Journal of Natural Products
Vol. 58, No. 2, pp. 233-238, February 1995

THREE NEW TAXANE DITERPENOIDS FROM NEEDLES AND STEMS OF *TAXUS CUSPIDATA*

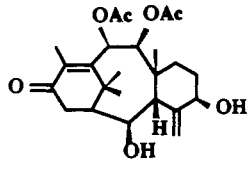
XIAO-JIE TONG, WEI-SHUO FANG, JIN-YUN ZHOU, CUN-HENG HE, WEI-MING CHEN, and QI-CHENG FANG*

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

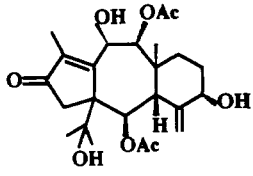
ABSTRACT.—In a continuing study on the chemical constituents of the needles and stems of *Taxus cuspidata*, three compounds were isolated. Based on spectral analysis, their structures were elucidated as the new taxane diterpenoids 2-deacetyltaxinine A [**1**], taxacustone [**2**], and 5α-*O*-(β-D-glucopyranosyl)-10β-benzoyltaxacustone [**3**], respectively.

Taxus cuspidata Sieb. et Zucc. (Taxaceae), is grown as an evergreen tree or cultivated as an ornamental shrub in China, Japan, and North America, and has been investigated since 1967 (1,2). Since the excellent antitumor activity and unique mechanism of action of the diterpenoid taxol (also known in the literature as paclitaxel) were discovered (3,4), efforts have continued to isolate taxol in high yields from biomass. The existence of taxol and its precursors in the regenerable needles of *Taxus* spp. plants (5) and the successful semi-synthesis of taxol and Taxotere® from 10-deacetylbaccatin III, obtained from the needles of *T. baccata* and *T. wallichiana* (6), have served to stimulate studies on the renewable parts of various *Taxus* spp. plants (7–10). Many taxoids and some other compounds, such as steroids and fatty acids, have been isolated from the needles, stems, seeds, and heartwood of *Taxus* spp. The discovery of an acceptable amount of taxol (ca. 0.006%) in the twigs and needles of *T. cuspidata* (11) encouraged us to study this species further.

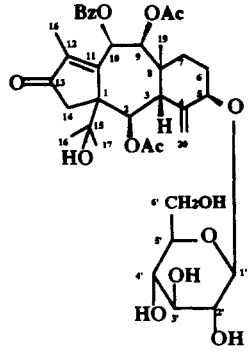
In a previous paper (12), we have reported twelve taxoids, including taxol and its precursor 10-deacetylbaccatin III, that were obtained from the CH_2Cl_2-partitioned portion of an EtOH extract of



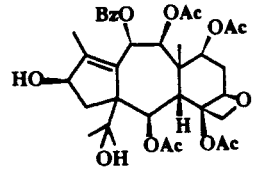
1



2



3



4

the needles and stems of *Taxus cuspidata*. In a continuing study of this extract, three new taxoids were isolated. The structures of these substances, namely, 2-deacetyltaxinine A [**1**], taxacustone [**2**], and 5α-*O*-(β-D-glucopyranosyl)-10β-benzoyltaxacustone [**3**], were elucidated on the basis of spectroscopic analysis and X-ray diffraction data.

Compound **1** was obtained as colorless plates when recrystallized from $CHCl_3$/MeOH. Its fabms showed an $[M+K]^+$ ion at *m/z* 473. Its composition $C_{24}H_{34}O_7$ was deduced by a combination of ^{1}H-nmr, ^{13}C-nmr, and fabms analysis. Comparing the spectroscopic data of compound **1** with those of taxinine A (7) and taxinine (1), we found that the ^{1}H-nmr spectrum of **1** was similar to that of taxinine A, except that an acetoxy group signal was not observed in **1** and the chemical shift of H-2 was shifted upfield from 5.54 ppm in taxinine to 4.31 ppm in compound **1** (see Tables 1 and 2).

Compound **2** occurred as colorless plates when recrystallized from MeOH. Its fabms showed an $[M+H]^+$ ion at *m/z* 451. This compound appeared as a dark-green spot on a Si gel plate after spraying with 10% H_2SO_4 in EtOH. We have found that only those taxoids whose skeletons consist of a 6/8/6 ring system with an oxirane ring at the C-4(20) position and those with a 5/7/6 ring system appear as dark-green spots after visualization in this manner. In the ^{1}H-nmr spectrum of **2**, two broad signals at δ 5.09 and 4.39 indicated the presence of an exomethylene moiety at the C-4(20) position, so we considered the possibility that **2** was a taxoid with a 5/7/6 ring system skeleton, in accordance with that of taxchinin A (13). We

TABLE 1. ^{1}H-Nmr (500 MHz, $CDCl_3$) Spectral Data of **1–3**.

Proton	Compound		
	1	**2**	**3**
H-1	2.34 dd (7.0, 2.4)		
H-2β	4.32 dd (6.5, 2.4)	5.67 br s	6.14 d (9.5)
H-3α	3.48 d (6.5)	3.17 br s	2.98 d (9.5)
H-5β	4.32 t (2.8)	4.23 br s	4.34 br s
H-6	1.64, 1.76 m	NA[a]	1.77, 1.93 m
H-7	1.64, 1.83 m	NA[a]	1.65, 1.82 m
H-9β	5.79 d (10.5)	5.94 d (9.0)	6.09 d (10.8)
H-10α	6.09 d (10.5)	4.69 br d (9.0)	6.58 d (10.8)
H-14α	2.26 d (19.8)	2.28 d (18.5)	2.48 d (18.5)
H-14β	2.80 dd (19.8, 7.0)	2.63 d (18.5)	2.76 d (18.5)
Me-16	1.69	1.05	1.22
Me-17	1.16	1.05	1.23
Me-18	2.21	2.03	1.88
Me-19	0.92	1.32	1.02
H-20	5.25 s, 5.57 s	4.39 s, 5.09 s	4.66 s, 5.21 s
OAc	2.04 s, 2.07 s	1.93 s, 2.13 s	1.78 s, 2.06 s
Ph-*p*			7.56 t (7.0)
Ph-*o*			7.88 d (7.5)
Ph-*m*			7.43 t (7.5)
H-1′			4.15 d (7.5)
H-2′			3.33 br t (7.5)
H-3′			3.45 br t (7.5)
H-4′			3.62 br t (7.5)
H-5′			3.24 m
H-6′			3.88 br s

[a]Could not be discerned because of overlapping signals.

TABLE 2. ^{13}C-Nmr Data (125 MHz, $CDCl_3$) of **1–3** and COLOC Nmr Spectral Data of **3**.

Carbon	Compound			
	1	**2**[a]	**3**	COLOC for **3**
C-1	51.52	65.07	65.02	H-3α, 10α, 14β, Me-16
C-2	68.44	68.90	68.09	
C-3	43.03	45.18	44.69	H-20
C-4	148.60	142.84	142.33	H-3α
C-5	76.22	80.29	78.82	H-20
C-6	31.22	31.49	28.89	
C-7	26.70	31.49	27.10	Me-19
C-8	45.02	42.92	42.41	Me-19
C-9	75.60	68.90	76.03	H-10α, Me-19
C-10	73.44	68.48	68.62	H-9β
C-11	149.78	150.24	161.32	H-14α, Me-18
C-12	138.21	135.80	147.33	H-14α, Me-18
C-13	200.21	207.57	209.67	H-14α, Me-18
C-14	35.82	44.06	44.49	
C-15	37.78	75.59	76.01	H-14α, 14β, Me-16, 17
C-16	25.49	27.91	27.90	Me-17
C-17	37.73	27.91	26.92	Me-16
C-18	14.39	8.32	8.98	
C-19	17.43	26.55	17.48	
C-20	114.70	109.46	115.60	H-5β
$COCH_3$	20.91	21.03	20.49	
$COCH_3$	170.22	170.75	170.04	
$COCH_3$	20.68	21.64	21.82	
$COCH_3$	169.64	171.17	171.33	
Ph-*p*			133.63	
Ph-*o*			129.57	
Ph-*m*			128.78	
PhCO			164.79	H-10α
C-1′			98.12	
C-2′			73.09	
C-3′			76.74	
C-4′			69.70	
C-5′			75.49	
C-6′			61.92	

[a]C_5D_5N was used as nmr solvent for **2**.

have isolated from *T. yunnanensis* four taxoids with the 5/7/6 ring system having an oxetane ring at the C-4(20), 5 position, named taxayuntin [**4**] and taxayuntins A, B, and C (14,15). The signals for C-1 and C-15 of the taxayuntins appear at about δ 68 and 75 ppm, respectively, whereas those of the taxoids with a 6/8/6 ring system with a hydroxy group at C-1 are observed at 70–75 ppm and C-15 at 40–45 ppm, respectively. A DEPT nmr spectrum of **2** showed the C-1 and C-15 signals, at 65.07 and 75.59 ppm, respectively. In the ^{1}H- and ^{13}C-nmr spectra, four methyl groups (δ 1.03, 1.03, 1.32, 2.03) and two acetyl groups (δ 1.93, 2.12) occurred, and a pair of doublets at δ 5.93 and 4.70 were attributed to protons at C-9 and C-10, respectively. The signal at δ 4.23 was ascribed to a hydroxy group attached to C-5. The structure of **2** was thus deduced to be that indicated, and this compound was named taxacustone. Its structure was confirmed by X-ray diffraction analysis (see Figure 1).

Compound **3** occurred as colorless needles when recrystallized from MeOH, and by fabms gave an $[M+Na]^+$ ion at *m/z* 739. Its spot on the Si gel plate after

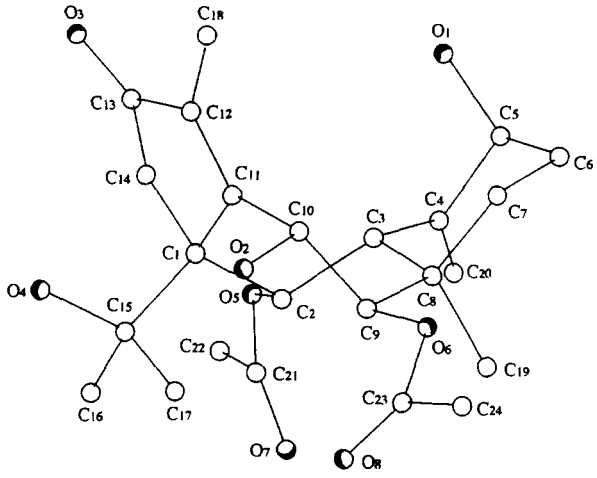


FIGURE 1. A computer-generated perspective drawing of the final model of compound **2**.

spraying with 10% H_2SO_4 in EtOH was the same color as those of taxacustone [**2**] and the taxayuntins. A detailed comparison between the ^{1}H- and ^{13}C-nmr spectra of **3** and those of taxacustone [**2**] revealed that the two compounds probably have the same skeleton with a 5/7/6 ring system and an exomethylene moiety at the C-4(20) position (see Tables 1 and 2). In a ^{13}C-^{1}H long-range correlation (COLOC) nmr experiment, correlations between C-15 and H-16, H-17, and C-1 and H-10 were observed, whereas a C-15 and H-10 correlation, always observed in taxoids with the 6/8/6 ring system, was not observed. A correlation between C-11, C-12, and H-14α was also observed, whereas only a correlation between C-12 and H-14 can be observed in taxoids with the 6/8/6 ring system. The appearance of signals for C-1 and C-15 of **3** at a similar position to those seen in taxacustone [**2**] made our deduction more reliable. A doublet at δ 6.14, correlated to the doublet at δ 2.98 (H-3α), was assigned as the signal of H-2β. A pair of doublets at δ 6.58 and 6.09, attributed to H-10α and H-9β, revealed the presence of an acetoxy or benzoyl group at C-10 and C-9. The COLOC spectrum indicated that the C-1 signal was correlated to a signal at the δ 6.58 doublet which in turn was associated with the signal at δ 68.62 in the ^{1}H-^{13}C correlation, so we designated the δ 6.58 doublet as the signal of H-10. The δ 6.09 signal, which correlated to the signal at δ 76.03, was thus ascribed to the signal of H-9. Rojas *et al.* have reported the ^{13}C-nmr spectra of several taxoids with oxirane and oxetane rings, in which the chemical shift of C-9 was at lower field than that of C-10 in all compounds when there are acetoxy groups at the C-9 and C-10 positions (16). In the spectra of the taxayuntins, the same phenomenon was observed. When C-9 and C-10 were both substituted with acetoxy or benzoyl groups, or if both have hydroxy groups, the signal of H-10 often appeared at lower field. The reason for this is probably that H-10 is deshielded by $\Delta^{11,12}$ (a similar rationale for H-18 appearing at lower field than the other methyl group holds). This is true for all taxoids, except for the taxagifine-type taxoids which lack a double bond between C-11 and C-12. Since the signal of -CO-Ph was correlated to the δ 6.58 doublet in the COLOC nmr spectrum of **3**, we assigned the benzoyl group as 10β. By comparison with the spectral data of **2**, we deduced that an oxy group was attached at the 5α position in **3**. The signals from δ 3.24 and 4.14 in the ^{1}H-nmr spectrum, representing six hydrogen atoms, showed the presence of a glucopyranosyl group. In the ^{1}H-^{1}H

COSY spectrum, we observed that these signals correlated to each other and not to other signals. The presence of a glucopyranosyl group in **3** was then confirmed by acidic hydrolysis of **3**. The R_f value for the spot of the sugar of **3** on a cellulose plate was the same as that of D-glucose. By comparing the ^{1}H-nmr spectral data of compound **3** with those of its acetylated derivative, we confirmed the existence of a glucopyranosyl group at the 5α position. The configuration of the glucoside was proposed as β-D-glucoside, because of the observed $J_{1',2'}$=7.5 Hz (J usually ca. 6–8 Hz) in **3**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on an Boetius melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Uv spectra were recorded on a Shimadzu UV-240 spectrophotometer and ir spectra on a Perkin-Elmer 683 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded using a Bruker AM-500 apparatus. Fabms spectra were recorded with a JMS-DX 300 MS spectrometer. Si gel for cc was obtained from Qingdao Marine Chemical Factory, People's Republic of China.

PLANT MATERIAL.—*T. cuspidata* was collected and identified by Mr. Yu-Min Huang, Shenyang Institute of Horticulture Sciences.

EXTRACTION AND ISOLATION.—A defatted 95% EtOH extract of the needles and stems of *T. cuspidata* was partitioned with CH_2Cl_2/H_2O. The CH_2Cl_2 extract (92 g) was eluted with a CH_2Cl_2/MeOH gradient on a Si gel column up to CH_2Cl_2-MeOH (95:5). Repeated low-pressure Si gel and medium-pressure reversed-phase cc gave compounds **1** (12 mg), **2** (80 mg), and **3** (50 mg).

2-Deacetyltaxinine A [**1**].—Obtained as colorless plates ($CHCl_3$/MeOH): mp 295–298°; $[\alpha]^{28}$D +147.8° (c=0.057, $CHCl_3$); uv (EtOH) λ max (log ε) 203 sh (3.82), 266 (3.78), 340 (2.57) nm; ir (dry film) ν max 3530, 3439, 2950–2850, 1737, 1677, 1374, 1237, 1180, 1024, 990, 920, 860, 605 cm^{-1}; fabms *m/z* $[M+K]^-$ 473 (100), $[M-H_2O]^+$ 416 (11), 297 (54), 165 (50), 163 (46), 161 (59), 154 (89), 151 (49); ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

Taxacustone [**2**].—Obtained as colorless plates (MeOH): mp 268–271°; $[\alpha]^{28}$D −14.6° (c=0.079, $CHCl_3$); uv (EtOH) λ max (log ε) 203 sh (3.73), 241 (4.04) nm; ir (dry film) ν max 3480, 3423, 2950–2830, 1735, 1710, 1687, 1434, 1374, 1261, 1176, 1023, 955, 909, 865, 848 cm^{-1}; fabms *m/z* $[M+Na]^+$ 473 (100), $[M+H]^+$ 451 (10), 273 (93), 255 (40), 235 (36); ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2, respectively; *anal.* found C 63.85%, H 7.33%, and O 28.82% (calcd C 63.96%, H 7.63%, and O 28.41%); hreims $[M-H_2O]^+$ *m/z* 432.2138 (calcd for $C_{24}H_{34}O_8$, 432.2138).

5α-O-(β-D-Glucopyranosyl)-10β-benzoyltaxacustone [**3**].—Obtained as colorless needles (MeOH); mp 178–180°; $[\alpha]^{28}$D −92.6° (c=0.074, $CHCl_3$); uv (EtOH) λ max (log ε) 202 sh (4.31), 233 (4.32), 283 sh (3.16) nm; ir (dry film) ν max 3510, 3427, 2938–2800, 1731, 1706, 1371, 1257, 1084, 1041, 1026, 715 cm^{-1}; fabms *m/z* $[M+Na]^+$ 739 (100), 663 (10), 621 (11), $[M-C_6H_{11}O_5+Na]^+$ 559 (15), 377 (35), 359 (41), 255 (98); ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

Hydrolysis of **3**.—One mg of compound **3** was added to 2 ml HCl in MeOH, and put in a H_2O bath for 30 min. The mixture was then spotted on a cellulose plate, and the H_2O solution of D-glucose was also spotted on the same plate. The plate was developed with *n*-BuOH-HOAc-H_2O (4:1:2) and then sprayed with aniline/oxalic acid in BuOH for visualization. The R_f value of spot sugar in **3** was the same as that of D-glucose.

Acetylation of **3**.—Three mg of compound **3** were acetylated using 0.5 ml of pyridine-Ac_2O (1:1) at room temperature for 24 h. ^{1}H nmr ($CDCl_3$, 500 MHz) δ 7.88 (2H, d, J=7.5 Hz, Ph-*o*), 7.56 (1H, t, J=7.0 Hz, Ph-*m*), 7.43 (2H, t, J=8.0 Hz, Ph-*p*), 6.58 (1H, br s, H-10α), 6.12 (1H, br s, H-2β), 6.08 (1H, br s, H-9β), 5.15 (1H, t, J=9.3 Hz, H-3′), 5.07 (1H, br s, H-20), 5.04 (1H, t, J=9 and 5 Hz, H-2′), 4.83 (1H, br s, H-4′), 4.48 (1H, br s, H-20), 4.47 (1H, d, J=9.5 Hz, H-1′), 4.22 (1H, dd, J=12.5 and 5.0 Hz, H-6′), 4.13 (1H, br d, J=12.5 Hz, H-6′), 3.66 (1H, m, H-5′), 2.94 (1H, br s, H-3α), 2.67 (1H, m, H-5β), 2.66 (1H, d, J=18.3 Hz, H-14β), 2.47 (1H, d, J=18.3 Hz, H-14α), 2.09 (6H, s, 2×OAc), 2.01 (6H, s, 2×OAc), 2.00 (6H, s, 2×OAc), 1.96 (6H, s, Me-18, 19), 1.19 (6H, s, Me-16, -17).

X-RAY CRYSTALLOGRAPHY.[1]—Taxacustone [**2**] crystallized in the orthorhombic system, space group $P2_12_12_1$ with one molecule of composition, $C_{24}H_{34}O_8$, forming the asymmetric unit. Accurate cell constants of a=10.547 (2) Å, b=12.198 (2) Å, c=18.076 (1) Å, v=2325.5 Å^3 were determined

[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB 2 1EZ, UK.

by a least-squares fit of 15 moderate angle 2σ values. All unique diffraction maxima with $2\theta<114°$ were collected on the Nicolet R3M/E X-ray system using graphite monochromated CuKα radiation (1.54178 Å) and variable speed ϖ scans. After correction for Lorentz, polarization, and background effects, 1520 of the 1803 unique reflections were judged observed ($|Fo|>3\sigma|Fo|$). The structure was solved routinely using direct method SAPI. Hydrogen atoms were located in difference electron density synthesis after least-squares refinement of the non-hydrogen atoms. The residual factors were R=0.075 for 1520 observed reflections.

ACKNOWLEDGMENTS

We acknowledge Mr. Yu-Min Huang for providing the plant material used for study. We also greatly thank Prof. Xiao-Tian Liang for helpful comments.

LITERATURE CITED

1. H.C. Chiang, M.C. Woods, Y. Nakadaira, and K. Nakanishi, *Chem. Commun.*, 1201 (1967).
2. M. Miyazaki, K. Shimizu, H. Mishima, and M. Kurabayashi, *Chem. Pharm. Bull.*, **16**, 546 (1968).
3. M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
4. P.B. Schiff, J. Fant, and S.B. Horwitz, *Nature*, **277**, 655 (1979).
5. K.M. Witherup, S.A. Look, M.W. Stasko, T.J. Ghiorzi, and G.M. Muschik, *J. Nat. Prod.*, **53**, 1249 (1990).
6. D.G.I. Kingston, *Pharmac. Ther.*, **52**, 1 (1991).
7. F. Yoshizaki, M. Madarame, C. Takahashi, and S. Hisamichi, *Shoyakugaku Zasshi*, **40**, 429 (1986).
8. F. Yoshizaki, M. Fukuda, S. Hisamichi, T. Ishida, and Y. In, *Chem. Pharm. Bull.*, **36**, 2098 (1988).
9. K. Nakano, T. Nohara, T. Tomimatsu, and M. Nishikawa, *Phytochemistry*, **21**, 2749 (1982).
10. X. Sun, X. Li, L. Wu, and Z. Xu, *Chin. J. Med. Chem.*, **2**, 31 (1992).
11. X.J. Tong, W.S. Fang, J.Y. Zhou, C.H. He, W.M. Chen, and Q.C. Fang, *Yaoxue Xuebao (Acta Pharm. Sin.)*, **29**, 55 (1994).
12. W.S. Fang, Y. Wu, J.Y. Zhou, W.M. Chen, and Q.C. Fang, *Phytochem. Anal.*, **4**, 115 (1993).
13. K. Fuji, K. Tanaka, B. Li, T. Shingu, H. Sun, and T. Taga, *Tetrahedron Lett.*, **33**, 7915 (1992).
14. C. Rao, J.Y. Zhou, W.M. Chen, Y. Lu, and Q.T. Zheng, *Chin. Chem. Lett.*, **4**, 693 (1993).
15. W.M. Chen, J.Y. Zhou, P.L. Zhang, and Q.C. Fang, *Chin. Chem. Lett.*, **4**, 699 (1993).
16. A.C. Rojas, D. de Marcarno, B. Mendez, and J. de Mendez, *Org. Magn. Reson.*, **21**, 257 (1983).

Received 1 December 1993