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A TAXOID FROM *TAXUS CUSPIDATA* VAR. *NANA*

HIROSHI MORITA, LAN WEI, AKIRA GONDA, KOICHI TAKEYA and HIDEJI ITOKAWA*

Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan

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Key Word Index—*Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder.; Taxaceae; taxuspinanane C; 7,9,10-deacetylbaccatin VI; taxoid.

Abstract—A new taxoid, named taxuspinanane C, has been isolated from the stems of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder. and the structure was elucidated by extensive 2D NMR methods. © 1997 Elsevier Science Ltd

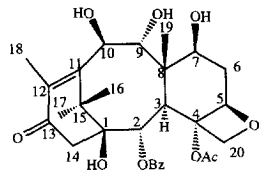
INTRODUCTION

The search for alternative sources of taxol has led to the development of a wide program devoted to the identification of *Taxus* species or varieties containing other taxanes, useful as key intermediates for the semi-synthesis of this drug [1]. The demand for this promising anticancer therapeutic agent and new important intermediate from a new source led to isolation of new various taxoids [1]. Recently, from Japanese yew, *Taxus cuspidata* Sieb. et. Zucc., new taxoids, named taxuspines, have been isolated, and some of them show the reduction of $CaCl_2$-induced depolymerization of microtubules and/or cytotoxicity [2–7]. In addition, taxuspinananes A and B have been isolated from toulene extract of the stems of *Taxus cuspidata* var. *nana* [8].

As a part of our continuing studies in the search for new bioactive compounds from higher plants, we have isolated a new taxoid, named taxuspinanane C (**1**) and 7,9,10-deacetylbaccatin VI (**2**), from the stems of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder., which has been often used as a garden tree in Japan. In this paper, we report the structure elucidation of taxuspinanane C (**1**), by extensive 2D NMR methods, together with the NMR data of 7,9,10-deacetylbaccatin VI (**2**), whose spectral data has not been fully reported previously.

RESULTS AND DISCUSSION

The chloroform extract obtained by solvent partition of the methanol extract of the stems of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder., showed cytotoxicity against P388 lymphocytic leukemia cells. Chromatography on a silica gel column followed by MPLC and HPLC on ODS yielded two taxoids, named taxuspinanane C (**1**) and 7,9,10-deacetylbaccatin VI (**2**) together with known taxoids, such as taxol, taxol B, and taxinine.



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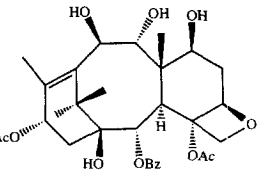

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Taxuspinanane C (**1**), colourless needles, mp. 152–154°, $[\alpha]_D$ +60° (*c* 0.13, $CHCl_3$), showed a high-resolution FAB-mass spectral quasimolecular ion peak at *m/z* 545.2388 [$(M+H)^+$, Δ +0.1 mmu], corresponding to molecular formula, $C_{29}H_{36}O_{10}$. The IR absorptions at 3445, 1737 and 1674 cm^{-1} were attributed to hydroxyl, ester and α,β-unsaturated ketone groups, respectively. In the NMR spectra (Table 1), the presence of an oxetane ring was suggested by ^1H signals (δ 4.16 and 4.35 J = 8.1 Hz) and a ^{13}C signal (δ 76.46). An acetyl group and a benzoyl group, characteristic of an ester of taxoids, were also verified by the observation of ^1H and ^{13}C signals (acetyl: δ_H 2.18, δ_C 21.93 and benzoyl: δ_H 7.50, 7.62, 8.08, δ_C 128.77, 128.99, 130.09, 133.91, 166.93). The ^1H-^1H COSY spectrum indicated the following three isolated spin systems. The hydroxy bearing methine at δ 4.38 assignable to H-7 was coupled with methylene protons (δ 1.90 and 2.51), being further coupled with an oxygen bearing methine at δ 4.86 assignable to H-5. The

* Author to whom correspondence should be addressed.

Table 1. ^1H and ^{13}C NMR signal assignments and HMBC correlations of taxuspinanane C (**1**) in $CDCl_3$

Position	δ_H	[int. mult, J (Hz)]	δ_C	HMBC
1			78.65	H-2, H-3, H-14α, H-14β, H-16, H-17
2	5.86	(1H, *d*, 5.8)	71.82	H-3, H-14α, H-14β
3	3.19	(1H, *d*, 5.8)	45.75	H-2, H-19
4			81.43	H-3, H-5, H-20a
5	4.86	(1H, *br d*, 9.2)	83.77	H-6β, H-20b
6α	2.51	(1H, *ddd*, 7.5, 9.2, 14.5)	38.11	
6β	1.90	(1H, *m*)		
7	4.38	(1H, *m*)	74.38	H-3, H-5, H-6α, H-19
8			44.48	H-2, H-3, H-6α, H-19
9	4.30	(1H, *m*)	77.66	H-10, H-19
10	5.00	(1H, *d*, 10.2)	72.33	
11			158.21	H-16, H-17, H-18
12			138.83	H-10, H-18
13			198.40	H-14α, H-14β, H-18
14α	2.87	(1H, *d*, 19.6)	42.92	H-2
14β	2.65	(1H, *d*, 19.6)		
15			43.40	H-10, H-14α, H-16, H-17
16	1.73	(3H, *s*)	20.23	H-17
17	1.31	(3H, *s*)	34.91	H-16
18	2.00	(3H, *s*)	13.88	
19	1.78	(3H, *s*)	11.94	H-3
20a	4.35	(1H, *d*, 8.1)	76.46	
20b	4.16	(1H, *d*, 8.1)		
4-AcO	2.18	(3H, *s*)	21.93	
			170.16	Me-4Ac
2-BzO	8.08	(2H, *dd*, 1.4, 7.5)	133.91	
	7.50	(2H, *t*, 7.5)	128.77	
	7.62	(1H, *t*, 7.5)	130.09	
			128.99	
			166.93	H-2, H-2Ph(p)

ester bearing proton at δ 5.86 was coupled with the methine proton at δ 3.19 assignable to H-3. In addition, two hydroxy bearing carbons each with coupled protons at δ 4.30 and 5.00, assignable to H-9 and H-10, were observed. Protons of a methyl group attached on the double bond, assignable to C-18, characteristic of many taxoids resonated at slightly lower field (δ 2.00), indicative of carbonyl group at C-13. This was also supported by the presence of methylene protons (δ 2.65 and 2.87) resonating at lower field, assignable to H-14. These ^1H and ^{13}C signal assignments were verified by HMBC correlations as shown in Table 1. In addition, the position of the benzoyl group was elucidated at C-2 from the correlations between the aromatic protons and carbonyl carbon, and between H-2 and the carbonyl carbon.

The stereostructure of **1** was elucidated by ROE correlations of a phase sensitive ROESY spectrum (Fig. 1) [9]. The ROE enhancements between H-2 and H-16, between H-2 and H-9, between H-2 and H-19, between H-3 and H-7, between H-9 and H-19, and between H-10 and H-18 implied the relative configuration as shown in **1**.

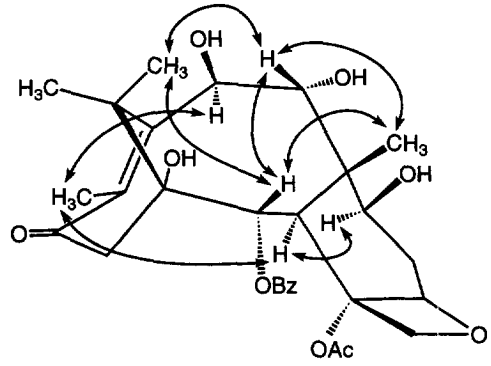

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Fig. 1. The arrows show selected ROE correlations.

Compound **2**, colourless powder, $[\alpha]_D$ +28.6° (*c* 0.07, $CHCl_3$), possess the molecular formula, $C_{31}H_{40}O_{11}$ by the pseudomolecular ion peak at *m/z* 611.2463 [$(M+Na)^+$, Δ −0.5 mmu] in the HR FAB-mass spectrum. The ^1H and ^{13}C signals of **2** showed a closely similar pattern to those of **1**. However, the ^{13}C

signal ascribable to carbonyl group at C-13 was absent and an acetyl group was observed. The proton signal at δ 6.19 attached to the acetoxy bearing carbon was coupled with methylene protons at δ 2.21 assignable to H-14. The deduced structure agreed with the ^{1}H-^{13}C long range correlation in the HMBC spectrum and the stereostructure of **2** was elucidated by the similar ROE correlations to those of **1** in a phase sensitive ROESY spectrum in $CDCl_3$. These data indicated that **2** was 7,9,10-deacetylbaccatin VI [10], which has been isolated from *Taxus canadensis*. However, the spectroscpic (^{13}C NMR, IR, and UV spectra) and physicochemical properties of **2** have not been reported previously.

Taxuspinanane C and 7,9,10-deacetylbaccatin VI showed moderate cell growth inhibitory activity against P388 cells (IC_{50}, **1**: 10 μg ml^{-1}; **2**: 35 μg ml^{-1}).

EXPERIMENTAL

General. IR and UV spectra were recorded on JASCO A-302 spectrometer and Hitachi 557 spectrophotometer, respectively. Optical rotation was measured with a JASCO DIP-4 spectrometer and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. FAB and high resolution mass spectra were taken with a VG Autospec spectrometer. HPLC was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm and 30 mm i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. All NMR spectroscopy were carried out on Bruker AM400, AM500, and Varian Unity 400 spectrometer. The spectra were recorded at 300 K in $CDCl_3$. The phase sensitive ROESY experiments were acquired with mixing times of 300 msec. The values of the delay to optimise one-bond correlations in the HMQC spectrum and suppress them in the HMBC spectrum was 150 msec and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 msec.

Plant material. The stems of *Taxus cuspidata* Sieb. et. Zucc, var. *nana* Rehder. were collected in Saitama, Japan in October, 1995. A voucher specimen has been deposited in the herbarium of the Tokyo University of Pharmacy and Life Science.

Extraction and isolation. The stems of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder. (20 kg) were extracted with hot MeOH at ×3 to give a MeOH extract which was treated with toluene and H_2O. The H_2O soluble fr. was further partitioned with $CHCl_3$. The $CHCl_3$ soluble fr. (130 g) was subjected to silica gel CC using $CHCl_3$–MeOH gradient system, followed by silica gel CC of the fr. eluted by 10% MeOH using hexane–EtOAc–MeOH gradient system. The fr. eluted by hexane–EtOAc–MeOH (6:4:1) solvent system was finally subjected to ODS HPLC with 50% MeOH solvent system to give taxuspinanane C (**1**) and 7,9,10-deacetylbaccatin VI (**2**) (**1**: 0.000009% and **2**: 0.00001%).

Taxuspinanane C (**1**). Colourless needles, mp 152–154°, $[\alpha]_D$ +60.0° (*c* 0.13, $CHCl_3$); *m/z* 545 (Found: $[M+H]^+$ 545.2388. $C_{29}H_{36}O_{10}$ requires, 545.2387). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3445 (OH), 1737 (ester C=O) and 1674 (α,β-unsaturated C=O). UV λ_{max}^{MeOH} nm: 230 (ε 14 000), 277 (ε 3100).

7,9,10-*deacetylbaccatin VI* (**2**). Colourless powder, $[\alpha]_D$ +28.6° (*c* 0.07, $CHCl_3$); *m/z* 611 (Found: $[M+Na]^+$, 611.2463. $C_{31}H_{40}O_{11}Na$ requires, 611.2468). $\nu_{max}^{CCl_4}$ cm^{-1}: 3448 (OH) and 1742 (ester C=O). UV λ_{max}^{MeOH} nm: 227 (ε 17 000), 273 (ε 1500). ^{13}C NMR ($CDCl_3$): δ 79.03 (C-1), 73.70 (C-2), 46.92 (C-3), 82.02 (C-4), 83.96 (C-5), 38.08 (C-6), 74.42 (C-7), 44.41 (C-8), 78.34 (C-9), 70.85 (C-10), 137.69 (C-11), 137.50 (C-12), 70.06 (C-13), 35.37 (C-14), 43.09 (C-15), 22.32 (C-16), 28.58 (C-17), 14.89 (C-18), 12.41 (C-19), 76.67 (C-20), 22.87 (4-Ac), 169.45 (4-Ac-CO), 21.3 (13-Ac), 170.56 (13-Ac-CO), 133.66, 128.63, 130.10, 129.37, 167.07 (2-Bz).

Cytotoxic activity on P388 cells. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay was performed in a 96-well plate. The blue formazan produced by the mitochondrial dehydrogenase of viable cells was measured spectrophotometrically. RPMI-1640 medium (100 μl) supplemented with 5% foetal calf serum and 100 μg ml^{-1} of kanamycin and containing mouse P388 leukemia cells (3×10^4 cells ml^{-1}) was added to each well. After overnight incubation (37°, 5% CO_2), 100, 30, 10, 3, 1, 0.3 and 0.1 μg ml^{-1} of sample sols were added to the wells and the plates were incubated for 48 hr. Then, 20 μl of MTT was added to each well and the plates were incubated for 4 hr. The resulting formazan was dissolved in 100 μl of 10% SDS (sodium dodecyl sulphate) containing 0.01 N HCl. Each well was mixed gently with a pipet for 1 or 2 min and the plate was read on a microplate reader (Tosoh MPR-A4i) at 540 nm. The IC_{50} (μg ml^{-1}) value was defined as the concn of sample which achieved 50% reduction of viable cells with respect to the control.

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