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TAXEZOPIDINES O AND P, NEW TAXOIDS FROM *TAXUS CUSPIDATA*

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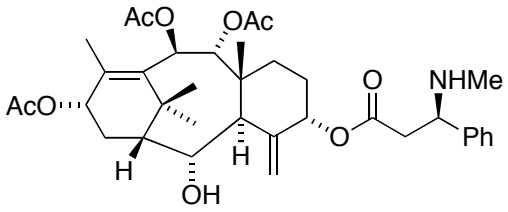
Abstract – Two new taxoids, taxezopidines O (**1**) and P (**2**), have been isolated from seeds of the Japanese yew *Taxus cuspidata*, and the structures and relative stereochemistry were elucidated on the basis of spectroscopic data. The absolute stereochemistry of a 3-*N*-methylamino-3-phenylpropanoyl group in **1** was determined by HPLC analysis for FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) derivative of hydrolysate of **1**.

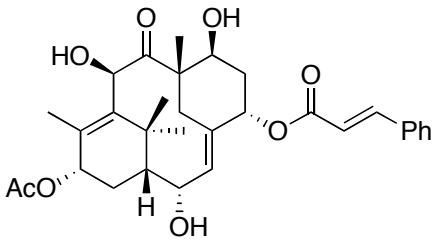
The discovery that taxol is an effective drug against ovarian and breast cancer has stimulated a renewed interest in the isolation of taxane diterpenoids from various species of yews.[1,2] In our continuing search for bioactive taxoids, we have isolated previously a series of new taxoids, taxuspines A~H and J-Z[3] and taxezopidines A-H and J-N,[4-7] from the stems, leaves, and seeds of the Japanese yew *Taxus cuspidata* Sieb. et Zucc. (Taxaceae). Further investigation on extracts of the seeds of this yew led to the isolation of two new taxoids, taxezopidines O (**1**) and P (**2**). In this paper, we describe the isolation and structure elucidation of **1** and **2**.

The methanolic extract of seeds of *T. cuspidata* collected in Sapporo was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. The $CHCl_3$ soluble portions were subjected to a silica gel column chromatography and then C_{18} HPLC to afford taxezopidine O (**1**) (0.00002%) together with known taxoids, taxinine, taxine II, 2-O-deacetyl taxine II,[8] and taxuspine Z.[3] The EtOAc soluble portions were subject to a silica gel column chromatography and C_{18} HPLC to give taxezopidine P (**2**) (0.00067%) together with a known taxoid, taxinine M.

Taxezopidine O (**1**) was obtained as a colorless amorphous solid, and the molecular formula was established to be $C_{36}H_{49}NO_9$ by HRESIMS [*m/z* 640.3486 $(M+H)^+$, Δ +0.0].



1



2

IR absorptions implied the presence of hydroxy (3460 cm^{-1}) and ester (1740 cm^{-1}) functionalities. Analysis of ^1H and ^{13}C NMR data and the HMQC spectrum revealed that **1** possessed eight sp^3 methines, four sp^3 methylenes, two sp^3 quaternary carbons, one sp^2 methylene, five sp^2 methines, eight sp^2 quaternary carbons, and eight methyl groups.

^1H and ^{13}C NMR data (Table 1) (δ_H 2.47, 3.05, 3.10, 4.37, and 7.48; δ_C 32.7, 42.8, 72.7, 129.8, 131.0, 131.1, 133.2, and 168.1) of **1** indicated the presence of a 3-*N*-(methylamino)-3-phenylpropanoyl group (nor-Winterstein's acid) at C-5, which was implied by an HMBC correlation for H-5 to C-21. Connectivities of C-1 to C-3, C-5 to C-7, C-9-C-10, C-13 to C-14, C-14 to C-1, and C-22 to C-23 were deduced from ^1H-^1H COSY and HOHAHA correlations (Figure 1). HMBC correlations (Figure 1) of H_3-16 to C-1 and C-15, H_3-17 to C-11 and C-15, and H_3-18 to C-11, C-12, and C-13 implied the presence of a cyclohexene moiety with Me-16 and Me-17 at C-15 and Me-18 at C-12 (ring A). Cross-peaks of H_3-19 to C-3, C-8, and C-9 and H-10 to C-15 in the HMBC spectrum provided the presence of an eight-membered ring with Me-19 at C-8 (ring B). ^1H-^{13}C long-range correlations of H-20 to C-3, C-4, and C-5 and H_3-19 to C-3, C-7 and C-8 were suggestive of the presence of a cyclohexane moiety with an exomethylene at C-4 (ring C). Three acetoxy groups were placed at C-9 (δ_H 5.86), C-10 (δ_H 5.99), and C-13 (δ_H 5.89) on the basis of the ^1H NMR chemical shifts, while an hydroxy group was attached to C-2 (δ_H 4.23) from comparison of the ^1H NMR chemical shifts of H-9, H-10, and H-13 with those of H-2. Thus, the gross structure of taxezopidine O was elucidated to be **1**.

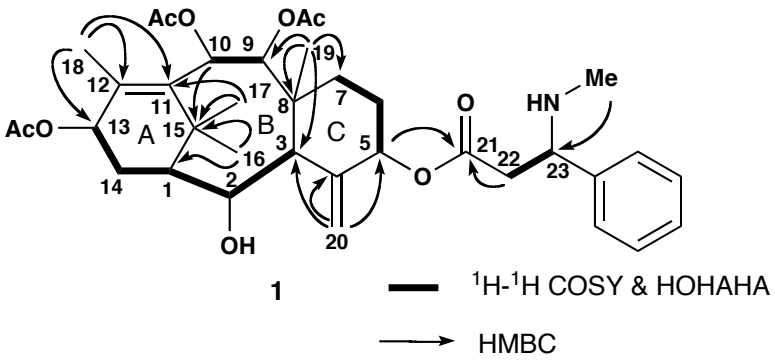


Figure 1. Selected two-dimensional NMR correlations for taxezopidine O (**1**)

The relative stereochemistry of **1** was deduced from NOESY correlations (Figure 2). A boat-chair conformation of the eight-membered ring (C-1—C-3, C-8—C-11, and C-15) was elucidated from the coupling constant (10.7 Hz) between H-9 and H-10 and NOESY correlations. NOESY correlations of H_3-18 to H-3 and H-22a indicated a cage-like backbone conformation.

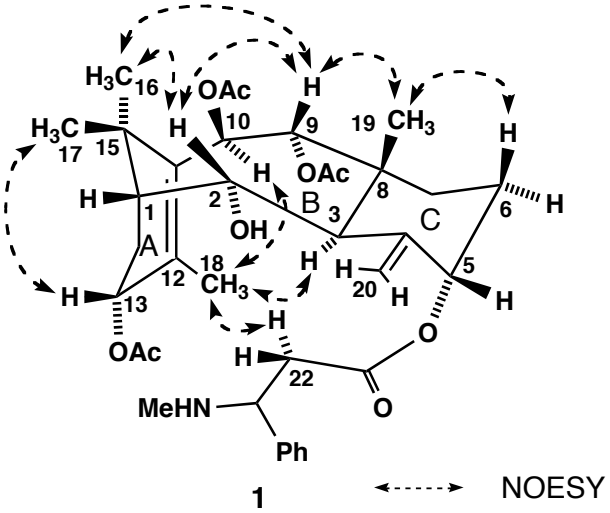


Figure 2. Selected NOESY correlations and relative stereochemistry for taxezopidine O (**1**)

The absolute stereochemistry of an 3-*N*-methylamino-3-phenylpropanoyl group in **1** was determined to be *R* by HPLC analysis for FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) derivative of hydrolysate of **1**.

Taxezopidine P (**2**) was obtained as a colorless amorphous solid, and HRESIMS analysis revealed the molecular formula to be $C_{31}H_{38}O_8$ [*m/z* 573.2280 $(M+Cl)^-$, Δ –2.6 mmu]. IR absorptions (3420 and 1720 cm^{-1}) implied the presence of hydroxy and ester functionalities. The ^1H NMR (Table 2) spectrum of **2** showed proton signals due to four methyls (δ_H 1.15, 1.18, 1.28, 1.90), one acetyl methyl (δ_H 1.96), and an olefin (δ_H 5.40). The ^{13}C NMR (Table 2) spectrum showed signals due to six sp^3 methines, three sp^3 methylenes, two sp^3 quaternary carbons, eight sp^2 methines, seven sp^2 quaternary carbons, and five methyl groups. Proton signals due to a cinnamoyl group appeared at δ_H 7.39-7.49 (5H, m), 7.79 (1H, d, *J* = 15.9 Hz), and 6.49 (1H, d, *J* = 15.9 Hz; *trans*-oriented). The UV absorption at 279 nm supported the presence of a cinnamoyl group. Detailed analysis of the ^1H-^1H COSY spectrum revealed connectiveities of C-14 to C-1, C-1 to C-3, C-5 to C-7, and C-22 to C-23. HMBC correlations (Fig. 3) of H-11 to C-12, C-13 and C-16, H-14 to C-12 and C-13, and H_3-19 to C-13 indicated the presence of a cyclohexene moiety with Me-19 at C-13 (ring A). Cross-peaks of H-3 to C-9, H-11 to C-10 (δ_C 214.2), H_3-17 to C-1 and C-16, H_3-18 to C-16, and H_3-20 to C-8 and C-10 in the HMBC spectrum indicated the presence of a ten-membered ring with Me-17 and Me-18 at C-16 (ring B). ^1H-^{13}C long-range correlations of H-5 to

C-4, H-9a to C-5 and C-7, and H_3-20 to C-7 indicated the presence of a cyclohexane moiety with Me-20 at C-8 (ring C). A carbonyl carbon at δ_C 166.4 (C-21) showed a correlation with H-5 in the HMBC spectrum, indicating the presence of a cinnamate group at C-5. An acetoxy carbonyl carbon (δ_C 170.5) showed an HMBC correlation with H-14, indicating that the acetoxy group was attached to C-14. Thus, the gross structure of taxezopidine P was assigned as **2**.

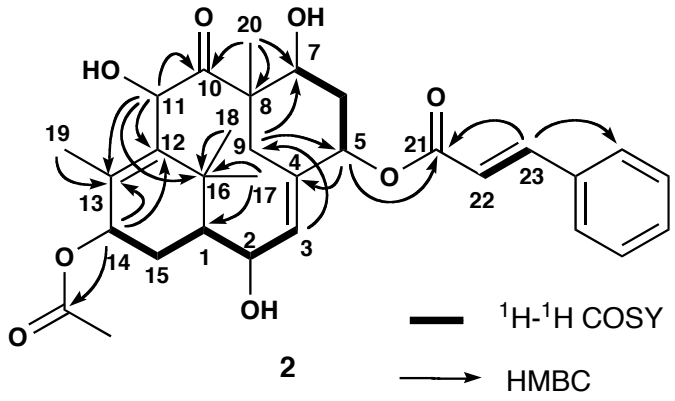


Figure 3. Selected two-dimensional NMR correlations for taxezopidine P (**2**)

Relative stereochemistry of **2** was elucidated by the NOESY spectrum. NOESY correlations of H_3-17/H-1, H_3-17/H-14, H-6b/H5, H-6b/H20, and H7/H-6a indicated pseudo boat conformations of rings A and C (Fig. 4).

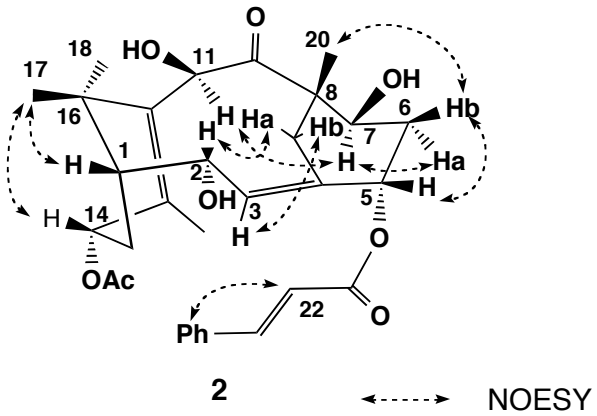


Figure 4. Selected NOESY correlations and relative stereochemistry for taxezopidine P (**2**)

To our knowledge, taxezopidine O (**1**) is the first taxoid in which the absolute stereochemistry of a 3-*N*-methylamino-3-phenylpropanoyl group in **1** was determined by degradation experiments.

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Taxezopidine O (**1**) in CD_3OD.

position	$^{1}H^{a}$	$^{13}C^{a}$
1	2.00 (1H, brs)	53.8
2	4.23 (1H, dd, 1.8, 6.0)	72.1
3	2.98 (1H, d, 5.9)	47.2
4		144.5
5	5.27 (1H, s)	82.3
6	1.50 (2H, m)	30.8
7a	1.64 (1H, m)	29.2
7b	1.41 (1H, m)	
8		47.8
9	5.86 (1H, d, 10.7)	79.0
10	5.99 (1H, d, 10.7)	74.4
11		135.2
13	5.89 (1H, t, 8.33)	63.7
14a	2.56 (1H, dt, 14.9, 7.6)	34.0
14b	2.56 (1H, dt, 14.9, 7.6)	
15		40.0
16	1.70 (3H, s)	28.4
17	1.17 (3H, s)	31.5
18	2.12 (3H, s)	16.9
19	0.91 (3H, s)	19.2
20a	5.75 (1H, s)	122.1
20b	5.34 (1H, s	
21		168.1
22a	3.05 (1H, dd, 7.5, 14.9)	42.8
22b	3.10 (1H, dd, 7.0, 14.9)	
23	4.37 (1H, t, 6.1)	72.7
2-OAc	1.93 (3H, s)	22.4, 173.2
9-OAc	2.08 (3H, s)	21.5, 173.0
10-OAc	2.12 (3H, s)	21.7, 172.5
Ph	7.48 (5H, m)	129.8, 131.0, 131.1, 133.2
23-NHMe	2.47 (3H, s)	32.7

a δ in ppm.

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

position	^{1}H[a]	^{13}C[a]
1	1.65 (1H, m)	49.6
2	4.55 (1H, d, 9.6)	67.8
3	5.40 (1H, d, 9.6)	47.2
4		131.1
5	5.55 (1H, d, 7.2)	70.7
6a	1.81 (1H, m)	36.3
6b	2.37 (1H, ddd, 15.6, 13.2, 7.2)	
7	4.21 (1H, dd, 13.2, 3.6)	
8		53.2
9a	1.81 (1H, m)	35.0
9b	2.67 (1H, d, 15.6)	
10		214.2
11	5.33 (1H, s)	77.1
12		132.9
13		134.7
14	5.43 (1H, d, 10.8)	69.7
15a	1.85 (1H, m)	26.2
15b	2.63 (1H, m)	
16		37.6
17	1.15 (3H, s)	24.6
18	1.18 (3H, s)	32.8
19	1.90 (3H, s)	16.6
20	1.28 (3H, s)	18.5
21		166.4
22	6.49 (1H, d, 15.9)	117.5
23	7.79 (1H, d, 15.9)	146.3
14-OAc	1.96 (3H, s)	21.3, 170.5
Ph	7.39-7.49 (5H, m)	128.0, 129,0, 130.8, 133.9

[a] δ in ppm.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were recorded on a Shimadzu UV1600PC spectrometer. ^{1}H and 2D NMR spectra were recorded on a 600 MHz NMR spectrometer at 300K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Samples were prepared by dissolving in 30 μL of CD_3OD or $CDCl_3$ in 2.5 mm micro cells (Shigemi Co. Ltd., Tokyo, Japan), and chemical shifts were reported using residual CH_3OH (δ_H 3.35: δ_C 49.5) and $CHCl_3$ (δ_H 7.26: δ_C 77.0) as internal standard. Standard pulse sequences were employed for 2D NMR experiments. ^{1}H-^{1}H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase-sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra, a 50 ms delay time was used for long-range C-H coupling. Zerofilling to 1 K for F_1 and multiplication with squared cosinebell windows shifted in both dimensions were performed prior to 2D Fourier transformations.

Plant Material. The Japanese yew *Taxus cuspidata* Sieb. et Zucc. was collected at Sapporo, Hokkaido, in 2003. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen (No. 030001) has been deposited in the herbarium of Hokkaido University.

Extraction and Isolation. The MeOH extract (1373 g) of seeds (10 kg) of the yew was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, after being adjusted at pH 10 with saturated aqueous Na_2CO_3, were partitioned with $CHCl_3$. The $CHCl_3$-soluble materials (3.8 g) were subjected to a silica gel column ($CHCl_3$/MeOH/EtOAc, 10:1:0.5→MeOH→MeOH/TFA, 100:1) and then C_{18} HPLC (LUNA C18(2), 5 μm, Phenomenex,10 x 250 mm; flow rate 2.0 mL/min; UV detection at 220 nm; eluent, 35% MeCN-0.1% TFA) to afford taxezopidine O (**1**) (0.3 mg, 0.00002%). The EtOAc-soluble materials (4.0 g) were separated by silica gel columns ($CHCl_3$/MeOH, 95:1→MeOH, and hexane/acetone, 9:1→acetone) and then C_{18} HPLC (YMC-Pack Pro C18, YMC Co., Ltd., 10 x 250 mm; flow rate 2.0 mL/min; UV detection at 220 nm; eluent, 70% CH_3OH) to yield taxezopidine P (**2**) (3.6 mg, 0.00067%).

Taxezopidine O (1): colorless amorphous solid; $[\alpha]^{23}_{D}$ +11 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.98), 210 nm (2.94); IR (film) ν_{max} 3460, 2930, 1740 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS *m/z* 640 $(M+H)^+$; HRESIMS *m/z* 640.3486 $(M+H)^+$, calcd for $C_{36}H_{50}NO_9$ 640.3486.

Taxezopidine P (2): colorless amorphous solid; $[\alpha]^{23}_{D}$ -17 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 279 (3.99), 203 nm (4.18); IR (film) ν_{max} 3420, 1720, 1240 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); ESIMS *m/z* 573 $(M+Cl)^-$; HRESIMS *m/z* 573.2280 $(M+Cl)^-$, calcd for $C_{31}H_{38}O_8$ 573.2306.

Determination of Absolute Configuration of 3-*N*-Dimethylamino-3-phenylpropionic Acid in Taxezopidine O (1). Taxezopidine O (**1**) (50 μg) was refluxed in 10% aqueous HCl (0.1 mL) for 1 h. The reaction mixture was extracted with Et_2O (0.1 mL x 2), and the aqueous layer was concentrated under reduced pressure. An aqueous solution of the hydrolysates of **1** in H_2O (25 μL) was treated with 1 M $NaHCO_3$ (10 μL) and 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone (50 μL) at 40 °C for 8h. The reaction was quenched by addition of 2 N HCl (10 μL). The reaction mixture was diluted with 100 μL of MeCN, and an aliquot was applied to C_{18} HPLC (YMC-Pack Pro C18, YMC Co., Ltd., 4.6 x 250 mm) using 45% MeCN/0.1% TFA as the mobile phase at flow rate 0.5 mL/min. FDAA derivatives of amino acids were detected by absorption at 340 nm and compared with authentic samples. Retention times (min) are given in parentheses: authentic (3*SR*)-3-*N*-(methylamino)-3-phenylpropanoic acids (19.2 and 23.2) and (3*S*)-3-*N*-(methylamino)-3-phenylpropanoic acid (23.2). The retention time (min) of FDAA derivative of hydrolysates of **1** was 19.2.

ACKNOWLEDGEMENTS

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