Taxezopidines M and N, Taxoids from the Japanese Yew, *Taxus cuspidata*

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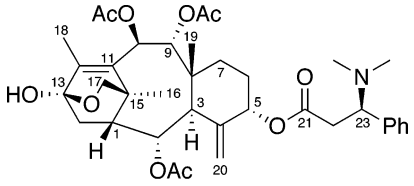
Two new taxoids, taxezopidines M (**1**) and N (**2**), have been isolated from seeds of the Japanese yew, *Taxus cuspidata*, and new structures were elucidated on the basis of spectroscopic data. The absolute configuration of a 3-*N,N*-(dimethylamino)-3-phenylpropanoyl group in **1** and **2** was determined to be *R* in each case by chiral HPLC analysis. The effect of **1** and **2** on the $CaCl_2$-induced depolymerization of microtubules was investigated.

Since the discovery of anticancer activity of Taxol (paclitaxel), much attention has been paid to the isolation of new 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−L,[4−6] from the stems, leaves, and seeds of the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. (Taxaceae). Further investigation on an extract of the seeds of this yew has led to the isolation of two new taxoids, taxezopidines M (**1**) and N (**2**). In this paper, the isolation, structure elucidation, and biological evaluation of **1** and **2** are described.

The methanolic extract of seeds of *T. cuspidata* collected at 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 materials were subjected to silica gel column chromatography and then C_{18} HPLC to afford taxezopidines M (**1**, 0.0001%) and N (**2**, 0.0001%) together with the known taxoids 2-*O*-deacetyl taxine II (0.0001%)[7] and 1-deoxytaxine B (0.0014%).[8]



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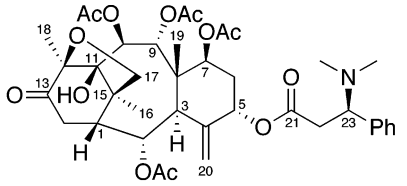

2

Taxezopidine M (**1**) was obtained as a colorless solid, and the molecular formula was established to be $C_{37}H_{49}NO_{10}$ by HRFABMS [*m/z* 668.3437 $(M + H)^+$, Δ +0.2 mmu]. IR absorptions implied that **1** possesses hydroxy (3440 cm^{-1}) and ester (1730 cm^{-1}) groups. Analysis of the ^1H and ^{13}C NMR data and the HMQC spectrum provided evidence that **1** possesses three acetyl groups, one tetrasubstituted olefin, one exomethylene, one hemiacetal carbon, four oxymethines, one oxymethylene, five methyl groups, four methylenes, three methines, one ester carbonyl, and six aromatic carbons.

The ^1H and ^{13}C NMR (Table 1) signals (δ_H 2.82, 3.28, 3.64, 4.83, and 7.53; δ_C 37.0, 41.8, 67.8, 132.1, and 170.5) of **1** indicated the presence of a 3-*N,N*-(dimethylamino)-3-phenylpropanoyl group (Winterstein's acid)[9] at C-5, which was implied by a HMBC correlation for H-5 to C-21. Connectivities of C-1 to C-3, C-5 to C-7, C-9 to C-10, C-14 to C-1, and C-22 to C-23 were deduced from the $^1H-^1H$ COSY and HOHAHA (Figure 1) spectra. HMBC correlations (Figure 1) of H_3-18 to C-11, C-12, and C-13 and from H_3-16 to C-1, C-11, and C-15 revealed that Me-18 and Me-16 are attached to C-12 and C-15, respectively. Three acetoxy groups were placed at C-2, C-9, and C-10 on the basis of HMBC correlations, while a hydroxy group was attached to C-13 from comparison of the ^{13}C NMR chemical shift of C-13 (δ 97.2) with those of hemiacetal carbons (δ 96−98) reported previously.[10] Connectivities among C-3, C-7, C-9, and Me-19 through C-8 were provided by HMBC correlations for H_3-19 to C-3, C-7, C-9, and C-8. The HMBC correlation of H-10 to C-15 revealed the connection between C-10 and C-11. The exomethylene was inserted between C-3 and C-5 by HMBC correlations of H_2-20 to C-3 and C-5. Thus, the gross structure of taxezopidine M was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY data (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. The NOESY correlation between H-1 and H-17a indicated that both protons were quasi-axial and the oxymethylene (C-17) at C-15 was β-oriented, while the correlations of H-3 to H-14a and H_3-18 revealed that **1** has a cage-like backbone conformation.

Taxezopidine N (**2**) was shown to have the molecular formula $C_{39}H_{51}NO_{13}$ by HRFABMS [*m/z* 742.3442 $(M + H)^+$, Δ −0.4 mmu]. The IR spectrum indicated the presence of hydroxy (3360 cm^{-1}) and ester (1740 cm^{-1}) groups. The ^1H and ^{13}C NMR (Table 1) spectroscopic data of **2** resembled those of taxezopidine L.[6]

An HMBC correlation (Figure 3) between H-17a and C-12 and the proton signals (δ_H 4.12 and 3.82, d, J = 8.1 Hz; H-17a and H-17b) at C-17 revealed the presence of a tetrahydrofuran ring (C-11, C-12, C-15, C-17, and O-1) fused to a six-membered ring (C-1 and C-11−C-15). The ^1H and ^{13}C NMR signals of C-21−C-29 (δ_H 2.88, 3.02, 3.64, 3.72, 5.00, and 7.61; δ_C 35.9, 40.8, 67.5, 130.6, 130.9, 132.9,

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Table 1. ^{1}H and ^{13}C NMR Data of Taxezopidines M (**1**) and N (**2**) in CD_3OD[a]

	1		**2**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	2.14 (1H, m)	49.2	2.54 (1H, m)	50.6
2	5.53 (1H, d, 4.9)	71.3	5.59 (1H, m)	69.9
3	2.96 (1H, d, 4.9)	43.7	3.29 (1H, m)	41.5
4		142.6		140.8
5	5.21 (1H, brs)	81.1	5.17 (1H, m)	77.9
6a	1.76 (1H, m)	28.9	1.93 (1H, m)	37.1
6b	1.76 (1H, m)		1.55 (1H, m)	
7	1.76 (2H, m)	28.6	5.29 (1H, m)	69.5
8		44.8		47.7
9	5.75 (1H, d, 10.7)	77.5	5.27 (1H, d, 2.6)	75.4
10	6.07 (1H, d, 10.7)	71.2	5.35 (1H, d, 2.6)	65.4
11		130.4		82.2
12		143.4		93.2
13		97.2		209.3
14a	2.14 (1H, m)	35.8	3.23 (1H, m)	36.6
14b	1.48 (1H, d, 15.4)		2.51 (1H, m)	
15		38.6		50.4
16	1.49 (3H, s)	17.6	1.53 (3H, s)	16.0
17a	3.52 (1H, d, 8.1)	75.2	4.12 (1H, d, 8.1)	82.7
17b	3.08 (1H, d, 8.1)		3.82 (1H, d, 8.1)	
18	2.27 (3H, s)	12.5	1.12 (3H, s)	12.3
19	0.89 (3H, s)	17.7	1.05 (3H, s)	13.2
20a	5.32 (1H, s)	119.9	5.37 (1H, s)	117.0
20b	5.12 (1H, s)		4.63 (1H, s)	
21		170.5		169.0
22a	3.64 (1H, m)	37.0	3.72 (1H, m)	35.9
22b	3.28 (1H, m)		3.64 (1H, m)	
23	4.83 (1H, m)	67.8	5.00 (1H, m)	67.5
OAc-2	2.04 (3H, s)	21.3	1.99 (3H, s)	21.2
		171.2		170.7
OAc-7			2.17 (3H, s)	21.4
				171.6
OAc-9	2.07 (3H, s)	20.8	2.12 (3H, s)	20.9
		172.0		172.4
OAc-10	2.00 (3H, s)	20.6	2.02 (3H, s)	21.2
		171.4		171.1
Ph	7.53 (5H, m)	132.1	7.61 (5H, m)	130.6
				130.9
				132.9
NMe_2-23	2.82 (6H, m)	41.8	3.02 (3H, m)	40.8
			2.88 (3H, m)	40.8

[a] δ in ppm.

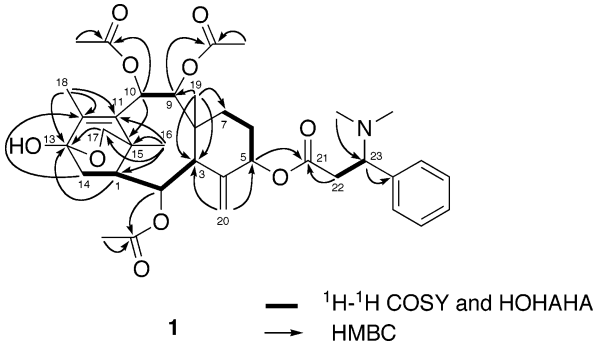

Figure 1. Selected two-dimensional NMR correlations for taxezopidine M (**1**).

and 169.0) indicated the presence of a 3-*N*,*N*-(dimethylamino)-3-phenylpropanoyl group (Winterstein's acid)[9] at C-5, which was implied by the HMBC correlation from H-5 to C-21. Four acetoxy groups were attached to C-2, C-7, C-9, and C-10 based on the HMBC correlations and oxymethine proton resonances (δ_H 5.59, H-2; 5.29, H-7; 5.27, H-9; and 5.35, H-10). The presence of a ketone (δ_C 209.3) at C-13 was deduced from HMBC correlations of H_3-18 and H-1 to C-13. HMBC correlations of H-20b to C-3 and C-5 indicated the presence of an exomethylene at C-4. Thus, the gross structure of taxezopidine N was assigned as **2**.

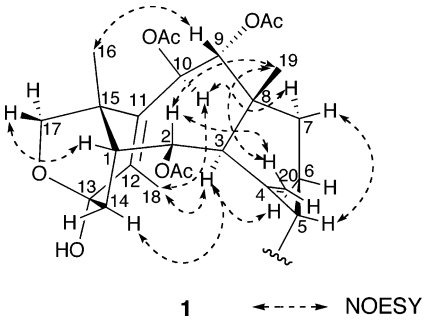

Figure 2. Selected NOESY correlations and relative stereochemistry for taxezopidine M (**1**).

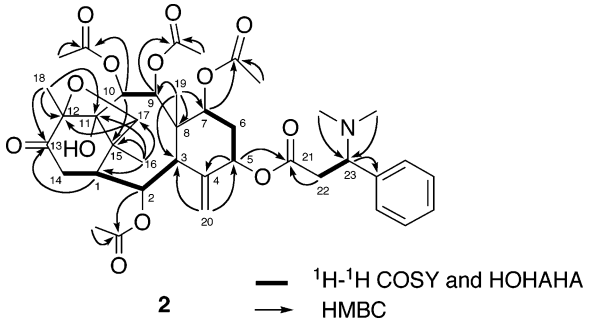

Figure 3. Selected two-dimensional NMR correlations for taxezopidine N (**2**).

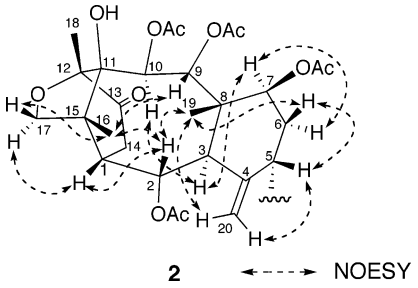

Figure 4. Selected NOESY correlations and relative stereochemistry for taxezopidine N (**2**).

The relative stereochemistry of **2** was elucidated by NOESY data (Figure 4) and ^{1}H−^{1}H coupling constants. The absolute stereochemistry of a 3-*N*,*N*-(dimethylamino)-3-phenylpropanoyl group (Winterstein's acid) in taxezopidines M (**1**) and N (**2**) was determined to be *R* in both cases by chiral HPLC analysis (Sumichiral OA-5000) of the acid hydrolysates of **1** and **2**.

Microtubules polymerized in the presence of paclitaxel are resistant to depolymerization by Ca^{2+} ions.[11] The effect of taxezopidines M (**1**) and N (**2**) was examined against the $CaCl_2$-induced depolymerization of microtubules. Microtubule proteins were polymerized under normal polymerization conditions[12] in the absence and the presence of paclitaxel, and after a 10 min incubation, $CaCl_2$ was added. Microtubule polymerization and depolymerization were monitored by the increase and decrease in turbidity. The results are summarized in Figure 5 as the changes in the relative absorbance at 400 nm. The $CaCl_2$-induced depolymerization of microtubules was inhibited by 5 μM paclitaxel. Taxezopidine M (**1**) reduced the depolymerization process at higher concentration (50 μM), while taxezopidine N (**2**) at the same concentration showed little effect. Taxezopidines M (**1**) and N (**2**) did not show cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells at 10 μg/mL.

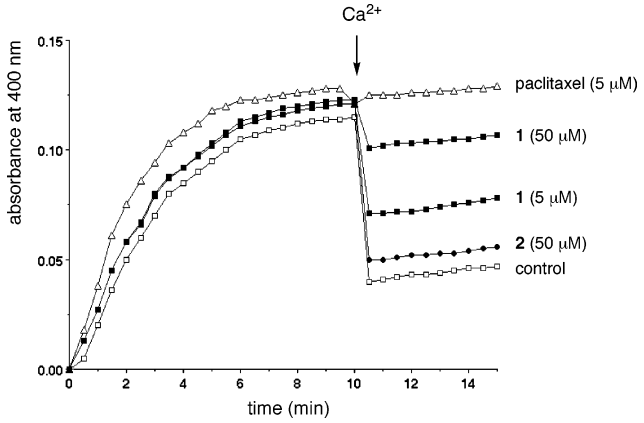

Figure 5. Effect of taxezopidines M (**1**) and N (**2**) on Ca^{2+}-induced microtubule depolymerization. Temperature was held at 37 °C, and changes in turbidity were monitored at 400 nm, with 4 mM $CaCl_2$ added at 10 min.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were recorded on a Shimadzu UV1600PC spectrophotometer and IR spectra on a JASCO FTIR-230 spectrometer. ^1H and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Compounds **1** and **2** were prepared by dissolving 1.0 mg in 30 μL of CD_3OD in 2.5 mm micro cells (Shigemi Co. Ltd., Tokyo, Japan), and chemical shifts were reported using residual CH_3OH (δ_H 3.31 and δ_C 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. $^1H-^1H$ COSY, HOHAHA, and NOESY NMR 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 with the *Z*-axis PFG, a 50 ms delay time was used for long-range C−H coupling. Zero-filling to 1 K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. FABMS was measured on a JEOL HX-110 spectrometer by using a glycerol matrix.

Plant Material. The Japanese yew *T. 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 the 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 Na_2CO_3, were partitioned with $CHCl_3$. The $CHCl_3$-soluble materials (3.8 g) were subjected to passage over a silica gel column (MeOH−$CHCl_3$−EtOAc, 100:1:0.5 → MeOH → MeOH−TFA, 100:1) and then C_{18} HPLC (YMC-Pack ODS AM-323, 5 μm, 20 × 250 mm; flow rate 8.0 mL/min; UV detection at 254 nm; eluent, 30−40% CH_3CN−0.1% TFA) to afford taxezopidines M (**1**, 1.2 mg) and N (**2**, 2.2 mg), 2-*O*-deacetyltaxine II (1.2 mg), and 1-deoxytaxine B (14.3 mg).

Taxezopidine M (1): colorless solid; $[\alpha]^{23}_D$ +30° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.0), 204 nm (4.0); IR (film) ν_{max} 3440, 1730, 1240 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 668 $(M + H)^+$; HRFABMS *m/z* 668.3437 $(M + H)^+$, calcd for $C_{37}H_{50}NO_{10}$ 668.3435.

Taxezopidine N (2): colorless solid; $[\alpha]^{23}_D$ +16° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.0), 205 nm (3.9); IR (film) ν_{max} 3360, 1740, 1230 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 742 $(M + H)^+$; HRFABMS *m/z* 742.3442 $(M + H)^+$, calcd for $C_{39}H_{52}NO_{13}$ 742.3446.

Determination of Absolute Configuration of 3-*N,N*-Dimethylamino-3-phenylpropionic Acid in Taxezopidines M (1) and N (2). Taxezopidine M (**1**; 0.2 mg) was refluxed in 10% aqueous HCl (0.1 mL) for 1 h. The reaction mixture was extracted with Et_2O (1 mL × 2), and the aqueous layer was concentrated under reduced pressure. The residue was dissolved in H_2O for chiral HPLC analysis. The chiral HPLC analysis was carried out using a Sumichiral OA-5000 column (Sumitomo Chemical Industry, 0.46 × 15 cm; flow rate 1.0 mL/min; UV detection at 254 nm; eluent 2.0 mM $CuSO_4$ in H_2O-CH_3OH, 97:3). The retention times of standard 3-(*S*)- and 3-(*R*)-*N,N*-(dimethylamino)-3-phenylpropanoic acids[13] were 12.8 and 14.7 min, respectively, and that of 3-*N,N*-(dimethylamino)-3-phenylpropanoic acid contained in the hydrolysate of **1** was found to be 14.7 min. According to the same procedure as described above, the retention time of 3-*N,N*-(dimethylamino)-3-phenylpropanoic acid contained in the hydrolysate of taxezopidine N (**2**) was found to be 14.7 min.

Microtubule Assembly Assay.[14] Microtubule assembly was monitored spectroscopically using a spectrophotometer equipped with a thermostatically regulated liquid circulator. The temperature was held at 37 °C, and changes in turbidity were monitored at 400 nm. The turbidity changes were monitored throughout the incubation time.

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