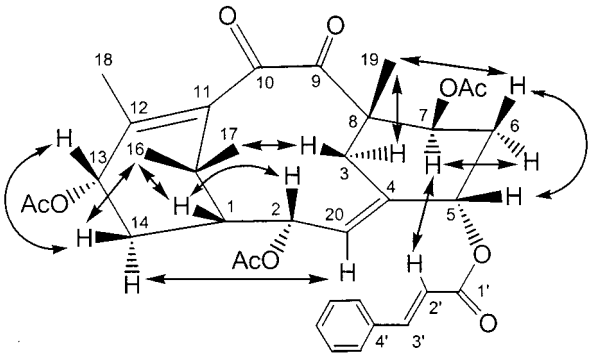

Figure 1. Relative stereochemistry of **1**, deduced from a NOESY experiment (400 MHz).

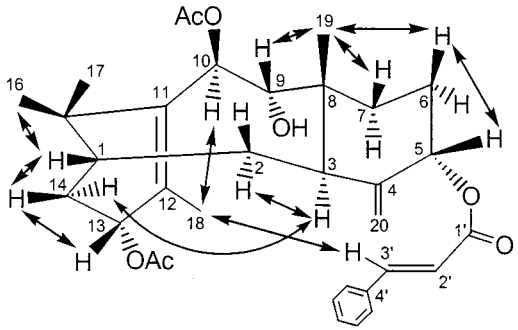

Figure 2. Relative stereochemistry of **2**, deduced from a NOESY experiment (400 MHz).

(3420 cm^{-1}), ester carbonyl (1740 cm^{-1}), and α,β-unsaturated carbonyl (1665 cm^{-1}) functionalities. Its UV spectrum showed an absorption maximum at 274 nm due to a conjugated aromatic ring. Its molecular formula was established as $C_{33}H_{42}O_7$ from HRMS (*m/z* 550.2918) and ^1H, ^{13}C, and DEPT spectral data.

The ^1H and ^{13}C NMR spectra of **2** showed the presence of characteristic taxane skeleton signals and were similar to those of 5α-cinnamoyloxy-7β,9α,10β,13α-tetraacetoxytaxa-4(20),11-diene. The presence was apparent of an exomethylene (δ 4.88 and 5.29), four methyl groups (δ 0.96, 1.07, 1.49, 2.24), two acetyl methyl groups (δ 1.72 and 2.12), and a cinnamoyloxy group (δ 6.58, 7.40, 7.50, and 7.75). However, the ^1H NMR spectra of **2** lacked two acetyl groups as found in 5α-cinnamoyloxy-7β,9α,10β,13α-tetraacetoxytaxa-4(20),11-diene, but contained an additional methine proton attached to the hydroxyl-bearing carbon.

The positions of the cinnamoyloxy and acetoxy groups in **2** were confirmed by long-range correlations with the respective ring protons in the HMBC spectrum (Table 1). The ester carbonyl carbon signals (C-1′ at δ 166.3, OAc-10 at δ 170.5, OAc-13 at δ 170.8) showed long-range correlations with the respective ring protons (H-5 at δ 5.54, H-10 at δ 5.92, and H-13 at δ 5.75). Additionally, correlations of the methine proton signal (H-9 at δ 4.28) were observed with the carbon signals at δ 76.2 (C-10), δ 26.1 (C-7), and δ 37.9 (C-3), respectively. These findings indicated that a hydroxyl group was attached at C-9. The DEPT and HMBC NMR spectra indicated that C-7 was a methylene carbon. The carbon signal at δ 28.2 (C-2) exhibited long-range correlations with the proton signals at δ 1.80 (H-1), δ 3.08 (H-3), and δ 2.74 (H-14). Other correlations agreed with the proposed structure. The relative stereochemistry of **2** was established using the information obtained from the NOESY experiment; arrows in Figure 2 show the NOE correlations observed. Thus the structure of **2** was elucidated as 5α-cinnamoyloxy-9α-hydroxy-10β,13α-diacetoxytaxa-4(20),11-diene and given the trivial name dantaxusin B.

Experimental Section

General Experimental Procedures. Melting points were determined on an MRK air-bath type melting point apparatus. 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 JEOL JNM-A400 instruments in $CDCl_3$ using TMS as 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. 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-80 Ts) using solvent mixtures of MeOH−H_2O. Preparative HPLC was carried out on Tosoh or Gilson liquid chromatographs equipped with a reversed-phase column (YMC-Pack-ODS-A) at 254 nm using the same solvents as employed for analytical HPLC.

Plant Material. The plant bark, twigs, and leaves of *T. yunnanensis* were collected in August 1993, in Yunnan Province, People's Republic of China, and verified by Prof. Daofeng Chen. The voucher specimen (YNLJ19930802) is deposited at Shanghai Medical University, Shanghai, People's Republic of China.

Extraction and Isolation. The plant bark, twigs, and leaves of *T. yunnanensis* (air-dried material, 7.3 kg) were extracted with EtOH. An EtOH extract (480 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 to give an *n*-hexane extract (53 g). The EtOH−H_2O layer then was extracted with CH_2Cl_2 and *n*-BuOH successively, to give a CH_2Cl_2 extract (111 g), an *n*-BuOH extract (156 g), and finally an H_2O-soluble residue (148 g). Si gel column chromatography of the CH_2Cl_2 extract eluting with benzene−EtOAc−*n*-hexane (14:5:6) (36 L) gave 13 fractions, that with EtOAc−Et_2O (1:1) (17 L) gave nine fractions, and that with $CHCl_3$−MeOH−H_2O (50:14:3) (41 L) gave four fractions. Each fraction was checked by analytical HPLC. The fractions that eluted with mixed solvents of benzene−EtOAc−*n*-hexane were as follows: fractions 1 (741 mg), 2 (232 mg), 3 (195 mg), 4 (277 mg), 5 (320 mg), 6 (683 mg), 7 (349 mg), 8 (316 mg), 9 (85 mg), 10 (319 mg), 11 (150 mg), 12 (189 mg), and 13 (288 mg).

Fractions 7 (349 mg) and 8 (316 mg) were suspended in MeOH and the insoluble materials (22 mg from fraction 7, 31 mg from fraction 8) removed by filtration. The remaining materials were combined (52.9 mg) and then subjected to preparative HPLC (MeOH−H_2O, 75:25) to give 48 subfractions. Fraction 41 was further purified with repeated preparative HPLC (MeOH−H_2O, 75:25) to provide the new taxane diterpene **1** (6.7 mg, 0.0014%) as a colorless amorphous powder. Purification of fraction 42 by preparative HPLC (MeOH−H_2O, 75:25) afforded the new taxane diterpene **2** (2.8 mg, 0.00058%) as a colorless amorphous powder.

Dantaxusin A (1): colorless amorphous powder; mp 114−116 °C; $[\alpha]^{27}_D$ +12° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 274 (4.00) nm; IR (KBr) ν_{max} 1740 (C=O), 1665 (α,β-unsaturated C=O) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 620.2602 (calcd for $C_{35}H_{40}O_{10}$, 620.2619).

Dantaxusin B (2): colorless amorphous powder; mp 245−246 °C; $[\alpha]^{27}_D$ −8° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 274 (5.30) 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; HREIMS *m/z* 550.2918 (calcd for $C_{35}H_{42}O_7$, 550.2929).

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