TAXANES FROM THE LEAVES OF *Taxus cuspidata*

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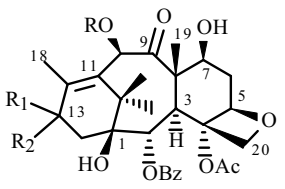
*A new taxane, 13-oxobaccatin III (**2**), and five known taxanes, 10-deacetyl-13-oxobaccatin III (**1**), baccatin III (**3**), 7-*epi*-10-deacetyltaxol (**4**), 19-debenzoyl-19-acetyltaxinine M (**5**), and 5α-decinnamoyltaxagifine (**6**), were isolated from the leaves of the Japanese yew,* Taxus cuspidate. *Their structures were established on the basis of the analysis of their spectral data.*

Keywords: *Taxus cuspidata*, Taxaceae, yew, leaves, taxane, diterpene, isolation.

The novel mechanism, limited natural source, and clinical effectiveness of Taxol® (paclitaxel), a diterpene with an unprecedented skeleton firstl isolated from the bark of *Taxus brevifolia*, against breast and ovarian cancers has stimulated a renewed interest in the isolation of taxane diterpenes from *Taxus* species (Taxaceae). As a result, more than 400 taxanes have been reported from various *Taxus* species [1, 2]. *Taxus cuspidata* is an evergreen tall tree or shrub distributed mainly in the northeast of China, Korea, and Japan; it is also a popular garden shrub in Japan. Previous chemical investigations on this yew have led to the identification of more than 100 taxanes including various skeletons [3–5], but there are still new taxanes being isolated [6]. In our continuing search for bioactive taxanes, we have isolated previously a series of new taxanes with various skeletons from the needles of *Taxus cuspidata* [7–10]. Further investigation on chemical constituents of the leaves of this plant harvested in different regions in Japan resulted in the isolation of six taxanes **1–6**. This paper will focus on the isolation and structure identification of these six taxanes.



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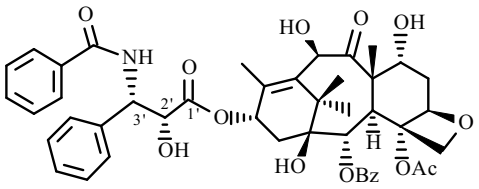

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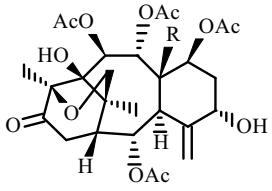

5, 6

1: R = H, R_1R_2 = O; **2:** R= Ac, R_1R_2 = O; **3:** R = Ac, R_1 = α-OH, R_2 = H; **5:** R = CH_2OAc; **6:** R = CH_3

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TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H (mult, J/Hz)	δ_C	δ_H (mult, J/Hz)	δ_C
1		78.5		79.4
2	5.68 (d, J = 7.0)	76.2	5.68 (d, J = 7.0)	77.0
3	4.02 (d, J = 7.0)	46.0	3.93 (d, J = 7.0)	46.0
4		80.5		81.4
5	4.94 (br.d, J = 8.0)	84.1	4.96 (br.d, J = 8.0)	84.8
6a	1.84 (m)	37.1	1.88 (m)	36.1
6b	2.57 (m)		2.57 (m)	
7	4.28 (m)	71.8	4.46 (br.t, J = 8.0)	72.8
8		58.3		60.2
9		209.3		209.2
10	5.41 (br.s)	72.8	6.46 (s)	73.4
10-OH	4.28 (br.s)			
11		156.1		155.5
12		139.4		135.6
13		198.0		199.2
14a	2.65 (d, J = 20.0)	42.4	2.70 (d, J = 20.0)	43.0
14b	2.95 (d, J = 20.0)		2.97 (d, J = 20.0)	
15		43.3		44.4
16	1.25 (s)	17.6	1.25 (s)	18.8
17	1.73 (s)	32.8	1.74 (s)	30.2
18	2.17 (s)	13.5	2.08 (s)	14.3
19	1.15 (s)	9.3	1.16 (s)	9.4
20a	4.14 (d, J = 8.0)	75.9	4.14 (d, J = 8.0)	77.2
20b	4.34 (d, J = 8.0)		4.35 (d, J = 8.0)	
OAc	2.10 (s)	21.8	1.98 (s)	21.8
		170.2		170.5
			2.31 (s)	22.1
				171.4
Bz-2				
o	8.07 (d, J = 8.0)	130.0	8.08 (d, J = 8.0)	131.1
m	7.43 (t, J = 7.5)	128.7	7.50 (t, J = 7.5)	129.2
p	7.50 (t, J = 7.5)	134.0	7.65 (t, J = 7.5)	134.5

Compound **1** was isolated as an amorphous powder. Its HRFAB-MS spectrum showed a peak at 543.2237 which implied this compound has a molecular formula of $C_{29}H_{35}O_{10}$ by analysis. The ^{1}H NMR (Table 1) and ^{1}H–^{1}H COSY spectra of **1** in $CDCl_3$ demonstrated characteristic signals of a taxane, including an AB system due to an oxane group of H-20a and H-20b (δ_H 4.14 and 4.34, d, J = 8.0 Hz) [11], four methyl groups at δ_H 1.15, 1.25, 1.73, and 2.17, an acetyl methyl at δ_H 2.10, a broad doublet at δ_H 4.94 (d, J = 8.0 Hz) due to the presence of the H-5α, and a second AB system of H-2 and H-3 at δ_H 5.68 (d, J = 7.0 Hz) and δ_H 4.02 (d, J = 7.0 Hz). Two broad singlets at δ_H 5.41 and 4.28 were attributed to H-10 and 10-OH protons. The signal at δ_H 4.28 dispeared after adding a drop of D_2O, which confirmed it as an exhangeable proton. The AB system with a large coupling constant and characteristic signals was caused by the geminal protons of H-14a at δ_H 2.65 (1H, d, J = 20.0 Hz) and H-14b at 2.95 (1H, d, J = 20.0 Hz). A set of phenolic protons at δ_H 7.50 (1H, t, J = 7.5 Hz), 7.43 (2H, t, J = 7.5 Hz), and 8.07 (2H, br.d, J = 8.0 Hz) due to a benzoxyl group was also observed. In the ^{13}C NMR spectrum, the characteristic signal indicated the existence of a ketone at δ_C 209.3 (C-9) and a conjugated ketone at δ_C 198.0 (C-13). Taking all the above data into account, the structure of **1** was identified as 10-deacetyl-13-oxobaccatin III. This compound was isolated previously from a yew tree *Taxus sumatrana* collected in Indonesia, but its full NMR data were not reported [12].

TABLE 2. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), HMBC, and NOESY Data of Compound **4** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H (mult, J/Hz)	δ_C	HMBC	NOESY
1		79.1		
2	5.73 (d, J = 7.4)	75.4	1, 8, 14, 166.9	3, 17, 19, 20
3	3.91 (d, J = 7.5)	40.3	1, 2, 8, 19	2, 6, 10, 18, 7-OH
4		81.9		
5	4.90 (dd, J = 9.0, 3.9)	82.6		6, 7-OH
6ab	2.32 (m)	35.4		
7	3.66 (br.ddd, J = 12.3, 4.8, 1.8)	75.8	7	6, 10, 19, 7-OH
7-OH	4.73 (d, J = 12.2)			3, 5, 6, 7, 10
8		57.1		
9		214.9		
10	5.42 (s)	77.7	9, 11, 12, 15	3, 18, 7-OH
10-OH	4.11 (br.s)			10
11		135.5		
12		137.7		
13	6.23 (m)	72.5		14b, 16
14a	2.38 (m)	36.3		3, 14b
14b	2.22 (m)			13, 14a
15		42.4		
16	1.205 (s)	26.0	1, 11, 15, Me	13, 14b
17	1.08 (s)	20.5	1, 11, 15, Me	2, 19
18	1.74 (s)	14.3	11, 12, 13	3, 10, 7-OH
19	1.72 (s)	16.6	3, 7, 8, 9	2, 6, 7, 17, 20
20ab	4.39 (s)	77.7	4	2, 5, 19, Bz-2*o*
OAc	2.51 (s)	22.5	172.3	
		172.3		
CO-2		166.9		
Bz-2				
o	8.16 (d, J = 7.8)	130.2	166.9	20, Bz-*m*
m	7.52 (t, J = 7.8)	128.8		
p	7.61 (t, J = 7.8)	133.7		
1′				
2′	4.79 (br.s)	73.1		
2′-OH	3.44 (br.)			
3′	5.79 (dd, J = 9.2, 2.7)	54.9		
Ph 3′				
o	7.47	126.5		
m, *p*	7.40	129–128		
NH 4′			166.9	Ph6′-*o*, Ph3′-*o*, 3′
CO 5′		166.9		
Ph 6′				
o	7.72 (d)	126.5	166.9	NH4′, Ph-*m*
m/p	7.37/7.36	128.6		

Compound **2** was isolated as an amorphous powder. Its molecular formula was assigned as $C_{31}H_{37}O_{11}$ because the HRFAB-MS showed a molecular ion peak at 585.2337. This suggested that **2** had one more acetyl group than **1**. The ^{1}H NMR spectrum of **2** in $CDCl_3$ (Table 1) closely resembled that of **1** except that the H-10 signal shifted to down-field compared to that of **1**, while the proton signal of 10-OH in **1** disappeared in the spectrum of **2**. Indeed, in the ^{13}C NMR spectrum of **2**, one more acetyl group signal was observed at δ 22.1 and δ 171.4. All these results indicated that **2** was the 10-OH acetylated derivative of **1**. Thus, the structure of **2** was characterized as 13-oxobaccatin III, and it is found for the first time in *Taxus cuspidata*.

The structures of baccatin III (**3**) [13], 7-*epi*-10-deacetyltaxol (**4**) [14], 19-debenzoyl-19-acetyltaxinine M (**5**) [15], and 5-decinnamoyltaxagifine (**6**) [16] were established on the basis of spectroscopic analysis (Tables 1 and 3) and further confirmed by comparing with reported spectroscopic data in the literature.

TABLE 3. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), HMBC Data of Compounds **5** and **6** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**5**			**6**			
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	NOESY
1	2.41 (br.d, J = 12.0)	48.8		2.36 (br.dd, J = 11.7, ~1.7)	49.3	2, 3, 13, 14, 15, 16	14a, 16b, 17
2	6.16 (dd, J = 10.2, 2.5)	69.4		5.50 (dd, J = 1.7, 9.3)	68.7	8, 14, 169.9	1, 9, 17, 19, 20b
3	3.63 (d, J = 10.2)	38.3	1, 2, 20	3.53 (d, J = 9.2)	38.2	1, 2, 4, 5, 8, 19, 20	7, 10, 14b
4		**144.4**			144.3		
5	4.37 (o.d)	72.6		4.33 (br.t, J = ~2.8)	73.2	3, 20	6b, 20a
6a	2.15 (o.m)	39.2		2.10 (o.m)	39.4		6b, 7
6b	1.61 (o.m)			1.58 (ddd, J = 13.5, 10.3, 3.7)			5, 6a, 19
7	5.44 (o.dd, J = 10.6, 6.1)	68.4		5.38 (dd, J = 6.1, 10.5)	68.2	6, 8, 9, 19, 168.4	3, 6a
8		49.0			46.7		
9	5.47 (o.d, J = 2.8)	70.3	11, 172.1	4.92 (d, J = 3.3)	75.9	3, 6, 7, 8, 11, 19, 172.4	19, 17
10	5.28 (o.d, J = 2.8)	64.0	168.5	5.34 (d, J = 3.4)	63.9	8, 9, 12, 168.4	3, 9, 18
11		**80.1**			80.3		
12		**91.2**			91.8		
13		**204.3**			205.4		
14a	2.99 (dd, J = 19.1, 12.2)	33.9	2, 15	3.02 (dd, J = 11.5, 18.3)	34.9	1, 2, 13	1, 14b, 16b
14b	2.69 (d, J = 19.1)		2, 15	2.63 (d, J = 18.3)		1, 2, 13, 15	3, 14a
15		**49.5**			49.7		
16a	4.18 (d, J = 8.2)	82.2	1, 2, 15, 17	4.18 (d, J = 8.1)	82.0	1, 2, 12, 17	16b, 17
16b	3.69 (d, J = 8.2)		11, 12, 15	3.68 (d, J = 8.0)		1, 11, 12	1, 14a, 16a, 17
17	1.58 (o.s)	14.9	1, 11, 15, 16	1.51 (s)	15.6	1, 11, 15, 16	1, 2, 9, 16a, 11-OH
18	1.17 (s)	12.1	11, 12, 13	1.16 (s)	11.8	11, 12, 13	
19				1.04 (s)	13.5	3, 7, 8, 9	
19a	4.39 (d, J = 12.0)	61.4	8, 9, 170.4				
19b	4.25 (o.d, J = 12.0)		3, 8				
20a	5.30 (s)	112.8	5	5.25 (s)	113.1	3, 4, 5, 8	2, 5, 20b
20b	4.44 (s)		3, 4, 5	4.49 (s)		3, 4, 5, 8	2, 19, 20a
OAc	2.176 (s)	20.7 170.3	170.3	2.10 (s)	20.5, 168.4	168.4	
	2.10 (2 × s)	20.6 172.1, 168.5	172.1, 168.5	2.09 (s) 2.02 (s)	20.5, 172.4 21.2	172.4 169.9	
	1.98 (s)	21.3 169.8	169.8	1.95 (s)	169.9 21.3	168.4	
	1.95 (s)	21.1 168.0	168.0		168.4		
11-OH				4.03 (br.s)		10, 11, 12	9, 17

EXPERIMENTAL

General Comments. Flash chromatography was performed on Silica gel 60 (230–400 mesh, EM Science). Thin layer chromatography was conducted on Silica Gel 60 F_{254} pre-coated TLC plates (0.25 mm or 0.5 mm, EM Science). The compounds were visualized on TLC plates with 10% sulfuric acid in ethanol and heated on a hot plate. Na_2SO_4 was the drying agent used in all work-up procedures. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 2996 detector. Preparative and semipreparative HPLC were carried out on a Waters Delta Prep 3000 instrument with a UV 2487 detector. Analytical HPLC was performed with two Whatman partisil 10 ODS-2 analytical columns (4.6 × 250 mm) in series. Semipreparative HPLC was performed with two Whatman partisil 10 ODS-2 Mag-9 semipreparative columns (9.4 × 250 mm) in series. A 50 min linear gradient of acetonitrile (25 to 100%) in water was used for HPLC with a flow rate of 18 mL/min for the preparative HPLC and 3 mL/min for the semipreparative HPLC runs. Melting points were measured on an MRK micromelting point apparatus and are uncorrected. Optical rotations were recorded on a SEPA-300 polarimeter. MS spectra were obtained

on a JMS-Dx 305 HF mass spectrometer, using the FAB method with glycerol as a matrix. NMR spectra were recorded on Varian GEMINI 2000/300 and Varian Unity Inova 500 spectrometers operating at 300 and 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$ at ambient temperature. Coupling constants are given in Hz, and the ^{1}H chemical shifts data were recorded in ppm with TMS as an internal reference. ^{13}C shifts were referred to the $CDCl_3$ signal at 77.0 ppm.

Plant Materials. The leaves of *Taxus cuspidata* were collected in the autumn of 1997 in Toyama Prefecture in the west of Japan. The botanical identification was made by Prof. T. Oritani of Toyama Prefectural University, Toyama, Japan. The voucher specimens were deposited in the Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan.

Extraction and Isolation. Air-dried leaves of *Taxus cuspidata* were chipped (5.4 kg) and fully submerged in 40 L of methanol for two weeks at room temperature. The plant residue was filtered and extracted again with fresh solvent (12 L) for another week. The combined organic solution was evaporated under reduced pressure. The residue was suspended in 3 L of water, and the lipid was removed by partition with hexane (3 × 3 L). The aqueous phase was then salted (NaCl, 200 g) and extracted with CH_2Cl_2 (4 × 3 L). The combined CH_2Cl_2 solutions were dried with anhydrous sodium sulfate and filtered. The CH_2Cl_2 solution was evaporated to dryness and yielded a dark extract (40 g). A portion of the methylene chloride extract (30 g) was absorbed onto 40 g silica gel and packed on a wet column chromatograph. Successive elution with CH_2Cl_2–MeOH gradient with increasing amounts of MeOH from 5 to 45% yielded 35 fractions (Fr_{D-1} to Fr_{D-35}). The Fr_{D-30} to Fr_{D-34} were combined (5 g) according to their TLC results and chromatographed over silica gel (150 g) and eluted with hexane–acetone and yielded 15 fractions (Fr_{D-30-1} to $Fr_{D-30-15}$). The Fr_{D-30-8} and Fr_{D-30-9} (0.88 g) were subjected to preparative HPLC and yielded **1** (4 mg, t_R = 26.38 min), **2** (2 mg, t_R = 28.12 min), and **5** (2 mg, t_R = 28.47 min). The $Fr_{D-30-10}$ and $Fr_{D-30-15}$ (1.28 g) were subjected to column chromatography followed by preparative TLC and gave **3** (5 mg), **4** (2 mg), and **6** (2 mg).

10-Deacetyl-13-oxobaccatin III (1). Amorphous powder; $[\alpha]_D^{22}$ –41.0° (*c* 0.1, $CHCl_3$). For ^{1}H and ^{13}C NMR data, see Table 1; HR-FAB-MS *m/z:* 543.2237 $[M+H]^+$ (calcd for $C_{29}H_{35}O_{10}$, 543.2230).

13-Oxobaccatin III (2). Amorphous powder; $[\alpha]_D^{22}$ –39.0° (*c* 0.05, $CHCl_3$). For ^{1}H and ^{13}C NMR data, see Table 1; HR-FAB-MS *m/z:* 585.2337 $[M+H]^+$ (calcd for $C_{31}H_{37}O_{11}$, 585.2336).

Baccatin III (3). Mp 237–238°C, $[\alpha]_D^{22}$ –53.0° (*c* 1.0, $CHCl_3$). HR-FAB-MS *m/z:* 545.2389 $[M+H]^+$ (calcd for $C_{31}H_{38}O_{11}$, 586.2387).

7-*epi*-10-Deacetyltaxol (4). Amorphous powder; $[\alpha]_D^{22}$ –33.0° (*c* 0.1, $CHCl_3$). HR-FAB-MS *m/z:* 854.3386 $[M+H]^+$ (calcd for $C_{47}H_{52}O_{14}$, 854.3388). For ^{1}H and ^{13}C NMR, HMBC, and NOESY spectral data, see Table 2.

19-Debenzoyl-19-acetyltaxinine M (5). $[\alpha]_D^{22}$ +3.0° (*c* 0.1, $CHCl_3$). HR-FAB-MS *m/z:* 625.2493 $[M+H]^+$ (calcd for $C_{30}H_{41}O_{14}$, 625.2496). For ^{1}H and ^{13}C NMR and HMBC spectral data, see Table 3.

5α-Decinnamoyltaxagifine (6). Mp 119–120°C, $[\alpha]_D^{22}$ +5.0° (*c* 1.0, $CHCl_3$). HR-FAB-MS *m/z:* 567.2441 $[M+H]^+$ (calcd for $C_{28}H_{39}O_{12}$, 567.2442). For ^{1}H and ^{13}C NMR, HMBC, and NOESY spectral data, see Table 3.

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