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Taxuspines N, O, and P, New Taxoids from Japanese Yew *Taxus cuspidata*

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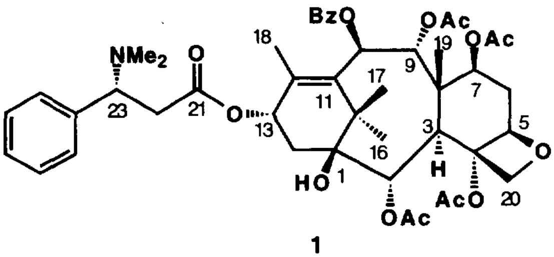
Abstract: Three new taxoids, taxuspines N ~ P (**1** ~ **3**), have been isolated from stems of the Japanese yew *Taxus cuspidata* Sieb. et Zucc. and the structures elucidated on the basis of spectroscopic data. Taxuspine N (**1**) is the first example of a taxane diterpene containing a 6/8/6-membered ring system with a 3-*N,N*-dimethylamino-3-phenylpropanoyl group (Winterstein's acid) at C-13 from yew trees. The absolute configuration of the 3-*N,N*-dimethylamino-3-phenylpropanoyl group in **1** and **3** was determined to be *R* by chiral HPLC analysis.

On account of promising anticancer activity of taxol and some related compounds, considerable attention has been given to the taxoids. Chemical studies on constituents of different yew trees have resulted in isolation of a large number of taxoids.[1] In our continuing search for bioactive natural products, we previously isolated some new taxoids, taxuspines A ~ H and J ~ M,[2~5] from the Japanese yew *Taxus cuspidata* Sieb. et Zucc. Further investigation on extracts of stems of this yew led to isolation of three new taxoids, named taxuspines N ~ P (**1** ~ **3**). In this paper we describe the isolation and structure elucidation of **1** ~ **3**.



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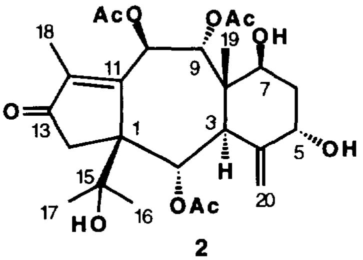

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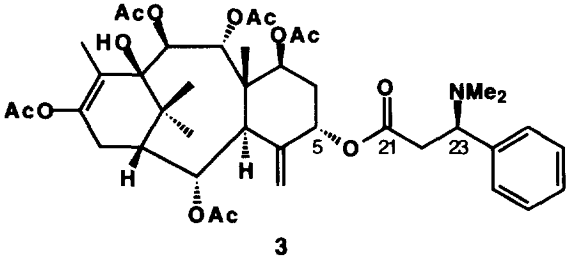

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Table 1. ^1H and ^{13}C NMR Data of Taxuspine N (**1**) in $CDCl_3$

position	$^1H^a$		J(Hz)	$^{13}C^a$		position	$^1H^a$		J(Hz)	$^{13}C^a$	
1				75.9	s	21				169.8	s
2	6.19	d	7.9	65.7	d	22(a)	2.80	dd	14.0, 8.1	38.6	t
3	3.03	d	7.9	45.0	d	(b)	3.09	dd	14.0, 8.1		
4				81.6	s	23	4.09	br.t	8.1	65.7	d
5	5.01	d	8.0	84.7	d	Ph	7.30	m		128.2	d
6(a)	1.69	m		37.1	t					128.3	d
(b)	2.35	m								128.3	s
7	5.53	t	7.8	80.4	d					128.6	d
8				44.0	s	NMe_2	2.22	s		41.8	q
9	6.12	d	10.9	76.3	d	AcO	1.75	s		21.4	q
10	6.50	d	10.9	68.6	d		2.00	s		21.6	q
11				132.4	s		2.09	s		22.6	q
12				135.6	s		2.17	s		22.6	q
13	5.57	m		70.7	d					170.1	s
14(a)	1.94	m		36.0	t					171.6	s
(b)	2.57	m								171.7	s
15				44.0	s					172.1	s
16	1.16	s		25.3	q	BzO	7.82	m		133.4	d
17	1.10	s		29.0	q		7.55	m		137.7	d
18	1.66	s		14.1	q		7.42	m		129.5	d
19	1.69	s		20.6	q					128.8	s
20(a)	4.39	d	7.7	75.5	t					164.4	s
(b)	4.48	d	7.7								

a) δ in ppm

The chloroform soluble portions of the methanolic extract of stems of the yew were subjected to silica gel and reversed-phase column chromatographies and centrifuged counter-current chromatography to afford taxuspines N (**1** 0.00073 %), O (**2**, 0.00062 %), and P (**3**, 0.0023 %).

Taxuspine N (**1**) was shown to have the molecular formula, $C_{46}H_{57}O_{14}N$, by HRFABMS [*m/z* 848.3839 $(M+H)^+$, Δ -1.8 mmu]. IR absorptions of 3450 and 1730 cm^{-1} implied that **1** possessed hydroxy and ester groups. Analyses of the ^1H and ^{13}C NMR data (Table 1) and HMQC spectrum of **1** provided four acetyls, one benzoyl, one ester carbonyl, one tetrasubstituted olefin, one phenyl, one oxymethylene, six oxymethines, two oxygenated quaternary carbons, two quaternary carbons, three methylenes, two methines, one dimethylamino group, and four methyls. The ^1H and ^{13}C NMR signals (δ_H 2.22, 2.80, 3.09, 4.09, and 7.30; δ_C 38.6, 41.8, 65.7, and 128.2~128.6) indicated the presence of a 3-*N*,*N*-dimethylamino-3-phenylpropanoyl group (Winterstein's acid[6]). Prominent fragment ion peaks at *m/z* 134 and 192, and 105 in the EIMS corresponded to fission of a 3-*N*,*N*-dimethylamino-3-phenylpropanoyl and a benzoyl groups from **1**, respectively. The signals at δ_H 4.39 and 4.48 (each 1H, d, J = 7.7 Hz, H-20a and H-20b) and δ_C 75.5 (C-20) indicated the presence of an oxetane ring. Detailed analysis of the ^1H-^1H COSY spectrum revealed connectivities of C-2 to C-3, C-5 to C-7, C-9 to C-10, and C-13 to C-14. HMBC correlations of H-14a to C-15 and H-13 to C-1 and the ^{13}C NMR signals (δ_C 132.4 and 135.6) indicated the presence of a cyclohexene moiety (ring A). A ^1H-^{13}C long-range correlation of H_3-18 to C-13 and a NOESY correlation of H_3-18 to H-10 revealed that Me-18 was attached at C-12. HMBC correlations of H_3-16 and H_3-17 to C-15 indicated that Me-16 and Me-17 were attached at C-15. Cross-peaks of H-2 to C-15, H-3 to C-8, and H_3-19 to C-8 and C-9 in the HMBC spectrum revealed the presence of an eight-membered ring (ring B). HMBC correlations of H-3 and H-5 to C-4 and

H_3-19 to C-7 implied the presence of a cyclohexane moiety (ring C). The presence of an oxetane ring fused to C-4 and C-5 was revealed by HMBC correlations of H-20a to C-4 and C-5 and H-20b to C-5. Three acetoxy groups were attached at C-2, C-7, and C-9 based on three oxymethine proton signals (δ_H 6.19, H-2; δ_H 5.53, H-7; and δ_H 6.12, H-9), which was confirmed by HMBC correlations between the oxymethine protons and the acetoxy carbonyl carbons. A remaining acetoxy group was attached at C-4, from comparison of the C-4 chemical shift (δ_C 81.6) of **1** with those of other taxoids containing an oxetane ring.[1] HMBC correlation of H-10 (δ_H 6.50) to a carbonyl carbon (δ_C 164.4) of a benzoyl indicated that the benzoyloxy group was located at C-10. The 3-*N*,*N*-dimethylamino-3-phenylpropanoyl group was attached at C-13 (δ_H 5.57), since a remaining carbonyl carbon (δ_C 169.8) was assigned to C-21 by HMBC correlation of H-22a to C-21. Thus the plane structure of taxuspine N was determined to be **1**. The relative stereochemistry of taxuspine N (**1**) was elucidated by the NOESY (Figure 1) spectrum and ^{1}H-^{1}H coupling constants. A boat-chair conformation of ring B was elucidated from the coupling constant (10.9 Hz) between H-9 and H-10, and NOESY correlations of H-2/H-9, H-3/H-10, and H-9/H_3-16, while a chair conformation of ring C was assigned from NOESY correlations of H-3/H-7 and H-6b/H-20a. The NOESY spectrum showed cross-peaks of H-2/H-9, H-3/H-7, and H-9/H_3-19, indicating *trans* junction between rings B and C. The β-orientation of H-13 and the oxetane ring was deduced from NOESY correlations of H-13/H_3-17 and H-6a/H-20b, while α-orientation of H-7 and H-10 was assigned on the basis of those of H-7/H-3 and H-7/H-10.

The molecular formula of taxuspine O (**2**) was established to be $C_{26}H_{36}O_{10}$ by HREIMS [*m/z* 508.2295 $(M+H)^+$, Δ -1.9 mmu]. The IR spectrum indicated the presence of hydroxyl (3380 cm^{-1}), ester (1730 cm^{-1}), and unsaturated carbonyl (1700 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** showed signals due to one α,β-unsaturated carbonyl, one exomethylene, three acetyls, one oxygenated quaternary carbon, two quaternary carbons, five oxymethines, one methine, two methylenes, and four methyls. The protonated carbons were all assigned by HMQC experiment. HMBC correlations of H-14b to C-1, C-11, and C-13 and H_3-18 to C-11, C-12, and C-13 revealed the presence of a cyclopentenone ring (ring A), which was supported by UV absorption at 241 nm (ε 7900). Two methyl proton (δ_H 1.21 and 1.31), a deuterium-exchangeable proton (δ_H 2.50), and an oxygenated quaternary carbon (δ_C 77.1, C-15) signals

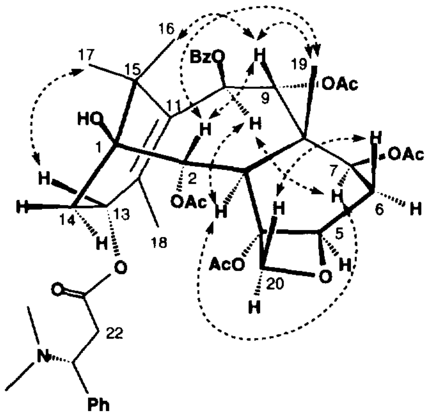

Figure 1. Relative Stereochemistry of Taxuspine N (1)
Dotted arrows denote NOESY correlations.

indicated the presence of a hydroxyisopropyl group, which was attached at C-1 from HMBC correlations of H_3-16 and H_3-17 to C-1. The hydroxyisopropyl group was inferred to be β-oriented, since NOESY correlations of H_3-16/H-2 and H_3-17/H-14b were observed. HMBC correlations of H-2 to C-8, H-6b to C-8, H-7 to C-9, and H_3-19 to C-8 and NOESY correlations of H-2/H_3-19, H-3/H-7, and H-7/H-10 revealed the presence of a seven-membered ring (ring B) and a six-membered ring (ring C). Three acetoxy groups were attached at C-2, C-9, and C-10, judging from the ^{1}H NMR chemical shifts of H-2, H-9, and H-10. Thus the structure of taxuspine O was assigned to be **2**. The relative stereochemistry of taxuspine O (**2**) was elucidated by the NOESY spectrum and ^{1}H-^{1}H coupling constants. The ^{1}H-^{1}H coupling constant (3.6 Hz) between H-9 and H-10 and NOESY correlations indicated a chair conformation of ring B.

Taxuspine P (**3**) was shown to have the molecular formula, $C_{41}H_{55}O_{13}N$, by HRFABMS [*m/z* 770.3782 $(M+H)^+$, Δ +3.0 mmu]. Fragment ion peaks at *m/z* 192 and 134 in the EIMS spectrum indicated the presence of a 3-*N,N*-dimethylamino-3-phenylpropanoyl group. Analyses of the ^{1}H and ^{13}C NMR data and HMQC spectrum of **3** provided five acetyls, one tetrasubstituted olefin, five oxymethines, one oxygenated quaternary carbons, and four methyl groups. The carbon chemical shift of C-11 (δ_C 79.4) indicated that a hydroxy group was attached at C-11. The chemical shifts of two olefin carbons (δ_C 125.4, C-12; δ_C 144.8, C-13) implied the presence of an enolacetate moiety in ring A.[3] HMBC correlations of H_3-18 to C-12 and C-13 revealed that Me-18 was attached at C-12. HMBC correlations of H_3-16 and H_3-17 to C-1, C-11, and C-15 indicated that Me-16 and Me-17 were attached at C-15. Cross-peaks of H-2 to C-1, H-3 to C-8, H-10 to C-8, H-10 to C-11, H-3 to C-4, and H-7 to C-8 in the HMBC spectrum revealed the presence of an eight-membered ring (ring B) and a cyclohexane moiety (ring C). Four acetoxy groups were attached at C-2, C-7, C-9, and C-10, judging from the ^{1}H NMR chemical shift of each oxymethine proton (δ_H 6.14, 5.57, 5.24, and 5.92, respectively). The 3-*N,N*-dimethylamino-3-phenylpropanoyl group was connected to C-5 by the ^{1}H NMR chemical shift of H-5 (δ_H 5.24). Thus the structure of taxuspine P was assigned to be **3**. The relative stereochemistry of taxuspine P (**3**) was elucidated by NOESY data.

The absolute stereochemistry of each 3-*N,N*-dimethylamino-3-phenylpropanoyl group (Winterstein's acid) in taxuspines N (**1**) and P (**3**) was determined to be *R* by chiral HPLC analysis (SUMICHIRAL OA-5000) of the acid hydrolysates of **1** and **3**.

Taxuspines N ~ P (**1** ~ **3**) are new taxoids isolated from stems of the Japanese yew *Taxus cuspidata* Sieb. et Zucc. Taxuspine N (**1**) is the first example of taxoids containing a 6/8/6-membred ring system with a 3-(*R*)-*N,N*-dimethylamino-3-phenylpropanoyl group at C-13 from yew trees. Taxuspine O (**2**) is a rare taxoid having rearranged 11(15→1)*abeo*-taxane skeleton.[7] Taxuspine P (**3**) is the second example of taxoids involving an enolacetate moiety in ring A.[3] Taxuspines N (**1**) and P (**3**) exhibited cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 7.8 and 2.5 μg/mL, respectively, and human epidermoid carcinoma KB cells with IC_{50} values of >10 and 2.6 μg/mL, respectively, while taxuspine O (**2**) showed no cytotoxicity (IC_{50}, >10 μg/mL).

Experimental Section

General Methods. Optical rotations were determined on a JASCO DIP-370 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO IR report-100 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL EX-400, Bruker ARX-500 and AMX-600 spectrometers. The 7.26 and 7.20 ppm resonance of residual $CHCl_3$ and C_6H_6 and 77.1 and 123.5 ppm of $CDCl_3$ and C_6D_6 were used as internal references, respectively. EIMS was obtained on a JEOL DX-303 spectrometer operating at 70 eV. FABMS was measured on an HX-110 spectrometer by using glycerol matrix.

Collection, Extraction, and Separation. The Japanese yew *Taxus cuspidata* Sieb. et Zucc. was collected at Sapporo, Hokkaido. The stems (4.6 kg) of the yew was extracted with MeOH (15 L x 4). The MeOH extract was partitioned between toluene (900 mL x 4) and H_2O (900 mL). The aqueous layer was extracted with $CHCl_3$ (1 L x 3). The $CHCl_3$ soluble portions were evaporated under reduced pressure to give a residue (5.2 g), which was subjected to a silica gel column (4.5 x 39 cm) eluted with $CHCl_3/CH_3OH$ (95:5) to afford two fractions **I** (300 ~ 340 mL) and **II** (340 ~ 440 mL). Fracton **I** was applied to a silica gel column (1.5 x 50 cm) eluted with $CHCl_3/CH_3CN$ [80:20 (200 mL) → 50:50 (300 mL) → 30:70 (200 mL)] to give fraction **a** (460 ~ 660 mL). Fraction **a** was subjected to silica gel HPLC [Develosil 100-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; UV detection at 260 nm; eluent, *n*-hexane/EtOAc, 1:1 (75 mL)] to give taxuspine N (**1**, 2.6 mg). Fraction **II** was applied to a reversed-phase column (YMC-GEL ODS-60A, YMC Co. Ltd., 2.4 x 7cm) eluted with CH_3OH/H_2O (60:40) to afford two fractions **b** (0 ~ 300 mL) and **c** (300 ~ 800 mL). Fraction **b** was applied to a counter-current chromatography using a Centrifugal Partition Chromatograph (CPC) [Model LLB-M, Sanki Laboratories, Inc.] in the descending mode (*n*-hexane/$CHCl_3/CH_3CN$, 15:3:8). Fraction **b** was equilibrated with the mobile (lower) phase of the solvent system at 2.5 mL/min, 1000 rpm to give a fraction (0 ~ 10 mL). The fraction was applied to a Sephadex LH-20 column (2 x 95 cm) eluted with $CH_3OH/CHCl_3$ (1:1) to afford one fraction (130 ~ 170 mL), which was separated by a reversed-phase column (Develosil Lop ODS 24S, flow rate, 3.0 mL/min; eluent, CH_3OH/H_2O, 75:25) to give a fraction (16.0 mg, t_R 45 min). The fraction was purified by reversed-phase HPLC (YMC-Pack ODS AM-323, 5 μm, 250 x 10 mm; flow rate 2.0 mL/min; UV detection at 227 nm; eluent, CH_3CN/H_2O, 40:60) to give taxuspine O (**2**, 3.1 mg, t_R 10.4 min). Fraction **c** was applied to a reversed-phase column (Develosil Lop ODS 24S; flow rate 2.5 mL/min; eluent, CH_3OH/H_2O, 95:5) to give a fraction (109.3 mg, t_R 37.6 min), which was subjected to a silica gel column (1.5 x 26 cm) eluted with $CHCl_3$/EtOAc [1:2 (240 mL)] to afford a fraction (75 ~ 190 mL). The fraction was applied to CPC [ascending mode (600 mL, 1800 rpm) using *n*-hexane/$CHCl_3/CH_3CN$, 25:7:15] to give taxuspine P (**3**, 9.6 mg, 405 ~ 430 mL).

Taxuspine N (1): A colorless amorphous solid; $[\alpha]^{25}_D$ -6.0° (*c* 0.26, $CHCl_3$); IR (film) ν_{max} 3450, 1730, and 1240 cm^{-1}; UV (MeOH) λ_{max} 278 (ε 4900) and 230 nm (sh): ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* (%) 788 (M^+-OAc, 0.5), 667 (0.2), 476 (0.5), 458 (0.2), 192 (10), 134 (100), and 105 (20); FABMS (positive ion) *m/z* 848 $(M+H)^+$; HRFABMS (positive ion) *m/z* 848.3839 $(M+H)^+$, calcd for $C_{46}H_{57}O_{14}N$, 848.3857; ^1H-^1H COSY correlations ($CDCl_3$, H/H): 2/3, 5/6a, 5/6b, 6a/7, 6b/7, 9/10, 13/14a, 13/14b, 22/23a, and 22/23b; HMBC correlations (H/C): 2/15, 2/OAc, 3/2, 3/4, 3/5, 3/7, 3/8, 3/19, 3/20, 5/3, 5/4, 6a/7, 6b/5, 7/OAc, 9/7, 9/8, 9/10, 9/OAc, 10/9, 10/15, 10/OBz, 13/1, 13/18, 14a/13, 14a/15, 16/15, 16/17, 17/15, 17/16, 18/13, 19/3, 19/7, 19/8, 19/9, 20a/3, 20a/4, 20a/5, 20b/5, 22a/21, and 22a/23; NOESY correlations ($CDCl_3$, H/H): 2/6a, 2/9, 3/6a, 3/7, 3/10, 5/14b, 6a/6b, 6a/9, 6a/20b, 7/10, 9/16, 9/19, 10/18, 16/19, 13/17, and 20a/20b.

Taxuspine O (2): A colorless amorphous solid; $[\alpha]^{26}_D$ +79.7° (*c* 0.51, CH_3OH); IR (film) ν_{max} 3380, 1730, 1700, and 1240 cm^{-1}; UV (CH_3OH) λ_{max} 241 (ε 7900) and 204 nm (5100); ^1H NMR ($CDCl_3$): δ 5.96 (1H, d, *J* = 9.0 Hz, H-2), 5.47 (1H, br s, H-20b), 5.09 (1H, br d, *J* = 3.6 Hz, H-10), 5.05 (1H, br d, *J* = 3.6 Hz, H-9), 4.78 (1H, br t, *J* = 9.0 Hz, H-5), 4.75 (1H, br s, H-20a), 4.66 (1H, m, H-7), 2.78 (1H, d, *J* = 18.7 Hz, H-14a), 2.70 (1H, d, *J* = 8.8 Hz, H-3), 2.50 (1H, brs, OH-15), 2.45 (1H, d, *J* = 18.7 Hz, H-14b), 2.10 (1H, m, H-6a), 1.99 (3H, s, $C\underline{H}_3CO$), 1.98 (3H, s, $C\underline{H}_3CO$), 1.95 (3H, s, $C\underline{H}_3CO$), 1.85 (1H, m, H-6b), 1.75 (3H, s, H-18), 1.68 (3H, s, H-19), 1.31 (3H, s, H-16), and 1.21 (3H, s, H-17); ^{13}C NMR ($CDCl_3$): δ 206.6 (s, C-13), 146.2 (s, C-12), 142.8 (s, C-4), 134.6 (s, C-11), 114.0 (t, C-20), 77.1 (s, C-15), 76.0 (d, C-9), 70.8 (d, C-5), 69.2 (d, C-10), 68.2 (d, C-2), 65.7 (d, C-7), 62.1 (s, C-1), 45.6 (d, C-3), 43.5 (t, C-14), 43.4 (s, C-8), 35.7 (t, C-6), 28.0 (q, C-16), 28.0 (q, C-17), 21.4 (s, $\underline{C}H_3CO$), 20.8 (s, $\underline{C}H_3CO$ x 2), 14.8 (q, C-19), and 9.60 (q, C-18) ; EIMS *m/z* (%) 508 (M^+, 0.3), 490 (0.2), 466 (0.3), 448 (0.2), 408 (0.4), 390 (1), 372 (3), and 43 (100); HREIMS *m/z* 508.2295 (M^+) calcd for $C_{26}H_{36}O_{10}$, 508.2314; ^1H-^1H COSY correlations ($CDCl_3$, H/H): 2/3, 3/20a, 5/6a, 5/6b, 5/20a, 5/5-OH, 6a/6b, 6a/7, 6b/7, 9/10, 14a/14b; HMBC correlations (H/C): 2/8, 6a/4, 6b/8, 7/9, 14a/11, 14b/1, 14b/11, 14b/13, 14b/15, 16/1, 16/15, 17/1, 17/15, 18/11, 18/12, 18/13, and 19/8;

NOESY correlations ($CDCl_3$, H/H): 2/9, 2/16, 2/19, 3/7, 3/14a, 5/6a, 5/6b, 5/20b, 6a/7, 7/10, 9/19, 14a/14b, and 14b/17.

Taxuspine P (3): A colorless amorphous solid; $[\alpha]^{19}_D$ +32.7° (*c* 1.27, CH_3OH); **IR** (film) ν_{max} 3510, 1735, 1680, and 1240 cm^{-1}; UV (CH_3OH) λ_{max} 261 (ε 960) and 207 nm (14500); ^1H NMR (C_6D_6) δ 7.20 (2H, m, H-25 and H-29), 7.10 (3H, m, H-26, H-27, and H-28), 6.14 (1H, d, J = 6.6 Hz, H-2), 5.92 (1H, d, J = 4.8 Hz, H-10), 5.57 (1H, t, J = 8.3 Hz, H-7), 5.46 (1H, s, H-20b), 5.44 (1H, s, H-20a), 5.24 (1H, m, H-5), 5.24 (1H, d, J = 4.8 Hz, H-9), 4.07 (1H, t, J = 7.8 Hz, H-23), 3.99 (1H, d, J = 6.6 Hz, H-3), 2.91 (1H, dd, J = 14.5 and 7.8 Hz, H-22b), 2.60 (2H, m, H-14), 2.49 (1H, dd, J = 14.5 and 7.8 Hz, H-22a), 2.22 (6H, s, NMe_2), 2.12 (1H, m, H-6), 2.10 (3H, s, CH_3CO), 2.06 (1H, m, H-1), 1.94 (3H, s, CH_3CO), 1.78 (3H, s, CH_3CO), 1.69 (3H, s, H-19), 1.62 (3H, s, H-17), 1.58 (6H, s, CH_3COx2), 1.50 (3H, s, H-18), and 1.26 (3H, s, H-16); ^{13}C NMR (C_6D_6) δ 170.8 (s, $CH_3\underline{C}O$), 170.6 (s, $CH_3\underline{C}O$), 170.5 (s, $CH_3\underline{C}O$x2), 169.9 (s, C-21), 168.6 (s, $CH_3\underline{C}O$), 144.8 (s, C-13), 144.1 (s, C-4), 138.4 (s, C-24), 128.5 (d, C-25~29), 125.4 (s, C-12), 111.4 (t, C-20), 79.4 (s, C-11), 77.8 (d, C-10), 75.5 (d, C-9), 71.2 (d, C-5), 68.9 (d, C-2), 68.3 (d, C-7), 66.7 (d, C-23), 51.6 (d, C-1), 44.1 (s, C-8), 42.1 (s, C-15), 41.9 (d, C-3), 32.1 (t, C-6), 38.8 (d, C-22), 30.5 (q, C-17), 25.0 (q, C-16), 21.4 (s, $\underline{C}H_3CO$), 20.8 (s, $\underline{C}H_3CO$ x 2), 20.4 (s, $\underline{C}H_3CO$ x 2), 15.6 (q, C-19), and 12.0 (q, C-18); EIMS *m/z* (%) 769 (M^+, 1), 710 (2), 668 (1), 650 (1), 608 (1), 516 (2), 192 (60), and 134 (100); FABMS (positive ion) *m/z* 770 $(M+H)^+$; HRFABMS (positive ion) *m/z* 770.3782 $(M+H)^+$, calcd for $C_{41}H_{56}O_{13}N$, 770.3752; ^1H-^1H COSY correlations (C_6D_6, H/H): 1/2, 1/14a, 2/3, 3/20, 5/6a, 5/20, 6a/6b, 6a/7, 9/10, 14a/18, 22a/22b, and 22/23; HMBC correlations (C_6D_6, H/C): 1/3, 2/1, 2/14, 3/1, 3/2, 3/4, 3/5, 3/7, 3/8, 3/9, 3/19, 3/20, 7/3, 7/8, 7/19, 9/3, 9/8, 9/10, 9/11, 9/19, 10/8, 10/9, 10/11, 14a/1, 14a/13, 14b/1, 14b/13, 16/1, 16/11, 16/15, 16/17, 17/1, 17/11, 17/15, 17/16, 18/12, 18/13, and 20a/5; NOESY correlations (C_6D_6, H/H): 1/2, 2/3, 2/19, 3/7, 3/14a, 3/20a, 5/6a, 6a/7, 6/22, 7/10, 9/19, 10/18, 14b/17, 14a/20, 16/17, 22a/22b, 22a/23, and 23/25.

Determination of absolute configuration of 3-*N*,*N*-dimethylamino-3-phenylpropionic acid in taxuspines N (1) and P (3). Compound **1** (0.1 mg) was refluxed in 10% HCl aq. (0.1 mL) for 1h. The reaction mixture was extracted with Et_2O (1 mL x 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 x 15 cm; flow rate 1.0 mL/min; UV detection at 254 nm; eluent: 2.0 mM $CuSO_4$ in H_2O/CH_3OH, 98:2). Retention times of standard 3-(*S*)- and 3-(*R*)-*N*,*N*-dimethylamino-3-phenylpropanoic acids[8] were 14.7 and 15.9 min, respectively, and that of 3-*N*,*N*-dimethylamino-3-phenylpropanoic acid contained in the hydrolysate of **1** was found to be 15.9 min. According to essentially the same procedure as described above, those of 3-*N*,*N*-dimethylamino-3-phenylpropanoic acid contained in the hydrolysates of taxuspine P (**3**) was found to be 15.9 min.

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