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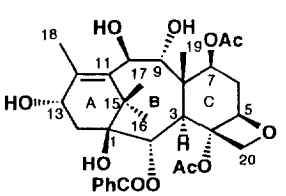
Taxuspines E ~ H and J, New Taxoids from the Japanese Yew *Taxus cuspidata*

Jun'ichi Kobayashi*, Akiko Inubushi, Hirokazu Hosoyama, Naotoshi Yoshida, Takuma Sasaki,[a] and Hideyuki Shigemori

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan,
[a]Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

Abstract: Five new taxoids, taxuspines E ~ H and J (**1** ~ **5**), have been isolated together with known taxoids (**6** ~ **23**) containing a series of taxol-related compounds (**15** ~ **20**) from stems and leaves of the Japanese yew *Taxus cuspidata* Sieb. et Zucc. and the structures elucidated on the basis of spectroscopic data. The structures and cytotoxicity of these taxoids (**1** ~ **23**) are described.

The discovery that taxol is an effective drug against ovarian and breast cancer has stimulated a renewed interest in the isolation of taxol-related compounds from various species of yews.[1] In our continuing search for bioactive natural products, we isolated previously new taxane diterpenoids, taxuspines A ~ C[2] and D[3], from stems of the Japanese yew *Taxus cuspidata* Sieb. et Zucc. Further investigation on extracts of stems and leaves of this yew led to isolation of five new taxane diterpenoids, taxuspines E ~ H and J (**1** ~ **5**), together with known taxoids (**6** ~ **23**) containing a series of taxol-related compounds (**15** ~ **20**) other than taxuspines A ~ D and eight known taxoids reported previously.[2] Here we describe the isolation and structure elucidation of taxuspines E ~ H and J (**1** ~ **5**) and cytotoxicity of the new and known taxoids (**1** ~ **23**) obtained.



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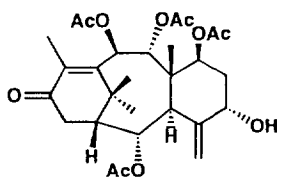

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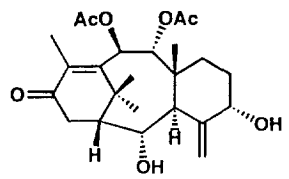

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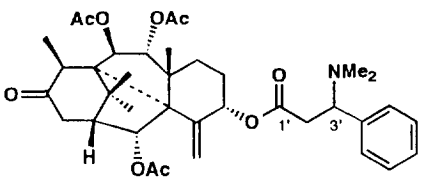

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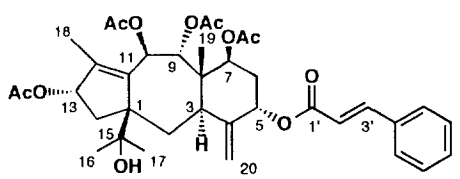

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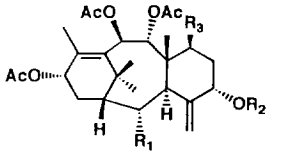
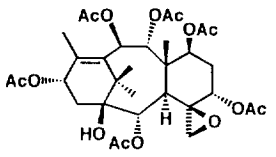
The methanolic extract of stems of the yew collected at Sapporo was partitioned between toluene and water. The toluene soluble portions were subjected to a silica gel column followed by reversed-phase and silica gel column chromatographies to afford taxuspines E (**1**, 0.00021%), F (**2**, 0.001%), and G (**3**, 0.00034%) together with known taxane diterpenoids, 7,2'-didesacetoxyaustrospicatine (**6**)[4], 12α-acetoxytaxusin (**7**)[5], decinnamoyltaxinine J (**8**)[6], 1β-hydroxybaccatin I (**9**)[7], taxagifine (**10**)[8], taxacin (**11**)[9], decinnamoyltaxagifine (**12**)[10], taxinine M (**13**)[11], taxol (**14**)[12], 10-deacetyltaxol (**15**)[13], cephalomannine (**16**)[16], 10-deacetylcephalomannine (**17**)[15], taxol C (**18**)[17], 10-deacetyltaxol C (**19**)[18], taxol D (**20**)[17], taxchinin B (**21**)[18], brevifoliol (**22**)[19, 21], 13-acetylbrevifoliol (**23**)[20, 21], and other known taxoids reported previously.[2] The toluene soluble portions of the methanolic extract of leaves of the yew were separated by a silica gel column, centrifuged counter-current chromatography, and a reversed-phase column to obtain taxuspines H (**4**, 0.0017%) and J (**5**, 0.0017%) together with known taxoids, taxinine, taxinines A and B, taxagifine (**10**), taxacin (**11**), taxine II, *O*-cinnamoyltaxicin I triacetate, 2'β-desacetoxyaustrospicatine, taxuspines A and D.[2,3]

Taxuspine E (**1**) was shown to have the molecular formula, $C_{31}H_{40}O_{11}$, by HRFABMS [*m/z* 589.2669 (M^++H), Δ +2.0 mmu]. IR absorptions at 3420 and 1720 cm^{-1} implied that **1** possessed hydroxy and ester groups, respectively. Analyses of the ^1H and ^{13}C NMR data (Table 1 and 2) and HMQC spectrum of **1** provided two acetyls, one benzoyl, one tetrasubstituted olefin, six oxymethines, two oxygenated quaternary carbons, and four methyl groups. Eight out of the twelve unsaturations were thus characterized, and compound **1** was therefore inferred to contain four rings. The signals at δ_H 4.13 and 4.50 (each 1H, d, J = 7.8 Hz) and δ_C 75.0 (t) indicated the presence of an oxetane ring in the molecule.[1] 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, C-13 to C-14, and C-18 to C-13. In the HMBC spectrum cross-peaks of H_3-16 and H_3-17 to C-1, C-11, and C-15 revealed that both Me-16 and Me-17 were attached at C-15, while cross-peaks of H_3-18 to C-11 and C-12 provided that Me-18 was attached at C-12. These HMBC correlations and a ^1H-^{13}C long-range cross-peak between H-14 and C-15 implied the presence of a cyclohexene moiety (ring A). Cross-



6 R_1=R_3=H, R_2=$COCH_2CH(NMe_2)Ph$
7 R_1=OAc, R_2=Ac, R_3=H
8 R_1=R_3=OAc, R_2=H



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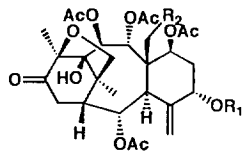
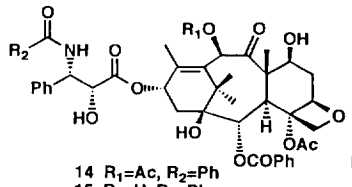
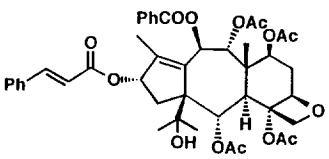


10 R_1=$COCH^{trans}=CHPh$, R_2=H
11 R_1=$COCH^{trans}=CHPh$, R_2=OCOPh
12 R_1=H, R_2=H
13 R_1=H, R_2=OCOPh



14 R_1=Ac, R_2=Ph
15 R_1=H, R_2=Ph
16 R_1=Ac, R_2=C_4H_7
17 R_1=H, R_2=C_4H_7
18 R_1=Ac, R_2=*n*-C_5H_{11}
19 R_1=H, R_2=*n*-C_5H_{11}
20 R_1=Ac, R_2=*n*-C_3H_7



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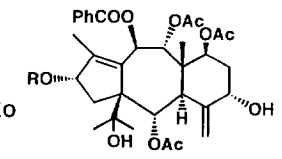

22 R=H
23 R=Ac

peaks of H-2 to C-1, H-3 to C-8 and C-9, and H-10 to C-15 in the HMBC spectrum indicated the presence of an eight-membered ring (ring B). ^1H-^{13}C long-range correlations of H-3 to C-4, H-5 to C-4, and H-7 to C-8 were suggestive of the presence of a cyclohexane moiety (ring C). HMBC correlations of H-20a and H-20b to C-4 and C-5 indicated that the oxetane ring was fused to the ring C at C-4 and C-5. A carbonyl carbon at δ_C 167.0 showed a correlation with H-2 in the HMBC spectrum, supporting the presence of a benzoyloxy group at C-2. An acetoxy carbonyl carbon (δ_C 170.8) showed an HMBC correlation with H-7, indicating that the acetoxy group was attached at C-7. Since two deuterium-exchangeable protons (δ_H 4.30 and 4.60) showed cross-peaks to two oxymethine protons (H-9 and H-10), respectively, in ^1H-^1H COSY spectrum of **1**, the two hydroxy groups were connected to C-9 and C-10, while the remaining acetoxy group was attached at C-4 (δ_C 81.0), like many other taxoids containing an oxetane ring.[1] Thus the structure of taxuspine E was concluded to be **1**. The relative stereochemistry of **1** was elucidated by the NOESY spectrum as shown in Fig. 1.

Taxuspine F (**2**) was shown to have the molecular formula, $C_{28}H_{38}O_{10}$, by HREIMS [*m/z* 534.2479 (M^+), Δ +1.4 mmu]. IR absorptions at 3440, 1740, and 1670 cm^{-1} indicated the presence of hydroxy, ester, and α,β-unsaturated carbonyl groups, respectively. ^1H and ^{13}C NMR data (Table 1 and 2) were suggestive of the presence of an exomethylene unit (δ_H 4.81 and 5.22, each 1H, s; δ_C 149.7 and 115.6). ^1H-^1H COSY connectivity between H-3 and H-20a and NOESY correlation of H-5 to H-20a provided that the exomethylene (C-20) was attached at C-4. The carbonyl (δ_C 199.3) and olefin (δ_C 139.1 and 144.7) carbon signals and UV absorption at 267 nm implied the presence of an α,β-unsaturated ketone group. The presence of four acetoxy groups in **2** was elucidated by ^1H NMR data and EIMS fragment ions [*m/z* 534 (M^+) and 294 $(M-4xAcOH)^+$]. The ^1H-^1H COSY spectrum of **2** revealed connectivities of C-1 to C-3, C-5 to C-7, C-9 to C-10, and C-14 to C-1. The protonated carbons were all assigned by HMQC experiment. Three out of four acetoxy groups were attached at C-2, C-9, and C-10 based on oxymethine proton signals (δ_H 5.59, H-2; 5.90, H-9; 6.26, H-10), while a hydroxy group was connected to C-5 (δ_H 4.21, H-5). Although the ^1H and ^{13}C NMR data were similar to those of taxinine A[23] having an usual 6/8/6-membered ring system, an acetoxy methyl (δ_H 2.05) and an oxymethine (δ_H 5.52) protons in **2** were observed in place of methylene protons (δ_H 1.60 and 1.85) in taxinine A. Since the oxymethine proton showed ^1H-^1H COSY cross-peaks for H_2-6, the acetoxy group was attached at C-7. Thus the structure of taxuspine F was assigned as **2**, in which the relative stereochemistry was elucidated by the NOESY spectrum.

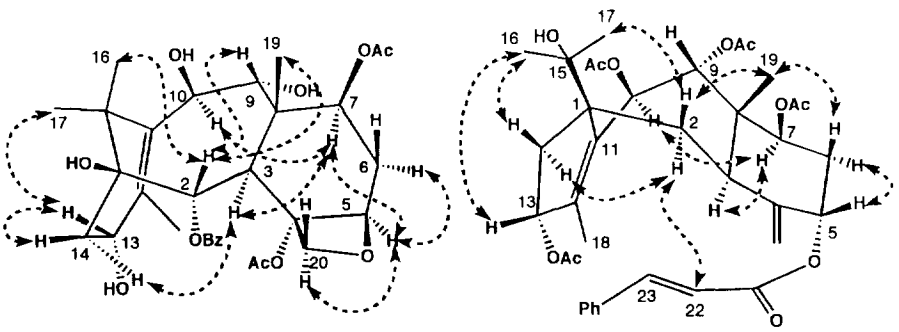

Figure 1. Relative Stereochemistries of Taxuspines E (1) and J (5)
Dotted arrows denote NOESY correlations.

Table 1. ^{1}H NMR Data of Taxuspines E ~ H and J (**1** ~ **5**) in $CDCl_3$

position	1	2	3	4	5
1		2.19 (dd, 7.0, 1.7)	2.34 (dd, 7.0, 1.7)	2.15 (m)	
2 (a)	6.10 (d, 7.4)	5.59 (dd, 6.1, 1.7)	4.30 (dd, 6.2, 1.7)	6.09 (d, 5.3)	2.35 (dd,14.2, 9.0)
(b)					1.45 (d, 14.2)
3	3.13 (d, 7.4)	3.54 (d, 6.1)	3.47 (d, 6.2)		2.71 (d, 9.0)
5	4.88 (d, 8.0)	4.21 (brs)	4.23 (brs)	5.35 (t, 4.0)	5.54 (t, 3.7)
6 (a)	1.87 (m)	1.66 (m)	1.64 (m)	1.62 (m)[c]	1.92 (dd, 14.5, 3.7)
(b)	2.59 (m)	1.93 (ddd, 13.6, 5.4, 2.6)	1.75 (m)		2.02 (m)
7 (a)	5.33 (t, 8.3)	5.52 (dd, 11.5, 5.4)	1.61 (m)	1.86 (m)[c]	5.62 (dd, 11.3, 5.2)
(b)			1.81 (m)		
9	4.30 (brd, 9.6)	5.90 (d, 10.7)	5.71 (d, 10.4)	5.59 (d, 9.5)	5.93 (d, 10.6)
10	4.60 (brd, 9.6)	6.26 (d, 10.7)	6.09 (d, 10.4)	5.68 (d, 9.5)	6.40 (brd, 10.6)
12				3.47 (q, 7.2)	
13	4.57 (brt, 6.9)				5.42 (t, 7.3)
14 (a)	1.75 (dd, 13.7, 5.7)	2.30 (d, 19.7)	2.23 (d, 19.7)	2.45 (dd, 20.5, 6.6)	1.19 (dd, 14.0, 8.0)
(b)	2.29 (dd, 13.7, 6.7)	2.77 (dd, 19.7, 7.0)	2.80 (dd, 19.7, 7.0)	2.56 (d, 20.5)	2.48 (dd, 14.0, 7.3)
16	1.08 (s)	1.74 (s)	1.69 (s)	1.68 (s)	1.31 (s)
17	1.04 (s)	1.12 (s)	1.16 (s)	1.22 (s)	1.12 (s)
18	1.97 (s)	2.32 (s)	2.21 (s)	1.31 (d, 7.2)	2.08 (s)
19	1.93 (s)	0.97 (s)	0.91 (s)	1.26 (s)	0.92 (s)
20 (a)	4.13 (d, 7.8)	4.81 (s)	5.25 (s)	5.43 (s)	5.35 (s)
(b)	4.50 (d, 7.8)	5.22 (s)	5.27 (s)	5.70 (s)	4.93 (s)
2'				2.72 (brdd, 15.0, 8.0)	6.39 (d, 16.0)
				3.00 (brdd, 15.0, 6.0)	
3'				3.91 (brdd, 8.0, 6.0)	7.69 (d, 16.0)
3'-Ph				7.30 (m)	7.39 (m)
					7.51 (m)
AcO	2.06 (s)	2.02 (s)	2.04 (s)	2.04 (s)	2.01 (s)
	2.23 (s)	2.05 (s)	2.07 (s)	2.06 (s)	1.98 (s)
		2.05 (s)		2.07 (s)	1.97 (s)
		2.07 (s)			1.53 (s)
BzO	7.47 (t, 7.6)				
	7.60 (t, 7.6)				
	8.01 (t, 7.6)				
Me_2N				2.22 (s)	
15-OH					2.60 (brs)

a) δ in ppm b) HMBC correlations c) 2H

The molecular formula of taxuspine G (**3**) was determined to be $C_{24}H_{34}O_7$ by HREIMS [*m/z* 434.2319 (M^+), Δ +1.4 mmu]. IR (ν_{max} 1670 cm^{-1}) and UV (λ_{max} 269 nm) data implied the presence of an α,β-unsaturated ketone group. The ^{1}H NMR (Table 1) spectrum of **3** showed proton signals due to an exomethylene, two acetyl, and four methyl groups. ^{1}H and ^{13}C (Table 2) NMR data of **3**, which were very close to those of taxinine A, were assigned on the basis of ^{1}H-^{1}H COSY and HMQC spectra. Two acetoxy groups (δ_H 2.04 and 2.07) were attached at C-9 and C-10 based on oxymethine proton signals (δ_H 5.71, H-9; δ_H 6.09, H-10), while two hydroxy groups were connected to C-2 (δ_H 4.30, H-2) and C-5 (δ_H 4.23, H-5). The presence of a hydroxy group on C-2 in **3** was deduced from comparison of the ^{1}H NMR data of **3** with those of taxinine A, since upfield shift of the C-2 oxymethine proton in **3** (δ_H 4.30 for **3**; δ_H 5.53 for taxinine A) was observed. Thus the structure of taxuspine G was determined to be **3**. The relative stereochemistry of **3** was provided from NOESY data.

Taxuspine H (**4**) was shown to have the molecular formula, $C_{37}H_{49}NO_9$, by HREIMS [*m/z* 651.3425 (M^+), Δ +1.8 mmu]. IR absorptions at 1740 and 1700 cm^{-1} implied that **4** possessed ester and ketone groups, respectively. The ^{1}H NMR (Table 1) spectrum of **4** showed proton signals due to three acetyl methyls, a dimethylamino group, and four methyls. Three acetoxy groups were attached at C-2, C-

Table 2. ^{13}C NMR Data of Taxuspines E ~ H and J (**1** ~ **5**) in $CDCl_3$

position	1	2	3	4	5	position	1	2	3	4	5
1	77.0	48.8	51.4	47.8	55.2	1'				170.8	165.6
2	68.2	68.9	68.3	76.5	29.2	2'				29.7	118.2
3	44.1	39.7	42.9	66.4	40.1	3'				65.9	145.1
4	81.0	149.7	149.6	141.8	145.2	3'-Ph				130.0	128.1
5	84.7	74.6	75.8	77.2	74.1					133.4	129.0
6	34.0	37.8	26.8	25.3	32.8						130.5
7	71.4	69.4	31.1	31.1	70.0	AcO	21.2	20.7	20.7	20.9	20.5
8	58.0	47.8	37.7	44.4	44.6		21.4	20.9	20.9	21.1	20.5
9	79.8	75.1	76.1	79.6	77.3		170.0	20.9	169.3	21.4	20.8
10	77.8	72.8	73.3	82.3	68.8		170.4	21.4	170.2	168.8	21.4
11	131.5	139.1	138.8	57.8	134.4			169.1		169.5	168.2
12	135.5	144.7	148.8	52.3	148.0			169.3		169.9	169.9
13	68.2	199.3	200.0	214.2	79.4			169.7			170.1
14	40.0	36.1	35.8	38.8	45.0			169.7			171.2
15	43.0	37.5	45.0	42.7	75.6	BzO	128.8				
16	22.3	25.4	25.5	28.8	26.7		129.8				
17	25.5	37.4	37.6	26.7	27.1		132.0				
18	21.8	14.4	14.0	15.7	11.8		167.0				
19	13.4	12.9	17.4	26.5	16.8	Me_2N				41.6	
20	75.0	115.6	114.7	130.1	114.9					42.2	

a) δ in ppm b) HMBC correlations c) 2H

9, and C-10 based on oxymethine proton signals (δ_H 6.09, H-2; 5.59, H-9; 5.68, H-10). ^1H and ^{13}C NMR (Table 2) data of **4** were similar to those of spicaledonine[24] having a 3,11-cyclotaxane skeleton, except for signals due to the acyl (β-dimethylamino-β-phenylpropanoic acid) side chain at C-5. The methylene protons (δ_H 2.72 and 3.00) at C-2' of the C-5 side chain in **4** were observed in place of an oxymethine proton (δ_H 4.64) in spicaledonine, indicating lack of a hydroxy group at C-2' in **4**. Thus the structure of taxuspine H was elucidated to be **4**. The relative stereochemistry of taxuspine H was established by derivatization of taxine II to **4** through the same photochemical reaction as that applied previously for taxinine.[2] All spectral data [^1H NMR, $[\alpha]_D$, etc.] of **4** derived from taxine II were identical with those of taxuspine H.

The molecular formula, $C_{37}H_{46}O_{11}$, of taxuspine J (**5**) was established by HRFABMS [*m/z* 667.3130 (M^++H), Δ +1.1 mmu]. The ^1H and ^{13}C NMR (Tables 1 and 2) spectra of **5** resembled those of taxuspine A[2] having an unusual 5/7/6-membered ring system. The olefin proton signals of the cinnamoyl group at C-5 appeared at δ_H 6.39 (1H, d, J = 16.0 Hz) and 7.69 (1H, d, J = 16.0 Hz; trans-oriented). Four acetoxy groups were attached at C-7, C-9, C-10, and C-13 based on oxymethine proton signals (δ_H 5.62, H-7; 5.93, H-9; 6.40, H-10; 5.42, H-13). Two methyl proton (δ_H 1.12 and 1.31), an deuterium-exchangeable proton (δ_H 2.60), and an oxygenated quaternary carbon (δ_C 75.6, C-15) signals indicated the presence of a dimethylcarbinol group at C-1 like taxuspine A.[2] The dimethylcarbinol group was inferred to be β-oriented on ring A, since NOESY correlations of H-16 to H-2b, H-13, and H-14b were revealed. The proton signal due to an acetoxy group at C-10 in **5** was observed in place of a benzoyloxy group in taxuspine A. Thus the structure of taxuspine J was assigned as **5**. The relative stereochemistry was elucidated by the NOESY spectrum as shown in Fig. 1. The coupling constant between H-9 and H-10 (J = 10.6 Hz) suggested that the B/C ring in **5** adopted a boat/chair conformation in solution.[18]

Taxuspines E ~ H and J (**1** ~ **5**) are new taxane diterpenoids from the Japanese yew *Taxus cuspidata* Sieb. et Zucc. Taxuspine E (**1**) contains an oxetane ring in addition to an usual 6/8/6-membered ring

system like taxol (**14**), while taxuspines H (**4**) and J (**5**) possess rearranged taxane skeletons, 6/5/5/6- and 5/7/6-membered ring system, respectively. This is the first isolation of taxol-related compounds **15** and **17** ~ **21** possessing both an oxetane ring and an *N*-acylphenylisoserine moiety from *T. cuspidata,* although these compounds have been previously isolated from *T. brevifolia, T. baccata,* or *T. chinensis.* Cytotoxic activity of taxoids **1** ~ **23** against murine leukemia L1210 and human epidermoid carcinoma KB cells *in vitro* is shown in Table 3. Among five new taxoids, taxuspine E (**1**) exhibited potent cytotoxicity against KB cells with an IC_{50} value of 0.08 μg/mL, while taxuspines F ~ H and J (**2** ~ **5**) showed weak or no cytotoxicity. It is noted that taxuspine E (**1**) lacking the side chain at C-13 showed potent cytotoxicity against KB cells, since the baccatin III-type compounds having an oxetane ring but no C-13 side chain were reported to be ca. 1700-fold less cytotoxic against KB cells than taxol (**14**).[25] The structures of taxuspine E (**1**) and the baccatin III derivatives differ in the functional group at C-9 (a hydroxy group for **1**; a ketone group for the baccatin III derivatives), indicating that the presence of the hydroxy group at C-9 in addition to an oxetane ring may be important for cytotoxicity. On the other hand, taxchinin B (**21**) containing an oxetane ring showed no cytotoxicity against KB cells (IC_{50} >10 μg/mL), indicating that contraction of ring A may reduce cytotoxicity due to instability of the contracted A ring in the tumor cell culture media.[25] The taxol-type compounds (**15** ~ **20**) possessing both an oxetane ring and an *N*-acylphenylisoserine moiety exhibited potent cytotoxicity against KB cells (IC_{50} 0.086 ~ 0.0016 μg/mL). This result indicates that the *N*-benzoyl group of taxol (**14**) is exchangeable into other aliphatic acyl groups without substantial loss of its potent cytotoxicity.[23] Taxagifine (**10**) and brevifoliol (**22**) showed modest cytotoxicity against KB cells although there is no oxetane ring in the molecules, while the other taxoids (**6** ~ **9**, **11** ~ **13**, **21**, and **23**) exhibited very weak or no cytotoxicity.

Table 3. Cytotoxicity of Taxoids (**1** ~ **23**) against L1210 Murine Leukemia Cells and KB Human Epidermoid Carcinoma Cells

compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)	compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)
1	0.27	0.08	**13**	>10	9.4
2	>10	>10	**14** (taxol)	0.33	0.0088
3	>10	>10	**15**	0.88	0.015
4	>10	1.6	**16**	0.25	0.086
5	>10	>10	**17**	0.95	0.0048
6	>10	>10	**18**	0.21	0.0053
7	>10	>10	**19**	0.24	0.0017
8	>10	>10	**20**	0.21	0.0016
9	>10	>10	**21**	3.8	>10
10	1.3	0.86	**22**	>10	0.4
11	>10	>10	**23**	>10	>10
12	>10	>10			

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 ppm resonance of residual $CHCl_3$ and 77.1 ppm of $CDCl_3$ 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 (1.2 kg) of the yew was extracted with MeOH (4 L x 4). The MeOH extract was partitioned between toluene (900 mL x 4) and H_2O (900 mL). The toluene soluble portions were evaporated under reduced pressure to give a residue (24.6 g), part of which (8.0 g) was subjected to a silica gel column (4.0 x 34 cm) with hexane/acetone (8:1 → 2:1) to afford two fractions **I** (820 ~ 1010 mL) and **II** (1010 ~ 1340 mL). Fraction **I** was applied to a silica gel column (2.0 x 30 cm) with $CHCl_3$/acetone (20:1) to afford two fractions **a** (20 ~ 310 mL) and **b** (470 ~ 540 mL). Fraction **a** was subjected to a silica gel column (1.0 x 29 cm) with hexane/acetone (3:1) and the fraction (65 ~ 170 mL) was applied to a reversed-phase HPLC column (YMC-Pack ODS AL-323, 5μm, 250 x 10 mm; flow rate 3.0 mL/min; UV detection at 254 nm; eluent: MeOH/H_2O, 70:30) to give taxuspine F (**2**, 4.0 mg, t_R 11 min). Fraction **b** was purified by the same reversed-phase HPLC column with MeOH/H_2O (70:30) to give taxuspine G (**3**, 1.3 mg, t_R 9 min). Fraction **II** was applied to a silica gel column (2.0 x 32 cm) eluted with $CHCl_3$/acetone (19:1 → 1:1) to afford a fraction (620 ~ 775 mL), which was separated by a reversed-phase column (Develosil Lop ODS 24S; flow rate 3.0 mL/min; eluent: MeOH/H_2O, 80:20) to give a fraction (18.4 mg, t_R 40 min), which was purified by the same reversed-phase HPLC column with MeOH/H_2O (65:15) to give taxuspine E (**1**, 0.8 mg, t_R 17 min). The leaves (0.5 kg) of the yew was extracted with MeOH (2 L x 3). The MeOH extract was partitioned between toluene (500 mL x 3) and H_2O (750 mL). The toluene soluble portions were evaporated under reduced pressure to give a residue (31.1 g), part of which (3.3 g) was subjected to a silica gel column (4.0 x 27 cm) eluted with hexane/acetone (8:1 → 3:7) to afford two fractions **III** (1320 ~ 1480 mL) and **IV** (1480 ~ 1900 mL). Fraction **III** was applied to a silica gel column (1.5 x 20 cm) with hexane/EtOAc (4:1) to give fraction **c** (140 ~ 175 mL), which was purified by a centrifuged counter-current chromatography [Model LLB-M, Sanki Laboratories, Inc.] in the descending mode (*n*-hexane/MeOH/H_2O, 5:4:1). The fraction **c** (18.4mg) was equilibrated with the mobile (lower) phase of the solvent system at 2.5mL/min, 1000 rpm to give a fraction (55 ~ 80 mL), which was subjected to a silica gel column [0.6 x 16 cm; CH_2Cl_2/acetone, 19:1] followed by the same reversed-phase HPLC column with MeOH/H_2O (75:25) to give taxuspine J (**5**, 1.0 mg, t_R 22.0 min). Fraction **IV** was purified by the same centrifuged counter-current chromatography in the ascending mode (*n*-hexane/$CHCl_3$/acetonitrile, 10:8:3). The fraction **IV** (153.8 mg) was equilibrated with the mobile (upper) phase of the solvent system at 3 mL/min, 1500 rpm to afford a fraction (655 ~ 760 mL), which was subjected to a silica gel column [0.6 x 16 cm; eluent: $CHCl_3$/CH_3CN (60:40) to give taxuspine H (**4**, 1.8 mg).

Taxuspine E (1): A colorless amorphous solid; $[\alpha]^{20}_D$ -17° (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3420, 1720, and 1260 cm^{-1}; UV (MeOH) λ_{max} 230 (ε 14100), 275 (sh), and 282 (sh); ^1H (Table 1) and ^{13}C (Table 2) NMR; FABMS (positive ion, glycerol matrix) *m/z* (%) 589 (M^++H, 10), 571 (16), 553 (15), 529 (3), 105 (100), and 43 (30); HRFABMS *m/z* 589.2669 (M^++H) calcd for $C_{31}H_{41}O_{11}$, 589.2649; ^1H-^1H COSY correlations ($CDCl_3$, H/H): 2/3, 5/6, 6a/6b, 6/7, 9/9-OH, 9/10, 10/10-OH, 13/14, 13/18, and 14a/14b; HMBC correlations ($CDCl_3$, H/C): 2/1, 2/3, 2/4, 2/8, 2/2-PhCO, 3/2, 3/4, 3/8, 3/9, 3/19, 5/4, 5/7, 7/5, 7/6a, 7/8, 7/9, 7/19, 7/7-AcO, 9/7, 9/10, 10/9, 10/15, 14a/3, 14a/15, 14b/3, 16/1, 16/11, 16/15, 17/1, 17/11, 17/15, 18/11, 18/12, 20a/4, 20b/4, and 20a/5, NOESY correlations ($CDCl_3$, H/H): 2/9, 2/16, 2/19, 3/7, 3/14a, 5/6b, 5/7, 5/20b, 7/10, 13/14b, 13/17, and 19/20a.

Taxuspine F (2): A colorless amorphous solid; $[\alpha]^{28}_D$ +50° (*c* 0.66, $CHCl_3$); IR (film) ν_{max} 3440, 1740, 1670, and 1240 cm^{-1}; UV (MeOH) λ_{max} 267 nm (ε 4500); ^1H (Table 1) and ^{13}C (Table 2) NMR; EIMS *m/z* (%) 534 (M^+, 2), 516 (1), 474 (1), 414 (2), 354 (2), 294 (3), and 43 (100); HREIMS *m/z* 534.2479 (M^+) calcd for $C_{28}H_{38}O_{10}$, 534.2465; ^1H-^1H COSY correlations ($CDCl_3$, H/H): 1/2, 2/3, 3/20a, 5/6, 6/7, 9/10, 14/1, and 16/17; NOESY correlations ($CDCl_3$, H/H): 1/2, 1/14b, 1/16, 1/17, 2/9, 2/16, 2/19, 3/7, 3/14a, 3/18, 5/6a, 5/6b, 5/20a, 7/10, 9/16, 9/19, 10/18, 14a/18, 14b/17, and 19/20b.

Taxuspine G (3): A colorless amorphous solid; $[\alpha]^{23}_D$ +97° (*c* 0.21, $CHCl_3$); IR (film) ν_{max} 3440, 1740, 1670, and 1240 cm^{-1}; UV (MeOH) λ_{max} 269 nm (ε 4300); ^1H (Table 1) and ^{13}C (Table 2) NMR; EIMS *m/z* (%) 434 (M^+, 1), 416 (1), 398 (1), 374 (2), 356 (2), 314 (3), and 43 (100); HREIMS *m/z* 434.2319 (M^+) calcd for $C_{24}H_{34}O_7$, 434.2305; ^1H-^1H COSY correlations ($CDCl_3$, H/H): 1/2, 2/3, 3/20a, 5/6, 6/7, 9/10, 14/1, and 16/17; NOESY correlations ($CDCl_3$, H/H): 1/2, 1/14b, 1/16, 1/17, 2/9, 2/16, 2/19, 3/7, 3/14a, 3/18, 5/6a, 5/6b, 5/20a, 6a/19, 7/10, 7/18, 9/16, 9/19, 10/18, 14b/17, 16/17, 19/20b.

Taxuspine H (4): A colorless amorphous solid; $[\alpha]^{23}_D$ +6.8° (*c* 0.29, $CHCl_3$); IR (film) ν_{max} 1740, 1700, and 1240 cm^{-1}; UV (MeOH) λ_{max} 230 (sh) and 281 nm (ε 3500); ^1H (Table 1) and ^{13}C

(Table 2) NMR; EIMS *m/z* (%) 651 (M^+, 8), 592 (1), 532 (1), 458 (4), 398 (4), 338 (5), 279 (6), 192 (86), and 134 (100); HREIMS *m/z* 651.3425 (M^+) calcd for $C_{37}H_{49}NO_9$, 651.3407.

Taxuspine J (5): A colorless amorphous solid; $[\alpha]^{23}_D$ -25° (*c* 0.46, $CHCl_3$); IR (film) ν_{max} 3550, 1730, 1710, 1630, and 1240 cm^{-1}; UV (MeOH) λ_{max} 218 (ε 21000), 223 (sh), and 279 nm (22000); ^1H (Table 1) and ^{13}C (Table 2) NMR; EIMS *m/z* (%) 548 (M^+-AcOx2, 1), 488 (15), 446 (17), 428 (10), 400 (4), 358 (6), 340 (6), 280 (9), 220 (73), and 131 (90); FABMS (positive ion, glycerol matrix) *m/z* 667 (M^++H); HRFABMS *m/z* 667.3130 (M^++H) calcd for $C_{37}H_{47}O_{11}$, 667.3119; COSY correlations ($CDCl_3$, H/H): 2/3, 5/6a, 5/6b, 6a/7, 6b/7, 9/10, 13/14a, 13/14b, and 22/23; NOESY correlations ($CDCl_3$, H/H): 2a/2b, 2a/3, 2a/14a, 2a/20a, 2a/22, 2b/17, 2b/19, 3/7, 3/13-AcO, 5/6a, 5/20b, 6a/6b, 6a/19, 6b/7, 7/10, 9/19, 13/14b, 13/16, 13/18, 14a/14b, 14a/16, and 19/7-AcO.

Photochemical Reaction of Taxine II. A solution of taxine II (1.5 mg) in 1.0 mL of degassed dioxane was irradiated using a mercury lamp (500 W) housed in a water-cooled Pyrex jacket at room teperature for 17 min. Evaporation under reduced pressure afforded a residue, which was purified by a silica gel column (1 x 10 cm, $CHCl_3$/EtOH, 97:3) to give compound **4** (0.7 mg); $[\alpha]^{23}_D$ +5.8° (*c* 0.12, $CHCl_3$); EIMS *m/z* 651 (M^+); ^1H NMR, IR, and UV spectra derived from taxine II were the same as those of taxuspine H.

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