Taxoids from *Taxus chinensis*

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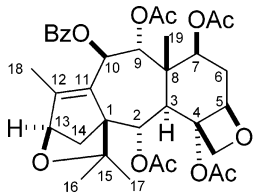
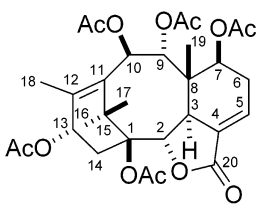
Two new taxoids, 13,15-epoxy-13-*epi*-taxayunnasin A (**1**) and taxchinin N (**2**), were isolated from the leaves and stems of *Taxus chinensis*. Compound **2** is the first taxoid to be reported with an α,*β*-unsaturated lactone at C-4, C-5, C-20, and C-2. The structure of **1** was elucidated by spectroscopic analysis and confirmed by semisynthesis from taxayunnasin A, while **2** was determined structurally using spectroscopic methods and by single-crystal X-ray diffraction.

The unique structure, novel mechanism of action, and clinical importance of the antitumor agent paclitaxel continues to stimulate a great deal of interest in the isolation of further taxoids from plants in the genus *Taxus*.[1−6] In a continuing search for new taxoids, we have previously isolated several new taxoids from *Taxus chinensis* (Pilger) Rehd. (Taxaceae).[7−9] On further investigation of an extract of the leaves and stems of *T. chinensis*, two minor new taxoids, 13,15-epoxy-13-*epi*-taxayunnasin A (**1**) and taxchinin N (**2**), were isolated. The isolation and structure elucidation of **1** and **2** are the subject of this report.



1



2

Compound **1** was obtained as a colorless, amorphous powder. Its molecular formula, $C_{35}H_{42}O_{12}$, was deduced from the HR-FABMS ($[M + H]^+$, *m*/*z* 655.2754, calcd 655.2755). Analysis of the ^1H and ^{13}C NMR spectroscopic data (Table 1) and the HMQC spectrum provided evidence that **1** possesses four acetyl groups, one tetrasubstituted olefin, six oxymethines, three methylenes (including one oxymethylene), four quaternary carbons (including two oxygenated ones), and four methyl groups. The signals at δ_C 82.3 (s, C-4), δ_C 77.9 (t, C-20), δ_H 4.54 (d, $J = 4.8$ Hz, H-20α), and δ_H 4.52 (d, $J = 4.8$ Hz, H-20*β*) indicated the presence of an oxetane moiety.[10,11] Comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** with those of taxayunnansin A showed that **1** is very similar to taxayunnansin A[12] except for C-13 and C-15. The HMBC correlation between H-13 (δ_H 4.32, d, $J = 0.6$ Hz) and C-15 (δ_C 79.0, s) suggested the presence of an oxygen bridge between C-13 and C-15, forming a C-13(15)-tetrahydrofuran ring moiety in **1**. Three acetoxy groups were placed at C-2, C-7, and C-9, while a benzoyloxy group was positioned at C-10 on the basis of HMBC correlations. The remaining acetoxy group was placed at C-4. Accordingly, compound **1** could be proposed as 13,15-epoxy-13-*epi*-taxayunnansin A. The relative stereochemistry of **1** was confirmed by a ROESY NMR experiment, in which correlations of H-2/H-9, Me-17, and Me-19 indicated that H-2 and H-9 are *β*-oriented, while correlations of H-3/H-7, H-14α, H-10/Me-18, and H-5/H-7 confirmed the α-configuration of H-3, H-7, H-10, and H-5 (Figure 1). A ROESY correlation between H-20*β* and Me-19 supported the *β*-configuration of the oxetane ring. ROESY correlations were observed for H-13/H-14α and H-14α/H-3, while there was no correlation between H-13 and H-14*β*, which suggested H-13 is in the α-orientation. Compound **1** resembles structurally 13-deacetoxy-13,15-epoxy-11(15→1)-*abeo*-13-*epi*-baccatin IV,[13] in which a benzoxy group is at C-2 while an acetyl group occurs at C-10. In order to confirm the structure of **1**, the chemical transformation from taxayunnansin A to **1** was conducted successfully and employed methanesulfonyl chloride in pyridine in excellent yield (99%). The mechanism of this transformation could be explained as an intramolecular S_N2 nucleophilic substitution involving OH-15 and C-13, which is accordance with an inversion of the configuration of H-13 in **1** (Scheme 1). Moreover, this transformation might also be the biosynthetic route of compound **1**.

Compound **2** was obtained as a colorless, amorphous powder. Its molecular formula, $C_{30}H_{38}O_{12}$, was determined by HRESIMS ($[M + Na]^+$, *m*/*z* 613.2260, calcd 613.2261). The IR spectrum showed strong absorptions at 1745, 1692, and 1631 cm^{-1}, corresponding to ester carbonyl groups and an α,*β*-unsaturated lactone, respectively. The UV spectrum showed an absorption at λ_{max} 241 nm, suggesting the presence of an α,*β*-unsaturated ester. The ^1H and ^{13}C NMR spectra of **2** showed the presence of a taxoid having nine methyls (including five acetyl methyls), two methylenes, seven methines (five oxygenated and one olefinic), and 12 quaternary carbons (including three olefinic carbons) (Table 1). The characteristic signals at δ_C 89.1 (s, C-1), 46.3 (d, C-3), 44.1 (s, C-8), 136.8 (s, C-11), 141.5 (s, C-12), and 43.7 (s, C-15) indicated that **2** has a 6/8/6 ring-system skeleton.[10] On the basis of an analysis of the $^1H-^1H$ COSY spectrum, the signals at δ_H 5.43 (1H, t, $J = 7.9$ Hz), 6.01 (1H, d, $J = 11.0$ Hz), 6.14 (1H, d, $J = 11.0$ Hz), and 5.79 (1H, m) were assigned to H-7, H-9, H-10, and H-13, respectively. The downfield chemical shift of H-7, H-9, H-10, and H-13 implied that four acetoxy groups were attached to C-7, C-9, C-10, and C-13, respectively, as confirmed by HMBC correlations of H-7, H-9, H-10, and H-13 with the corresponding carbonyl carbons of these acetyl groups. The remaining acetoxy group was assigned to C-1, as deduced from the relative downfield chemical shift (δ_C 89.1, s) of C-1. There were two double bonds [δ_C 136.8 (s, C-11), 141.5 (s, C-12), 128.7 (s, C-4), 134.7 (d, C-5)] in **2**. Except for the double bond between C-11 and C-12, another double bond was assigned to C-4 and C-5 on the basis of the HMBC

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for **1** and **2**[a]

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
1	68.0 s		89.1 s	
2	66.3 d	6.61 (1H, d, 7.5, H-2β)	79.8 d	5.54 (1H, dd, 7.6, 1.2, H-2β)
3	45.8 d	3.23 (1H, d, 7.5, H-3α)	46.3 d	3.53 (1H, m, H-3α)
4	82.3 s		128.7 s	
5	84.5 d	4.91 (1H, dd, 9.6, 5.0, H-5α)	134.7 d	6.70 (1H, dd, 3.8, 7.6, H-5)
6	32.8 t	2.40 (1H, m, H-6β)	33.2 t	2.34 (1H, m, H-6β)
		1.93 (1H, m, H-6α)		2.79 (1H, m, H-6α)
7	71.6 d	5.37 (1H, dd, 12.4, 6.0, H-7α)	71.2 d	5.43 (1H, t, 7.9, H-7α)
8	44.2 s		44.1 s	
9	74.6 d	5.01 (1H, d, 5.7, H-9β)	75.8 d	6.01 (1H, d, 11.0, H-9β)
10	67.3 d	6.31 (1H, d, 5.7, H-10α)	73.2 d	6.14 (1H, d, 11.0, H-10α)
11	134.5 s		136.8 s	
12	149.2 s		141.5 s	
13	82.1 d	4.32 (1H, d, 0.6, H-13α)	70.7 d	5.79 (1H, m, H-13α)
14	46.8 t	1.60 (1H, brd, 8.3, H-14α)	34.4 t	3.03 (1H, m, H-14α)
		2.14 (1H, overlap, H-14β)		2.07 (1H, m, H-14β)
15	79.0 s		43.7 s	
16	26.8 q	0.73 (3H, s, Me-16)	30.7 q	1.16 (3H, s, Me-16)
17	28.8 q	1.16 (3H, s, Me-17)	22.9 q	1.67 (3H, s, Me-17)
18	11.9 q	1.74 (3H, s, Me-18)	16.3 q	2.07 (3H, s, Me-18)
19	13.8 q	1.79 (3H, s, Me-19)	13.5 q	1.01 (3H, s, Me-19)
20	77.9 t	4.54 (1H, d, 4.8, H-20α)	166.9 s	
		4.52 (1H, d, 4.8, H-20β)		
OBz-10	165.4 s			
i	130.5 s			
o	130.8 d	8.22 (2H, dd, 8.3, 1.2)		
m	129.7 d	7.59 (2H, t, 7.4)		
p	134.5 d	7.69 (1H, t, 7.4)		
OAc-1			169.6 s	
OAc-2	172.2 s			
OAc-4	169.9 s s			
OAc-7	170.9 s		170.6 s	
OAc-9	170.3 s		170.6 s	
OAc-10			169.9 s	
OAc-13			170.3 s	
OAc	22.0 q	2.14 (3H, s)	22.8 q	2.09 (3H, s)
OAc	21.7 q	2.12 (3H, s)	22.7 q	2.06 (3H, s)
OAc	21.0 q	1.93 (3H, s)	21.4 q	2.02 (3H, s)
OAc	20.7 q	1.87 (3H, s)	20.8 q	2.01 (3H, s)
OAc			20.8 q	1.96 (3H, s)

[a] Data were recorded in acetone-d_6 on a Bruker AM-400 MHz spectrometer; δ in ppm and J in Hz.

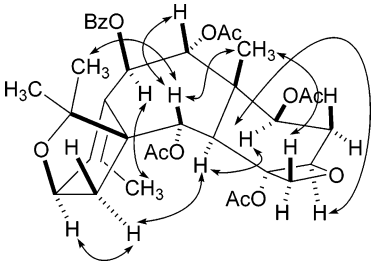


Figure 1. Selected ROESY correlations of **1**.

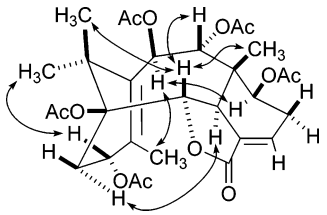
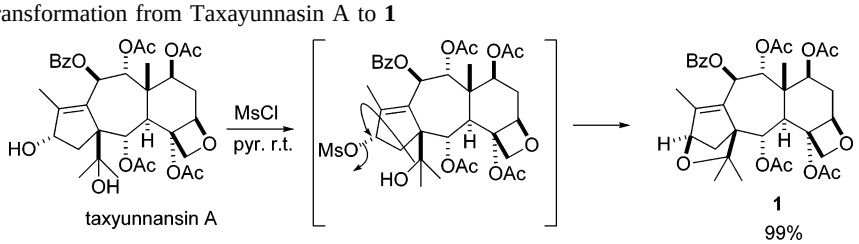


Figure 2. Selected ROESY correlations of **2**.

correlations of H-3 with C-4 and C-5. The signal at δ_H 5.54 (1H, dd, J = 7.6, 1.2 Hz) was ascribed to H-2, as judged from the HMBC correlations of H-2/C-1, C-8, and C-3. In turn, the signal at δ_C 166.9 (s) was assigned to C-20 from the HMBC correlations between H-2 and C-20, which suggested the presence of a lactone moiety between C-20 and C-2. The relative stereochemistry of **2** was established by a ROESY NMR experiment (Figure 2). The β-orientations of H-2, H-9, and H-13 were apparent by the correlations of H-2/H-9, Me-17, and Me-19, and H-13/Me-16, and the α-orientations of H-3, H-7, and H-10 were evident from the correlations of H-3/H-7 and H-14α, H-7/H-10, and H-10/Me-18 and H-7. Therefore, taxchinin N was determined as **2**. X-ray crystallographic analysis (Figure 3) of **2** confirmed the structure deduced above and the relative configuration of **2**.

Scheme 1. Chemical Transformation from Taxayunnasin A to **1**



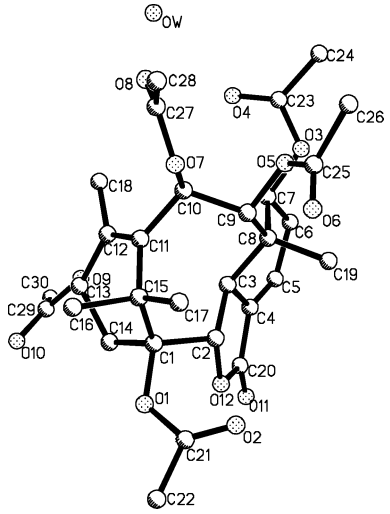


Figure 3. X-ray crystallographic structure of **2**.

Experimental Section

General Experimental Procedures. Melting points were determined on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained on a UV 2401 PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. ^{1}H and ^{13}C NMR experiments were performed on a Bruker AM-400 spectrometer, while 2D NMR spectra were recorded using a Bruker DRX-500 NMR instrument. FABMS and HRFABMS were taken on a VG Auto Spec-3000 or on a Finnigan-MAT 90 instrument. Column chromatography was performed using silica gel (Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 (Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves and stems of *Taxus chinensis* (Taxaceae) were collected in Liangshang of Sichuan Province, People's Republic of China, in March 2000, and identified by Prof. Lin Zhongwen. A voucher specimen (No. 20012) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The dried leaves and stems (15 kg) of *T. chinensis* were extracted three times with 95% ethanol to give a crude extract after concentrating under a vacuum. The residue was dissolved in MeOH−H_2O (9:1) and divided into MeOH-soluble and -insoluble parts. The MeOH-soluble part was further extracted with chloroform to give 250 g of extract. This was chromatographed over a silica gel column employing solvents of increasing polarity (petroleum−EtOAc, 9:1−1:9, and acetone) to give 10 fractions, of which three fractions (20 g) (petroleum−EtOAc, 9:1, 8:2, 7:3) were further chromatographed over a silica gel column eluted by $CHCl_3$−MeOH (100:1−50:1) to afford two subfractions, 1−10 (5 g) and 11−25 (12.5 g). Subfraction 11−25 (12.5 g) was chromatographed on a silica gel column eluted with cyclohexane−EtOAc (7:3) to give subfractions 1′−30′. Fractions 20′−30′ were combined and chromatographed on Sephadex LH-20 eluted with MeOH, and a mixture of compound **1** and **2** was obtained. The mixture was purified employing Lichroprep RP-18 eluted by MeOH−H_2O (5.5:4.5) to give compounds **1** (7 mg) and **2** (6 mg).

13,15-Epoxy-13-*epi*-taxayunnasin A (1): colorless, amorphous powder; $[\alpha]^{29}_D$ +25.0 (*c* 0.26, $CHCl_3$); UV λ_{max} (log ϵ) ($CHCl_3$) 241 (3.91), 275 (3.14) nm; IR (KBr) ν_{max} 2959, 2925, 2853, 1734, 1631, 1450, 1371, 1229 cm^{-1}; ^{13}C NMR (100 MHz, acetone-d_6) and ^{1}H NMR (400 MHz, acetone-d_6) data, see Table 1; positive FABMS *m*/*z* 655 $[M + H]^+$; positive HRFABMS *m*/*z* 655.2754 $[M + H]^+$ (calcd for $C_{35}H_{43}O_{12}$, 655.2755).

Taxchinin N (2): colorless, amorphous powder; $[\alpha]^{29}_D$ +40.0 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 241 (4.44) nm; IR (KBr) ν_{max} 2933, 1745, 1692, 1631,1429, 1374, 1324, 1233, 1145 cm^{-1}; ^{13}C NMR (100 MHz, acetone-d_6) and ^{1}H NMR (400 MHz, acetone-d_6) data, see Table 1; ESIMS *m*/*z* 613 $[M + Na]^+$; HRESIMS *m*/*z* 613.2260 $[M + Na]^+$ (calcd for $C_{30}H_{38}O_{12}Na$, 613.2261).

Chemical Transformation of Taxayunnasin A to 1. Taxayunnasin A (80 mg, 0.119 mmol) was dissolved in dry pyridine (3 mL). To this solution was added MsCl (27.25 mg, 0.238 mmol). The reaction was stirred at room temperature and monitored by TLC until all starting material was consumed. The reaction mixture was diluted with EtOAc and washed with diluted hydrochloric acid (10%), water, and brine. The organic layer was then dried (Na_2SO_4) and concentrated. The residue was chromatographed (10% ethyl acetate in chloroform) to afford **1** (77.3 mg, 99%) as a colorless, amorphous powder; $[\alpha]^{27}_D$ +31.8 (*c* 0.67, $CHCl_3$); ^{13}C NMR (100 MHz, acetone-d_6) and ^{1}H NMR (400 MHz, acetone-d_6) data, see Table 1.

X-ray Structural Determination of Taxchinin N (2). Crystallographic data for **2**: $C_{30}H_{38}O_{12}$, $M = 590.62$, monoclinic, space group $P3_2$ (or $P3_1$), $a = b = 8.978(1)$ Å, $c = 33.622(2)$ Å, $V = 2347.0(4)$ Å^3, $Z = 3$, $d = 1.292$ g/cm^3, crystal dimensions 0.20 × 0.20 × 0.50 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, $2\theta_{max} = 50.0°$), Mo Kα radiation. The total number of reflections measured was 13 263, which yielded an average number of reflections of 3342, of which 3076 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_1 = 0.067$, $wR_2 = 0.219$, and the goodness-of-fit is 1.197. The crystal structure (**2**) was solved by the direct method SHELXS-97[14] and expanded using difference Fourier techniques, refined by the program and method SHELXL-97[14] and the full-matrix least-squares calculations. In the absence of significant anomalous scattering, Friedel pairs were merged, and the absolute configuration of this compound was not determined. Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 613734). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223-336-033; or deposit@ ccdc.cam.ac.uk).

Supporting Information Available: Crystallographic data in cif format. This material is available free of charge via the Internet at http://pubs.acs.org.

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