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Absolute Structure of 2′-Desacetoxyaustrospicatine Established by X-Ray Analysis and Circular Dichroism Spectroscopy

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Three taxane diterpenes, taxinine (1), taxinine B (2), and 2′-desacetoxyaustrospicatin (3), have been isolated from *Taxus cuspidata* SIEB *et* ZUCC. The absolute stereostructure of 3 has been determined by a combination of X-ray analysis and circular dichroism (CD) spectroscopy. Complete assignments of ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) signals for 1 and 2 have been made on the basis of two-dimensional (2D) NMR techniques.

Keywords 2′-desacetoxyaustrospicatin; absolute structure; X-ray analysis; CD spectrum; taxane diterpene

The genus *Taxus* is famous for producing taxol,[1-4] which has potent anticancer activities. We have examined the constituents of *Taxus cuspidata* SIEB *et* ZUCC, which grows in Jilin province and is used as a traditional folk medicine for diabetes, kidney trouble, and diuresis in China, and isolated three taxane diterpenes, taxinine (**1**),[5,6] taxinine B (**2**),[7-9] and 2′-desacetoxyaustrospicatin (**3**),[10] together with biflavones,[11] ginkgetin and sciadopitysin, from the soft branches of this plants. Taxinine (**1**) and taxinine B (**2**) are representative taxane diterpenes and their stereostructures have been determined by X-ray analysis of a related compound[12] or NMR analysis.[7,13] So far, only partial assignments of ^{1}H-NMR and nuclear Overhauser effect (NOE) data for **1**[13] and **2**[7] have been reported. 2′-Desacetoxyaustrospicatin (**3**) was first isolated from *Austrotaxus spicata* by Ettouati *et al.* in 1988[10] and its structure was determined by NMR data and chemical degradation. Its absolute stereostructure has not yet been established.

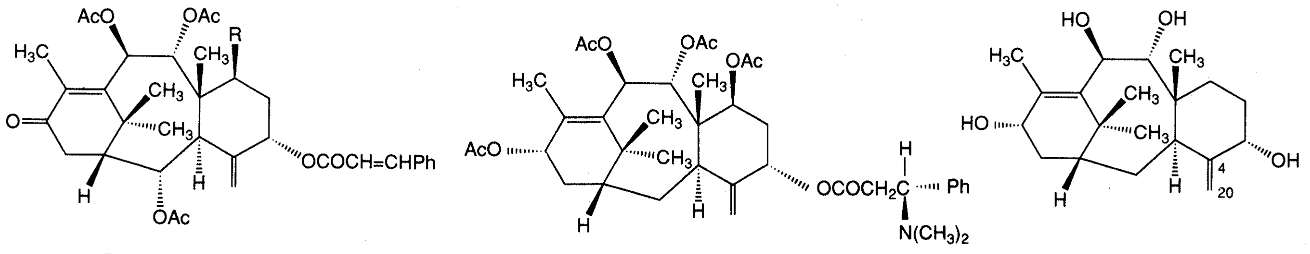
In this paper we wish to report determination of the absolute stereostructure of **3** by means of X-ray analysis and from the circular dichroism (CD) spectrum, together with complete assignments of the ^{1}H- and ^{13}C-NMR signals of **1** and **2**.

Results and Discussion

Compound **1** exhibited the molecular formula $C_{35}H_{42}O_9$ on the basis of high-resolution electron impact-MS (HREIMS). From the detailed examination of ^{1}H-, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H, ^{13}C–^{1}H, ^{13}C–^{1}H long-range correlation spectroscopy (COSY) spectra, **1** was determined to be taxinine. Complete assignments of NMR data are given in Table I. The values of melting point and $[\alpha]_D$ were coincident with the literature values.[14] Compound **2** exhibited the molecular formula $C_{37}H_{44}O_{11}$ in HREIMS. Two-dimensional NMR (2D-NMR) data showed **2** to be taxinine B. Complete assignments of the ^{1}H- and ^{13}C-NMR data are given in Table I. The values of melting point and $[\alpha]_D$ coincided with the literature values.[14] Compound **3** has the molecular formula $C_{39}H_{51}NO_{10}$ in HREIMS. Detailed analysis of 2D-NMR (Table I) and NOE spectra (Fig. 1) indicated **3** to be 2′-desacetoxyaustrospicatin. This was confirmed by a comparison of the NMR data with reported values,[10] though the value of $[\alpha]_D$ is smaller than the literature value.[10] This is the first report of isolation of 2′-desacetoxyaustrospicatin from the genus *Taxus*.

We next examined the relative stereochemistry of **3**. One-dimensional NOE data are shown in Fig. 1. NOEs observed between H-13 and H_3-17 indicated that ring A adopts boat form, and those between Ha-14 and H-3; H_3-18 and H-7 showed that **3** has a cage-form conformation with ring B centered and rings A and C attached on either side of ring B.

We conducted an X-ray analysis to establish the stereochemistry of **3**. A single crystal of **3** was grown as colorless needles from a saturated solution in methanol. Crystallographic and other data are summarized in Table II. An ORTEP drawing in Fig. 2 shows the relative stereostructure of **3**. The substituted group at C-5 and 18-H_3 in ring A are located in very close to each other and hence **3** adopts a cage conformation. Ring A adopts a half boat form and ring C adopts a chair form as judged



1 : R = H
2 : R = OAc

3

4

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Data[a] for **1** and **2**

Position	δ_C 1	δ_C 2		δ_H 1	δ_H 2
1	48.55 d	48.72 d	H-1	2.23 dd (7.0, 1.5)	2.24 dd (7.0, 2.0)
2	69.63 d	68.60 d	H-2	5.55 dd (6.5, 1.5)	5.61 dd (6.2, 2.0)
3	37.68 d	37.68 d	H-3	3.41 d (6.5)	3.32 d (6.0)
4	141.94 s	139.81 s			
5	78.20 d	76.13 d	H-5	5.35 m	5.38 dd (3.5, 2.0)
6	28.37 t	35.09 t	H_a-6	1.73 m	1.76 m
			H_b-6	1.99 m	1.76 m
7	27.51 t	69.56 d	H-7	1.75 m	5.44 dd (11.0, 6.0)
8	44.46 s	47.62 s			
9	75.88 d	74.94 d	H-9	5.89 d (10.0)	5.94 d (10.0)
10	73.46 d	72.77 d	H-10	6.04 d (10.0)	6.24 d (10.0)
11	150.60 s	150.51 s			
12	137.97 s	138.57 s			
13	199.41 s	199.26 s			
14	36.04 t	36.17 t	H_a-14	2.43 d (20.0)	2.36 d (20.0)
			H_b-14	2.84 dd (20.0, 7.0)	2.84 dd (20.0, 7.0)
15	37.63 s	37.34 s			
16	25.18 q	37.73 q	H_3-16	1.77 s	1.15 s
17	37.37 q	25.33 q	H_3-17	1.16 s	1.76 s
18	13.98 q	14.33 q	H_3-18	2.28 s	2.38 s
19	17.47 q	13.22 q	H_3-19	0.93 s	1.03 s
20	117.22 t	118.78 t	H_a-20	4.84 br s	4.91 s
			H_b-20	5.35 s	5.44 s
1′	166.28 s	166.18 s			
2′	117.83 d	117.37 d	H-2′	6.43 d (16.0)	6.44 d (16.0)
3′	145.74 d	67.06 d	H-3′	7.66 d (16.0)	7.68 d (16.0)
1″	134.52 s	134.94 s			
2″	128.47 d	128.57 d	H-2″	7.76 d (7.0)	7.77 dd (8.0, 1.0)
3″	128.94 d	128.95 d	H-3″	7.44 m	7.44 m
4″	130.35 d	130.45 d	H-4″	7.41 m	7.41 m
5″	128.94 d	128.95 d	H-5″	7.44 m	7.44 m
6″	128.47 d	128.57 d	H-6″	7.76 d (7.0)	7.77 dd (8.0, 1.0)
2-O$\underline{\text{C}}$O	169.44 s	169.33 s			
7-O$\underline{\text{C}}$O		176.54 s			
9-O$\underline{\text{C}}$O	169.89 s	169.67 s			
10-O$\underline{\text{C}}$O	169.70 s	169.14 s			
2-CO$\underline{\text{C}}$H$_3$	21.39 q	21.39 q	2-COC$\underline{\text{H}}_3$	2.07 s	2.07 s
7-CO$\underline{\text{C}}$H$_3$		21.28 q	7-COC$\underline{\text{H}}_3$		2.07 s
9-CO$\underline{\text{C}}$H$_3$	20.88 q	20.69 q	9-COC$\underline{\text{H}}_3$	2.07 s	2.06 s
10-CO$\underline{\text{C}}$H$_3$	20.71 q	20.86 q	10-COC$\underline{\text{H}}_3$	2.08 s	2.03 s

[a] Chemical shift in CDCl$_3$ δ (ppm). The assignments are based on DEPT, ^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC).

TABLE II. Crystallographic Data of **3**

Formula	$C_{39}H_{53}NO_{10}$
Formula weight	695.85
Crystal system	Orthorhombic
Space group	$P2_12_12_1$ (#19)
Size (mm)	0.1 × 0.1 × 0.1
Lattice parameters	
a (Å)	12.273 (3)
b (Å)	37.945 (3)
c (Å)	8.298 (3)
V (Å^3)	3864 (2)
Z value	4
D_{calcd} (g/cm^3)	1.196
μ (CuK_α) (cm^{-1})	6.64
R (R_w)	0.081 (0.057)
No. of reflections used	1091

TABLE III. Selected Torsional Angles of **3**

Ring A	Angle (°)	Ring B	Angle (°)
C1–C14–C13–C12	31	C3–C4–C5–C6	59
C11–C12–C13–C14	−40	C4–C5–C6–C7	−45
C13–C12–C11–C14	−5	C5–C6–C7–C8	48
C1–C15–C11–C15	57	C3–C8–C7–C6	−58
C11–C15–C1–C14	−61	C4–C3–C8–C7	66
C13–C14–C1–C15	21	C5–C4–C3–C8	−69

TABLE IV. Selected Intramolecular Distances of **3**

Position		Distance (Å)	Position		Distance (Å)
H_a-2	H_a-20	2.071	H-9	H_3-16	1.695—3.229
H-3	H-7	2.383	H-9	H_3-19	2.335—3.435
H-3	H_a-14	2.350	H-10	H_3-18	1.968—3.225
H-3	H_3-18	2.934—4.024	H-13	H_3-17	1.712—3.144
H-5	H_b-20	2.417	H_3-18	H-3′	2.807
H-7	H_3-18	2.459—3.842	AcO-13	Arom-H	2.950—4.980

from the torsion angles (Table III). As shown in Table IV, the distances between H-3 and Ha-14 (2.350 Å), H-3 and H_3-18 (2.934—4.204 Å), H_3-18 and H-3′ (2.807 Å) confirmed that ring A adopts boat form and also showed that ring A and substituted group at C-5 are located very near to each other.

The absolute structures of **3** together with **1** and **2** were next examined by CD spectroscopy. The absolute structure of taxinine (**1**) has already been determined by X-ray analysis of a related compound[5,6] and also by application of the dibenzoate chirality rule to the 9,10-dibenzoate of **1** by Nakanishi and Harada.[15] CD spectra (MeOH) of **1**, **2** and **3** are shown in Fig. 3. A weak negative Cotton effect at 342 nm and a strong positive Cotton effect at 267 nm in the CD of **1** result from n–π^*, and π–π^* transitions of the enone carbonyl group, respectively. As the CD spectrum of **2** showed the same Cotton effects as **1**, taxinine B (**2**) must have the same absolute configurations as taxinine (**1**). The absolute structure of the taxane diterpene **4** and its derivatives which have no enone group in the molecule have already been reported on the basis of CD examination by Halsall *et al.*[16] The CD spectrum of **4** showed a strong positive Cotton effect at 227 nm, which results from π–π^* transition of the olefin group at C-4. They applied the olefin octant rule to **4** and its derivatives. The CD spectrum of **3**, having the same structure as **4**, showed strong positive Cotton effect at 218 nm resulting from a π–π^* transition of the olefin at C-4. From these results, the absolute configurations of 2′-desacetoxyaustrospicatin (**3**) have been determined as 1*R*, 3*R*, 5*S*, 7*S*, 8*S*, 9*R*, 10*R*, 13*S*, 3′*R*.

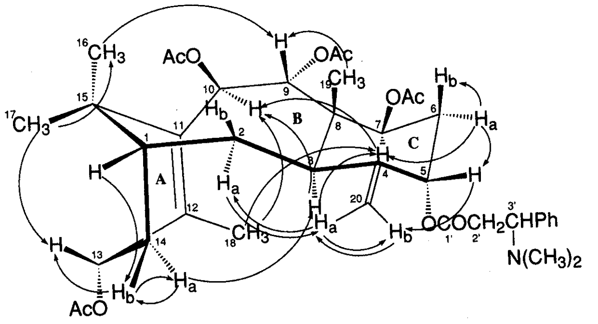


Fig. 1. Observed NOEs of **3**

In conclusion, the absolute stereostructure of **3** was determined for the first time by a combination of X-ray analysis and CD spectroscopy, and complete assignments of the ^{1}H- and ^{13}C-NMR signals of **1** and **2** have been made.

Experimental

General Procedures Melting points were determined on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DPI-181 polarimeter. CD spectra were measured on a JASCO J-20. MS were recorded on a JEOL JMS DX-300 spectrometer. ^{1}H- and ^{13}C-NMR were taken on Varian XL-400 (400 MHz), and XL-400 (100.6 MHz) spectrometers, respectively. ^{1}H–^{1}H and ^{1}H–^{13}C COSY and nuclear Overhauser effect spectroscopy (NOESY) spectra were obtained with the usual pulse sequences and data processing was performed with Varian standard software.

Extraction and Isolation of 1, 2 and 3 The branches of *Taxus cuspidata* Sieb *et* Zucc were collected in Jiling province, China. Plant material was identified by Prof. Y. Guo, Shenyang Pharmaceutical University, and a voucher specimen has been deposited at the Herbarium of Shenyang Pharmaceutical University. The air-dried, powdered plant material (3.0 kg) was extracted with boiling EtOH (36 l) for 2 h to give the extract (620 g). The EtOH extract was suspended in water and extracted with petroleum ether to give the extract (68 g). The petroleum ether extract (30 g) was chromatographed on silica gel and eluted with a mixture of $CHCl_3$ and MeOH. The $CHCl_3$–MeOH (7 : 3) eluate afforded taxinine (**1**) (200 mg) and the $CHCl_3$–MeOH (6 : 4) eluate afforded taxinine B (**2**) (15 mg).

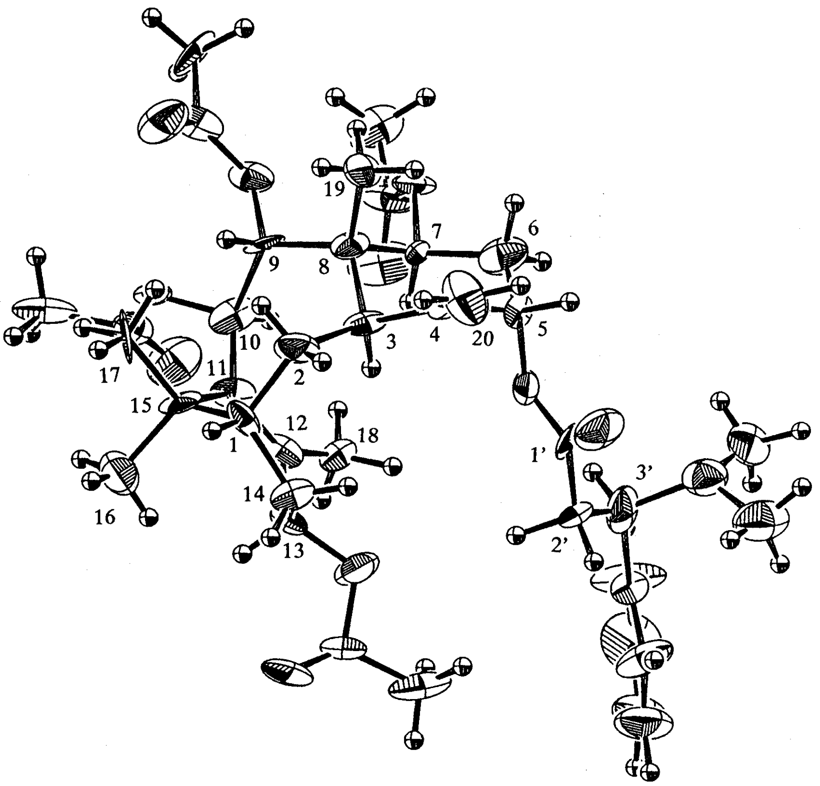


Fig. 2. ORTEP Drawing of **3**

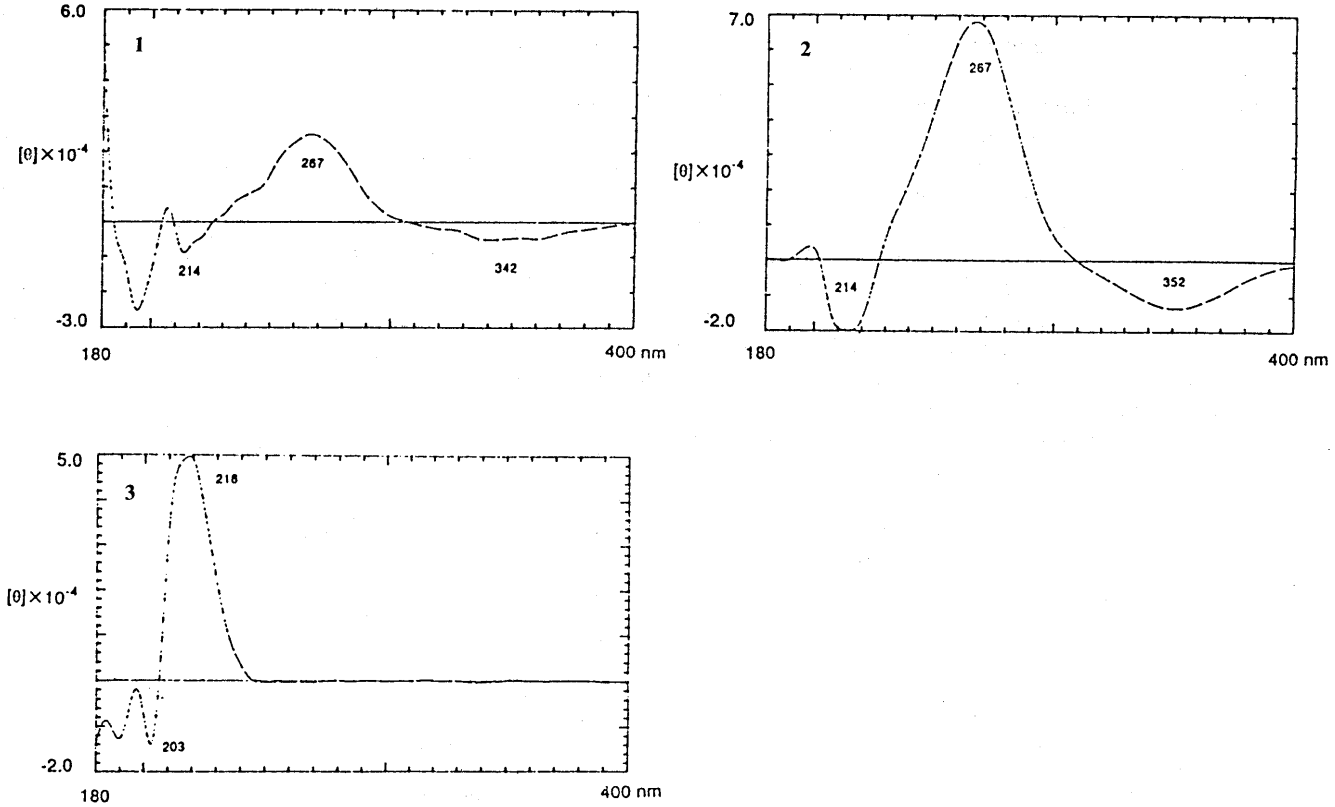


Fig. 3. CD Spectra of **1**, **2** and **3** (MeOH)

Next, the EtOH extract was further extracted with $CHCl_3$ to give the extract (58 g). The $CHCl_3$ extract (30 g) was chromatographed on silica gel with $CHCl_3$–MeOH (98 : 2) to give 2′-desacetoxyaustrospicatin (**3**) (30 mg). **1**: colorless needles, mp 273—277 °C (MeOH) (lit.,[14] 266—268 °C), $[\alpha]_D$ +126.4° (c=0.25, $CHCl_3$) (lit.,[14] +136.0°). HREIMS: Found m/z: 606.2827. Calcd for $C_{35}H_{42}O_9$: 606.2828 (M^+). **2**: colorless needles, mp 258—263 °C (MeOH) (lit.,[14] 265—266 °C), $[\alpha]_D$ +84.0° (c=0.10, $CHCl_3$) (lit.,[14] +93.8°). HREIMS: Found m/z: 664.2921. Calcd for $C_{37}H_{44}O_{11}$: 664.2884 (M^+). **3**: colorless needles, mp 269—271 °C (MeOH), $[\alpha]_D$ +33.6° (c=0.08, CH_2Cl_2) (lit.,[10] +71.0°). HREIMS: Found m/z: 695.3664. Calcd for $C_{39}H_{53}NO_{10}$: 695.3668 (M^+).

X-Ray Data of 3 The diffraction intensities were measured on a Rigaku AFC-5R diffractometer with graphite-monochromated CuK_α (λ=1.54178 Å) radiation at 20 °C. Crystal data and other information are summarized in Table II. The ω-2θ scan mode with a scan rate of 8°/min was employed in the ω scan range of $0.94+0.30\tan\theta°$. A total of 3123 reflections with $|F_O|>3\sigma|F_O|$ were collected. The collected reflection intensities were corrected for Lorenz and polarization factors. The structure was solved by Patterson and direct methods using the programs DIRDIF and MITHRIL, respectively. The non-hydrogen atoms were refined by the full-matrix, least-squares method with anisotropic temperature factors. The positions of all hydrogen atoms were calculated. At the final refinement, 1091 reflections, out of 3123 unique reflections with $|F_O|>3\sigma|F_O|$ were used. Final R was 0.081 (R_w=0.057). Atomic scattering factors were taken from International Tables for X-Ray Crystallography. All calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, U.K.

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