A New Basic Taxoid from *Taxus cuspidata*

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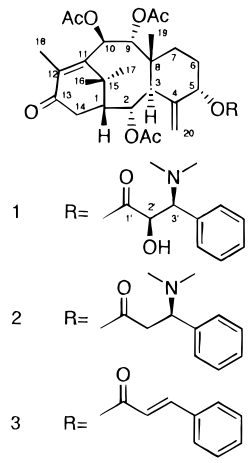
The new basic taxoid 2′-hydroxytaxine II (**1**) was isolated from the needles of *Taxus cuspidata* as the major component of the crude alkaloid fraction. An HPLC method for the analysis and separation of the alkaloid fraction was developed. The structure of **1** was determined mainly by spectroscopic analysis.

Since the discovery of the anticancer activity of Taxol against ovarian and breast cancer, much attention has been paid to the isolation of new taxane diterpenoids from various species of yews.[1] Current interest in the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. (Taxaceae), focuses on the nonalkaloid diterpenoids from the needles, stems, heartwood, and bark of this plant for the purpose of finding improved biological sources for paclitazel analogues and for precursors for the partial synthesis of paclitaxel.[2−4] *T. cuspidata* is also known as a poisonous plant, and the most common form of poisoning is by swallowing the seeds, which are surrounded by the red sweet-tasting aril. In 1986, Yoshizaki and his co-workers examined the constituents of the seeds of this plant and reported the isolation of taxine II.[5]

Because of our interest in the biological activities of taxane-type diterpene alkaloids we initiated an investigation of the constituents of the alkaloidal fraction of needles of the Japanese yew, *T. cuspidata*. Because an efficient method for the analysis and separation of the alkaloid fraction of this plant has not been reported, we attempted to develop a simple HPLC method for these purposes at the start of this work.

A defatted EtOAc extract of *T. cuspidata* needles was subjected to an acid−base extraction scheme to give a crude alkaloid fraction (0.16% from fresh needles). After various attempts it was found that an Inertsil ODS column (25 cm × 1 cm i.d.) in combination with 0.05 M ammonium acetate buffer (pH 4)−MeOH−CH_3CN (1:1:2) at a flow rate of 5 mL/min gave a satisfactory separation of the crude alkaloid fraction (Figure 1). The use of a buffer solution and the pH value of the buffer solution proved to be absolutely essential (Figure 2). Peaks 1−3 in this chromatogram correspond to compounds **1**−**3**.

The main constituent was a new compound **1**, which was isolated in 0.026% yield from fresh needles and in 16.29% yield from the crude alkaloidal fraction. Compound **1** had the composition $C_{37}H_{49}NO_{10}$, which was determined by a combination of HREIMS, and ^{1}H- and ^{13}C- NMR spectra. The IR spectrum of compound **1** showed the existence of a hydroxyl group (3480 cm^{-1}), an ester carbonyl group (1748 cm^{-1}), and an α,*β*-unsaturated carbonyl group (1678 cm^{-1}). Its ^{1}H-NMR spectra (Table 1) showed the presence of a taxane skeleton with four C-Me groups (0.83, 1.15, 1.74, and 2.33 ppm), three acetoxy methyls (2.05, 2.059, and 2.062 ppm), and one *N*,*N*-dimethyl group (2.29 ppm, 6 H).



The ^{1}H−^{1}H correlations, H-1 and H-2; H-1 and H-14*β*; H-2 and H-3; H-3 and H-20; H-5 and H-6a,b; H-6a and H-6b; H-7a and H-7b; H-6a,b and H-7a,b; H-9 and H-10; H-14α and H-14*β*; H-16 and H-17; H-20a and H-20b; and H-2′ and H-3′, were determined by analysis of a ^{1}H−^{1}H COSY spectrum. The assignments of all protonated carbons were determined by DEPT and HMQC experiments.

An HMBC experiment was used to assign the quaternary carbons and the attachment of ester functions. A correlation of the signal due to the *N*,*N*-dimethyl-3-phenylisoseryl carbonyl (C-1′) at 171.91 ppm with those of H-5 (4.98 ppm), and with H-2′ (4.39 ppm) and H-3′ (3.67 ppm) indicated the location of the (*N*,*N*-dimethyl-3-phenylisoseryl)oxy group at C-5. The multiple-bond ^{1}H−^{13}C correlations of the remaining six nonprotonated carbons of **1**, C-4 with H-3, 5, 20a,b; C-8 with H-2, 3, 7a,b, 9, 19; C-11 with H-1, 9, 10, 16, 17, 18; C-12 with H-10, 14*β*, 18; C-13 with H-1, 14α,*β*, 18; and C-15 with H-1, 10, 14α, 16, 17, were determined by an HMBC experiment and allowed unambiguous carbon skeletal connections.

The full stereostructure of the taxane skeleton of **1** was determined by CYCLENOE and NOESY experiments as well as by a consideration of vicinal coupling constants. The NOE correlations of compound **1** determined by the NOESY experiment are summarized in Table 2, and the results are in good agreement with the

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Table 1. NMR Spectral Data of Compound **1** in $CDCl_3$

position	^{13}C[a]	connected ^{1}H[b]	H-H COSY	HMBC[c]
1	48.64 (d)	2.19 (m)		H3,H14αβ,Me17,Me16
2	69.44 (d)	5.49 (dd, 6.6, 2.2)		H1,H3,H14αβ
3	43.27 (d)	3.04 (brd, 6.6)		H1,H2,H5,Me19,H20ab
4	141.02 (s)	-		H3,H5,H20ab
5	78.20 (d)	4.98 (dd, 3.0, 3.0)		H3,H7a,H20ab
6	27.78 (d)	a)1.47 (m)		H7b
		b)1.04 (m)		
7	27.50 (t)	a)1.62 (m)		H3,H5,H9,Me19
		b)1.50 (m)		
8	44.43 (s)	-		H2,H3,H7ab,H9,Me19
9	75.78 (d)	5.85 (d, 10.3)		H10,Me19
10	73.35 (d)	6.01 (d, 10.3)		H9
11	150.43 (s)	-		H1,H9,H10,Me16,Me17,Me18
12	137.82 (s)	-		H10,H14β,Me18
13	199.27 (s)	-		H1,H14αβ,Me18
14	35.82 (t)	β)2.81 (dd, 20.0, 6.8)		H1,H2
		α)2.36 (d, 20.0)		
15	37.50 (s)	-		H1,H10,H14α,Me16,Me17
16	37.39 (q)	1.15 (s)		H1,Me17
17	25.12 (q)	1.74 (s)		H1,Me16
18	14.26 (q)	2.33 (s)		-
19	17.52 (q)	0.83 (s)		H3,H9
20	118.34 (t)	a) 5.26 (brs)		H3,H5
		b) 4.80 (brs)		
OAc	169.78 (s)			H9
	169.78 (s)			H10
	169.48 (s)			H2
	21.40 (q)	2.05 (s)		
	20.89 (q)	2.059 (s)		
	20.68 (q)	2.062 (s)		
1'	171.91 (s)	-		H5,H2',H3'
2'	70.44 (d)	4.39 (d, 9.5)		H3'
3'	71.49 (d)	3.67 (d, 9.5)		H2,NMe2
q-Ph	133.00 (s)	-		H2',H3'
o-	129.85 (d)	7.22-7.19 (m)		H3'
m-	127.86 (d)	7.35-7.29 (m)		
p-	128.09 (d)			
NMe_2	41.15 (q)	2.29 (s)		H3'

[a] Multiplicities were determined by the DEPT. [b] Multiplicities and coupling constants in Hz are in parentheses. [c] Correlations from C to the indicated protons.

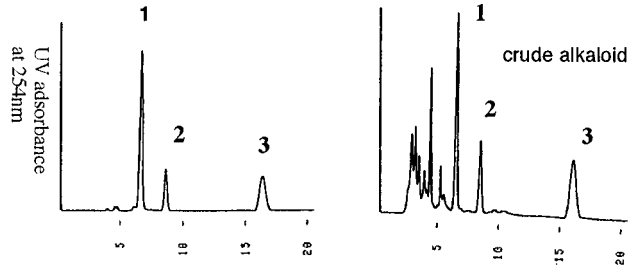

Figure 1. HPLC chromatogram of alkaloid fraction and of pure compounds **1**−**3** with ammonium acetate buffer (pH4)−MeOH−CH_3CN (1:1:2).

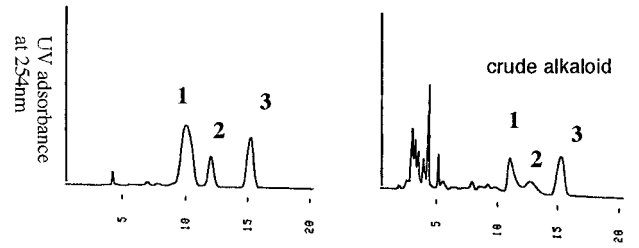

Figure 2. HPLC chromatogram of alkaloid fraction and of pure compounds **1**−**3** with H_2O−MeOH−CH_3CN (1:1:2).

stereostructure of compound **1**. Considering the co-occurrence of compound **1** and Taxol in the same plant and their biosynthetic pathway,[6] the C-5 side chain of compound **1** was assumed to have the same (2*R*,3*S*) stereochemistry as that of Taxol. In support of this assumption, the coupling constant between H-2′ and H-3′ of **1** (J = 9.5 Hz) is in good agreement with that of taxine A (J 2′,3′ = 10.0 Hz)[7,8] which possess a (2*R*,3*S*)-*N*,*N*-dimethyl-3-phenylisoseryl side chain.

Table 2. NOE Correlations of Compound **1**

proton	NOESY cross peaks[a]
1	16Me (m), 17Me (w), 14β-H (s), 2-H (s)
2	19Me (s), 17Me (s), 1-H (m), 9-H (s)
3	6-H (m), 14α-H (m)
5	20a-H (s)
9	19Me (s), 17Me (s), 2-H (s), 10-H (m)
10	7-H (w), 9-H (m), 18Me (s)
14α	3-H (s), 14β-H (s)
14β	1-H (m), 14α-H (s), 16Me (w)
16Me	1-H (m), 17Me (s)
17Me	1-H (m), 2-H (s), 9-H (s), 16Me (s)
18Me	3-H (s), 7-H (m), 10-H (s), 2′-H (m)
19Me	2-H (m), 9-H (m), 20b-H (w)
20a	5-H (s), 20b-H (s)
20b	19Me (w), 20a-H (s)
2′	18Me (w), 3′-H (w), NMe (m), *o*-Ph (m)
3′	2′-H (m), NMe (s), *o*-Ph (m)
o-Ph	14β-H (w), 2′-H (m), 3′-H (m), NMe (s)
NMe_2	2′-H (m), 3′-H (m)

[a] s = strong; m = medium; w = weak.

Appendino has recently reported that the most stable conformations of the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoseryl side chain of Taxol and the (2*R*,3*S*)-*N*,*N*-dimethyl-3-phenylisoseryl side chain of taxine A in $CDCl_3$ are

different.[8] The dihedral angle between H-2′ and H-3′ of the former is near 60° and in good agreement with the observed *J* 2′,3′ values (2.7 Hz). On the other hand, the dihedral angle between H-2′ and H-3′ of the latter is near 180°, and the observed value of *J* 2′,3′ (10.0 Hz) is reasonable for this conformation. The analysis of the most stable conformation of the phenylisoseryl side chain of **1** based on MM 2 calculations gave the same conclusion.

Compound **2** was isolated in 0.005% yield from fresh needles and in 2.98% yield from the crude alkaloid fraction. The ^{1}H- and ^{13}C- NMR spectra of **2** were identical with those of taxine II reported in the literature.[5,9]

Compound **3** was isolated in 0.002% yield from fresh needles and in 1.36% yield from the crude alkaloid fraction. The ^{1}H- and ^{13}C-NMR spectra of **3** were identical with those of taxinine.[10,11] Taxinine (**3**) may be produced from taxine II (**2**) or other alkaloids in the crude alkaloid fraction by acid−base extraction or other separation procedures.

Experimental Section

General Experimental Procedures. The melting point was determined with a Yanagimoto micro-melting point apparatus and is uncorrected. IR spectrum was recorded on a Hitachi 270−30 spectrometer. Optical rotation was measured by a Horiba Polarimeter SEPA-200. HREIMS was taken on a JEOL JMS HX-110 spectrometer. ^{1}H-NMR (499.87 MHz) and ^{13}C-NMR (125.70 MHz) spectra were run on a Varian UNITY-PS 500 spectrometer.

Plant Material. The needles of *T. cuspidata* were collected from trees of 3-m height grown in Aobayama, Sendai, Japan, on October 1, 1995.

Extraction. Fresh needles (1674 g) were defatted with hexane (2 × 3.5 L), and the remaining plant material was extracted with EtOAc (2 × 3.5 L). The residue (29.30 g) remaining after removal of the solvent was stirred with a mixture of EtOAc and hexane (1:1, 1 L). The insoluble portion (20.8 g) was dissolved in a mixture of MeOH−EtOAc (1:3, 400 mL) and extracted with a 0.489 M aqueous solution of H_2SO_4 (3 × 100 mL). Subsequently, the combined acid solution was brought to pH 9 by addition of a 29% aqueous solution of NH_4OH (300 mL) and then extracted with $CHCl_3$ (3 × 100 mL). The combined extracts were dried (Na_2SO_4), filtered, and concentrated to give a crude basic taxoid fraction [(2.72g; 0.16% from fresh needles; 9.28% from defatted EtOAc extracts; and 13.08% from the insoluble portion in a mixture of EtOAc−hexane (1:1)].

Isolation. The crude alkaloid fraction (2.72 g) was chromatographed over alumina (neutral, activity I, 272 g) and eluted with a mixture of EtOAc and MeOH (9:1). The combined eluent was separated by HPLC [Inertsil ODS column (25 cm × 1 cm i.d.), 0.05 M NH_4OAc buffer (pH 4)−MeOH−CH_3CN (1:1:2), flow rate 5 mL/min].

The first peak (t_R 6.6 min) gave 2′-hydroxytaxine II (**1**) (443 mg, 16.29% from crude alkaloid fraction) as a colorless amorphous product: mp 84−88 °C; $[\alpha]^{20}_D$ +43.0 (*c* 1, $CHCl_3$); HREIMS *m*/*z* 667.3349, calcd for $C_{37}H_{49}NO_{10}$ 667.3357.

The second peak (t_R 8.6 min) gave taxine II (81 mg, 2.9 8% from crude alkaloid fraction).

The third peak (t_R 16.3 min) gave taxinine (37 mg, 1.3 6% from crude alkaloid fraction).

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