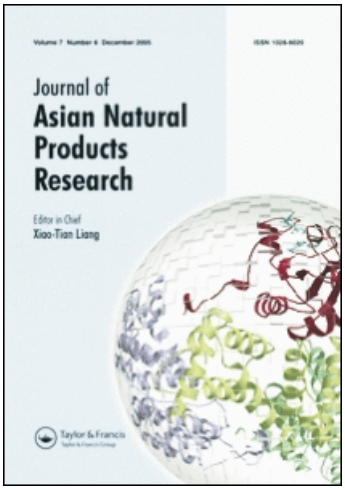

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THREE NEW BICYCLIC TAXANE DITERPENOIDS FROM THE NEEDLES OF JAPANESE YEW, *TAXUS CUSPIDATA* SIEB. ET ZUCC

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Three new bicyclic taxane diterpenes were isolated from the needles of the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. Their structures were established to be 7,9,10,13-tetraacetoxy-5-cinnamoyloxy-4-hydroxy-methyl-8,12,15,15-tetramethyl-bicyclo[9.3.1] pentadeca-3,8,11-trien-2-ol (2,20-dideacetyl taxuspine X), 7,9,10,13,20-pentaacetoxy-5-cinnamoyloxy-8,12,15,15-tetramethyl bicyclo[9.3.1] pentadeca-3,8,11-trien-2-ol (2-deacetyl taxuspine X), and 9,10,13,20-tetraacetoxy-5-cinnamoyloxy-8,12,15,15-tetramethyl-bicyclo[9.3.1] pentadeca-3,8,11-trien-2,7-diol (2,7-dideacetyl taxuspine X) with the aid of spectroscopic techniques and by comparing with taxuspine X.

Keywords: *Taxus cuspidata* Sieb. et Zucc; Taxaccae; Taxoid diterpenes; Bicyclic taxanes; Needles

INTRODUCTION

Taxol® (paclitaxel) is one of the most important anti-cancer drugs currently on the market for ovarian and breast cancers, and its promising effects for a variety of other cancers such as head and neck, lung, gastrointestinal

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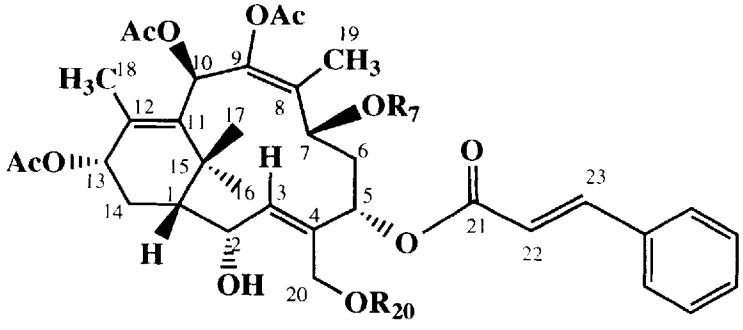
and bladder, have generated world wide interest in various areas, and extensive chemical studies have been carried out [1–4]. *Taxus cuspidata* Sieb. et Zucc was one of the yew trees distributed in Japan, in the past investigations, several groups isolated more than 50 new taxane diterpenoids from the needles, bark, seed and heart wood of the Japanese yew [5–22]. In the previous papers, we have reported several bicyclic taxoids and rearranged taxane diterpenoids from the needles and bark of the Chinese yew, *Taxus mairei* [23–31]. As a continuation of this work, recently we investigated components of the residues after removing taxinine of methanolic extracts of the needles of *T. cuspidata* Sieb. et Zucc, and more than 30 compounds were isolated, three of them were new compounds. This communication deals with the isolation and the structure elucidation of three new compounds.



1 R_{20}=H R_7=Ac
2 R_{20}=Ac R_7=Ac
3 R_{20}=Ac R_7=H

RESULTS AND DISCUSSION

A methanolic extract of the needles of *Taxus cuspidata* Sieb. et Zucc was processed as described in the experimental section to afford three new bicyclic taxane diterpenoids. Compound **1** was isolated as a white gum in a 0.00015% yield from the dried material. FAB-MS gave ion peaks at *m/z* 705 ($[M+Na]^+$) and 683 ($[M+H]^+$), indicating that the molecular weight of **1** was 682. By combination of FAB-MS, ^1H NMR, and ^{13}C NMR data, its molecular formula was proposed as $C_{37}H_{46}O_{12}$. HR-FAB-MS also gave the same formula. The unsaturation number of **1** was calculated as 15.

IR absorptions at 3450, 1740, and 1670 cm^{-1} implied that **1** possessed hydroxy, ester and unsaturated ester groups. The ^1H NMR spectrum of **1** showed the four characteristic tertiary methyl groups (1.62, 1.09, 1.25, and 2.18 ppm, each 3H, *s* or *br.s*) of taxoids skeleton, assignable to the 8-CH_3, 15-$(CH_3)_2$, and 12-CH_3 groups, respectively. Four acetyl groups (1.96, 1.77, 2.20, and 2.03 ppm, each 3H, *s*) at relatively low field; five oxy-bearing methine groups (4.72 ppm, *dd*; 5.75 ppm, *br.s*; 5.52 ppm, *br.d*; 7.25 ppm, *br.s*; and 5.31 ppm, *br.d*), one oxy-bearing methylene group (4.43 ppm, 1H, *d*, $J=$ 12.6 Hz; 3.81 ppm, 1H, *d*, $J=12.6$ Hz), one trisubstituted olefin (5.71 ppm, 1H, *br.d*, $J=10.1$ Hz) were also involved. Proton signals due to a cinnamoyl group appeared at 7.41 ppm (3H, *m*), 7.56 ppm (2H, *m*), 6.54 ppm (1H, *d*, $J=16.2$ Hz), and 7.95 ppm (1H, *d*, $J=16.2$ Hz). UV absorption at 278 nm also supported the presence of the cinnamoyl group. In addition to the cinnamoyl group, six olefinic carbons were also suggested in the ^{13}C NMR spectrum. Since 13 out of 15 unsaturations were accounted for, only two unsaturation equivalents were left. The carbon skeleton of **1** was thus deduced to be composed only with two rings. The spectral data of **1** were highly comparable with those from taxuspine X [6]. The ^1H and ^{13}C NMR spectra of **1** are closely similar to those from taxuspine X except for H-2, H-20a, and H-20b shift to upfield, and signals corresponding to two acetyl groups were absent. Therefore, the structure of **1** was established as 2,20-dideacetyl taxuspine X. Relative stereochemistry of **1** was deduced from the nuclear Overhauser effect spectroscopy (NOESY) spectrum as shown in Fig. 1. The NOESY correlation between H-13 and 17-CH_3 revealed that the ring A adopted a boat conformation and H-13 was β-orientation, while the correlations of H-2/16-CH_3 and H-14a/17-CH_3 indicated the β-orientation of H-14a and H-2. NOESY correlations of H-20b/H-2 and H-7/H-10 implied that the two double bonds at C-3 and C-8 had *E*-configuration. NOESY cross-peaks of H-7/18-CH_3, H-10/18-CH_3, and H-23/18-CH_3 revealed that H-7, H-10, and the cinnamoyl group were α-oriented, which was also in good agreement with that of taxuspine X.

Compound **2** was obtained as a white gum. The extensive absorptions at 3450 and 1740 cm^{-1} in the IR spectrum implied that **2** possessed hydroxyl and ester groups, respectively. ^1H NMR spectrum of **2** showed the characteristic signals of taxoids, including four tertiary methyl groups at 1.11, 1.29, 2.22, and 1.64 ppm (each 3H, *s*). ^1H NMR spectrum of **2** also indicated that there were four acetyl groups and a cinnamoyl group connected to the skeleton. Interpretation of $^1H-^1H$ correlation spectroscopy (COSY) spectrum can make assignment of all the protons in the skeleton and in the cinnamoyl group. Four acetyl groups were attached at C-7, C-9, C-10, C-13,

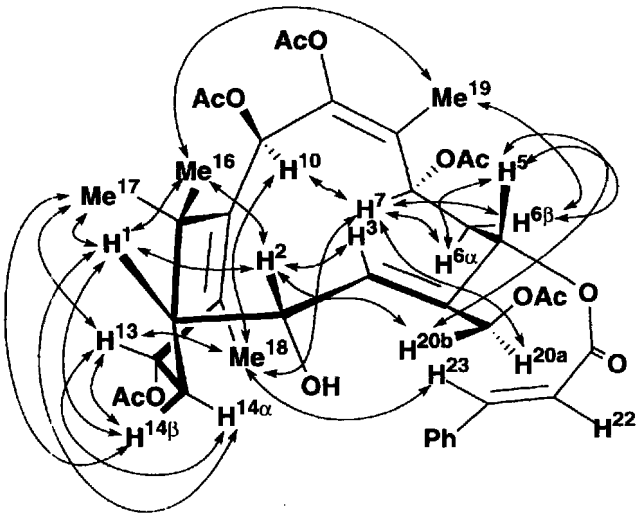


FIGURE 1 Relative stereochemistry of **1**, deduced from NOESY correlations.

and C-20 according to the chemical shifts of the corresponding protons. FAB-MS yielded an ion peak at *m/z* 747 ($[M \cdot Na]^+$), which indicated that **2** was 42 mass units higher than that of **1** and 42 mass units less than that of taxuspine X [6]. The ^1H spectrum of **2** closely resembled those of taxuspine X with the exception of H-2 shifted upfield to 4.78 ppm. Thus, the structure of **2** was established as 2-deacetyl taxuspine X. The relative stereochemistry of **2** was determined as same as **1** by NOESY experiment and coupling constants. Unfortunately, we could not isolate sufficient sample for running ^{13}C NMR spectrum.

Compound **3** was obtained as a white gum. The presence of a bicyclic taxane skeleton was suggested by the ^1H NMR spectrum. Proton signals due to four acetyl and a cinnamoyl groups appeared at 2.05, 2.12, 2.18, and 2.18 ppm (each 3H, *s*), and 7.56 (2H, *m*), 7.40 (3H, *m*), 6.41 (1H, *d*, $J = 16.2$ Hz), and 7.70 ppm (1H, *d*, $J = 16.2$ Hz, *trans*-oriented). Detailed analysis of the ^1H–^1H COSY spectrum revealed the connectivities of C-1 to C-3, C-5 to C-7, C-13 to C-1, and C-22 to C-23. FAB-MS gave an ion peak at *m/z* 721 ($[M+K]^+$), indicating that its molecular weight was 682, which was as same as that of **1** and 84 mass units less than that of taxuspine X [6]. The ^1H spectrum of **3** was closely resembled that of taxuspine X with the exception of H-2 shifted upfield to 4.85 ppm, and H-7 shifted upfield to 4.40 ppm. These data together with the IR absorption band at 3450 cm^{-1} due to hydroxy group established the structure of **3** as 2,7-dideacetyl

taxuspine X. ^{13}C NMR spectral data was not obtained due to the paucity of the sample.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were recorded on a Horiba SEPA-300 digital polarimeter. UV spectra were recorded on a Shimadzu UV-1600 spectrophotometer. IR spectra were obtained on a Jasco IR-810 instrument. MS were measured on a Jeol JMS-700 spectrometer using EI modes. ^{1}H and ^{13}C NMR spectra were obtained on Varian GEMINI 2000/300 and Varian Unity Inova 500 spectrometers operating at 300 MHz for ^{1}H and 125 MHz for ^{13}C nucleus, in $CDCl_3$ at ambient temperature, chemical shifts are reported in ppm scale relative to that of tetramethylsilane (TMS, $\delta = 0$) as an internal standard, and coupling constants are given in Hertz. Open column chromatography was performed using Merck silica gel 60 (100–200 mesh). Analytical thin layer chromatography was carried out with the precoated Merck silica gel 60 F_{254} plates. TLC separations and purifications were performed on glass plates coated with Merck silica gel 60 F_{254} (20×20 cm, 0.85 mm thickness) and visualized by UV (254 nm) and/or by spraying with 10% sulfuric acid and then heating on a hot plate.

Plant Material

The needles of *Taxus cuspidata* Sieb. et Zucc were collected in Sendai, in the north-east of Japan, in the autumn of 1996. The botanical identification was made by Prof. Oritani, T., Toyama Prefectural University. A voucher specimen has been deposited in our laboratory at Graduate School of Agricultural Science, Tohoku University, Japan.

Extraction and Isolation

Air dried needles (7.0 kg) were extracted with MeOH (25 l) twice at room temperature. The MeOH extracts were condensed to residue under reduced pressure. This residue was diluted with water and the aqueous solvent was extracted with EtOAc (each 1000 ml) three times. The combined EtOAc extract, upon evaporation, yielded 134 g of dark syrup. Part of which (66 g) was subjected to a silica gel (1100 g) column chromatography, eluted successively with $CHCl_3$–EtOAc (20 : 1, 10 : 1, 5 : 1, 2 : 1 and EtOAc, each

2500 ml), five fractions were obtained. Fractions 1 and 2 were repeatedly chromatographed on silica gel column and plates, eluted or developed with hexane–acetone (Prep. TLC 5:3), hexane–EtOAc (Prep. TLC 1 : 1), and $CHCl_3$–MeOH (Prep. TLC 100 : 3), finally afforded compounds **1** (3 mg), **2** (1 mg), and **3** (0.8 mg).

7,9,10,13-Tetraacetoxy-5-cinnamoyloxy-4-hydroxymethyl-8,12,15,15-tetramethyl-bicyclo[9.3.1] pentadeca-3,8,11-trien-2-ol (**1**). $[\alpha]_D^{24}$ + 14.52 (*c* 0.03, $CHCl_3$). IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3450, 3010, 2930, 2865, 1740, 1670, 1640, 1580, 1450, 1370, 1240, 1200, 1100, 1020, and 750; UV ($CHCl_3$) λ_{max} 278 nm (log ε 3.44); FAB-MS: *m/z* 705 ($[M+Na]^+$), 683 ($[M+H]^+$), 665 ($[M+H-H_2O]^+$), 623 ($[M+H-AcOH]^+$), 605 ($[M+H-H_2O-AcOH]^+$), 653 ($[M+H-2AcOH]^+$), 503 ($[M+H-3AcOH]^+$), 461 ($[M-3AcOH-COCH_3]^+$), 437, 415, 373, 355, 307, 289, 219, 154, 131, 107, 77, and 43. HR-FAB-MS: 665.2957 ($[M+H-H_2O]^+$) (calcd for $C_{37}H_{45}O_{11}$ 665.2959); ^1H NMR data: see Table I, ^{13}C NMR data (125 MHz, ppm, $CDCl_3$): 48.02 (C-1), 68.58 (C-2), 124.25 (C-3), 133.92 (C-4), 69.81 (C-5), 35.28 (C-6), 66.94

TABLE I ^1H NMR spectral data of **1**, **2**, and **3** ($CDCl_3$, ppm, 300 MHz)

Position	**1**		**2**		**3**	
	^1H	*J*	^1H	*J*	^1H	*J*
1	1.75 *m*		1.80 *m*		1.86 *m*	
2	4.72 *dd*	4.6,10.1	4.78 *dd*	4.6,10.7	4.85 *dd*	4.9,10.2
3	5.71 *br.d*	10.1	5.93 *br.d*	10.7	5.78 *br.d*	10.2
5	5.75 *br.s*		5.67 *br.s*		5.58 *br.s*	
6a	2.68 *ddd*	2.3,9.6,12.6	2.56 *m*		2.57 *m*	
6b	2.13 *br.d*	12.6	2.15 *m*		2.14 *m*	
7	5.52 *br.d*	9.6	5.47 *br.d*	8.8	4.40 *br.d*	8.2
10	7.25 *br.s*		7.25 *br.s*		7.22 *br.s*	
13	5.31 *br.d*	8.2	5.32 *br.d*	8.2	5.32 *br.d*	8.2
14α	2.05 *br.d*	16.4	2.02 *br.d*	16.5	2.05 *br.d*	16.4
14β	2.54 *br.dd*	8.8,16.4	2.47 *m*		2.58 *m*	
16	1.09 *s*		1.11 *s*		1.12 *s*	
17	1.25 *s*		1.29 *s*		1.28 *s*	
18	2.18 *br.s*		2.22 *br.s*		2.23 *br.s*	
19	1.62 *s*		1.64 *s*		1.63 *s*	
20a	4.43 *d*	12.6	5.09 *d*	12.9	5.16 *d*	12.4
20b	3.81 *d*	12.6	4.12 *d*	12.9	4.20 *d*	12.4
22	6.54 *d*	16.2	6.57 *d*	16.2	6.41 *d*	16.2
23	7.95 *d*	16.2	7.89 *d*	16.2	7.70 *d*	16.2
25	7.56 *m*		7.54 *m*		7.56 *m*	
26	7.41 *m*		7.42 *m*		7.40 *m*	
27	7.41 *m*		7.42 *m*		7.40 *m*	
AcO	1.77 *s*		1.80 *s*		2.05 *s*	
AcO	1.96 *s*		1.96 *s*		2.12 *s*	
AcO	2.03 *s*		2.01 *s*		2.18 *s*	
AcO	2.20 *s*		2.10 *s*		2.18 *s*	
AcO			2.21 *s*			

(C-7), 127.42 (C-8), 146.45 (C-9), 68.58 (C-10), 135.77 (C-11), 136.81 (C-12), 70.74 (C-13), 25.45 (C-14), 36.12 (C-15), 32.98 (C-16), 24.87 (C-17), 16.60 (C-18), 13.23 (C-19), 58.84 (C-20), 166.32 (C-21), 117.44 (C-22), 146.45 (C-23), 134.50 (C-24), 128.22 (C-25), 129.07 (C-26), 130.87 (C-27), 169.26, 170.62, 168.00, 168.02, 21.19, 21.03, 20.82, 20.48 (AcO). The assignment of ^{13}C NMR data were made by comparing with those of taxuspins X.

7,9,10,13,20-Pentaacetoxy-5-cinnamoyloxy-8,12,15,15-tetramethy bicyclo-[9.3.1] pentadeca-3,8,11-trien-2-ol (**2**). White gum; $[\alpha]_D^{25} + 10.33$ (*c* 0.03, $CHCl_3$); IR (film, $CHCl_3$) $\nu_{max}\,cm^{-1}$: 3450, 3010, 2950, 2905, 2850, 1740, 1640, 1580, 1450, 1370, 1240, 1200, 1100, 1020, 960, and 760; FAB-MS: *m/z* 747 ($[M+Na]^+$), 665 ($[M+H-AcOH]^+$), 605 ($[M+H-2AcOH]^+$); ^1H NMR (300 MHz) see Table I.

9,10,13,20-Tetraacetoxy-5-cinnamoyloxy-8,12,15,15-tetramethyl-bicyclo-[9.3.1] pentadeca-3,8,11-trien-2,7-diol (**3**). White gum; $[\alpha]_D^{25} + 7.33$ (*c* 0.002, $CHCl_3$); IR (film, $CHCl_3$) $\nu_{max}\,cm^{-1}$: 3450, 3010, 2910, 2850, 1740, 1720, 1640, 1450, 1370, 1240, 1200, 1160, 1100, 1020, and 755; FAB-MS: *m/z* 721 ($[M+K]^+$), 665 ($[M+H-H_2O]^+$), 605 ($[M+H-H_2O-AcOH]^+$), 545 ($[M+H-H_2O-2AcOH]^+$), 485, 443, 411, 395, 307, 289, 154, 135, and 43; ^1H NMR (300 MHz) see Table I.

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