New Glycosides from *Ajuga decumbens*

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A new phenethyl alcohol glycoside, galactosylmartynoside (**1**), and a new abietatriene-type diterpene glycoside, ajugaside A (**2**), were isolated from the whole plants of *Ajuga decumbens*, together with known phenethyl alcohol glycosides (**3** and **4**) and iridoid glycosides (**5**−**7**). Chemical structures were elucidated on the basis of spectral data. Of these compounds, 8-acetylharpagide (**6**) exhibited the strongest inhibitory effect on Epstein−Barr virus activation induced by 12-*O*-tetradecanoylphorbol-13-acetate.

Ajuga decumbens Thunb. (Labiatae) is distributed widely in China, Korea, and Japan.[1,2] The whole body of this plant has been used as a folk medicine for its antiinflammatory, antitussive, and expectorant effects in China and Japan.[3] From the *Ajuga* species, several ecdysteroids,[4−6] neoclerodane diterpenes,[7−9] and iridoid glycosides[10,11] had been isolated and reported. The activities as insect antifeedants and insect molting inhibitors had also been reported.[4−9] In the course of our continuing chemical and biological studies on antitumor promoters (chemopreventive agents),[12−15] we investigated the constituents of whole body of *A. decumbens*. Consequently, a new phenethyl alcohol glycoside, galactosylmartynoside (**1**), and diterpene glycoside, ajugaside A (**2**), were isolated from the EtOAc- and *n*-BuOH-soluble fractions of MeOH extract, together with two known phenethyl alcohol glycosides (**3** and **4**) and three iridoid glycosides (**5**−**7**). In this paper, we report the structure elucidations of these compounds and their inhibitory effects on Epstein−Barr virus (EBV) activation induced by a strong tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[16,17] Compounds **3**−**7** were identified as martynoside,[18,19] darendoside B,[20] reptoside, 8-acetylharpagide, and harpagide,[10] respectively, based on comparison with reported data (mp, IR, UV, $[\alpha]_D$, ^{1}H and ^{13}C NMR).

Results and Discussion

Compound **1** was obtained as a pale yellow amorphous powder, and its molecular formula was determined to be $C_{37}H_{50}O_{20}$ by HRFABMS. The IR (ν_{max} at 3500, 1710, 1635, 1515 cm^{-1}) and UV (λ_{max} at 329 and 220 nm) spectra of **1** showed the presence of hydroxyl and phenyl groups and an α,β-unsaturated ester. Further, in the positive FABMS of **1**, quasimolecular ion peaks were observed at *m*/*z* 837 $[M + Na]^+$ and 815 $[M + H]^+$; fragment ion peaks were observed at *m*/*z* 653 $[M -$ hexosyl$]^+$ and 507 $[653 -$ deoxyhexosyl$]^+$. On acid hydrolysis, **1** afforded D-glucose, D-galactose, and L-rhamnose. The ^{1}H NMR spectrum of **1** showed three anomeric proton signals [at δ 5.58 (br s), 4.38 (d, J = 7.5 Hz), and 4.36 (d, J = 7.5 Hz)], two sets of 1,3,4-trisubstituted phenyl proton signals [at δ 7.20 (d, J = 2.0 Hz), 7.08 (dd, J = 8.0, 2.0 Hz), 6.81 (d, J = 8.0 Hz), δ 6.83 (d, J = 8.0 Hz), 6.75 (d, J = 2.2 Hz), and 6.69 (dd, J = 8.0, 2.2 Hz)], and two olefinic proton signals [at δ 7.66 (d, J = 16.5 Hz) and 6.37 (d, J = 16.5 Hz)] indicating the *E* configuration. The HMBC spectrum of **1** showed long-range correlations from H-8 to C-1″, from H-3″ to C-1‴, from H-2‴ to C-1⁗, and from H-4″ to C-9′. Further, the substitutions in a phenethyl alcohol and a phenyl propanoid of **1** were determined by a NOE difference experiment. On the basis of these spectral data, the structure of **1** was deduced to be a trisaccharide glycoside of 3-hydroxy-4-methoxyphenethyl alcohol with a feruloyl ester group on the trisaccharide portion. The chemical shift values in the ^{13}C NMR spectrum of **1** were compared with those of compounds **3** and **4**, which were identified as martynoside and darendoside B, respectively. The glycosylation shift of the oxymethine carbon at C-2 of rhamnose was observed, and the ^{13}C NMR data of 3-hydroxy-4-methoxyphenethyl alcohol, feruloyl, and D-glucose moieties were superimposable on those of compound **3**. Therefore, the structure of **1** was characterized as 3-hydroxy-4-methoxyphenethyl 4′-feruloyl-3′-(2″-β-D-galactopyranosyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside, named galactosylmartynoside.

Ajugaside A (**2**) was obtained as colorless crystals, mp 195−197 °C, and its molecular formula was determined to be $C_{32}H_{50}O_{14}$ on the basis of HRFABMS. In the positive FABMS of **2**, quasimolecular ion peaks were observed at *m*/*z* 681 $[M + Na]^+$ and 659 $[M + H]^+$, and fragment ion peaks were observed at *m*/*z* 497 $[M -$ hexosyl$]^+$ and 335 $[497 -$ hexosyl$]^+$. The ^{13}C NMR spectra and DEPT experiment on **2** disclosed the presence of six sp^2 carbons in which there were five quaternary carbons, two sp^3 quaternary carbons, 12 sp^3 methine carbons (including 10 oxymethine carbons), nine sp^3 methylene carbons (including four oxygenated methylenes), and three sp^3 methyl carbons. In the ^{1}H NMR spectrum, three methyl proton signals at δ 1.30 (s), 1.12 (d, J = 7.2 Hz), and 1.07 (s); two anomeric proton signals at δ 4.38 (d, J = 7.8 Hz) and 4.22 (d, J = 8.4 Hz), indicating β-linkages; and one phenyl proton signal at δ 6.34 (s) were observed.

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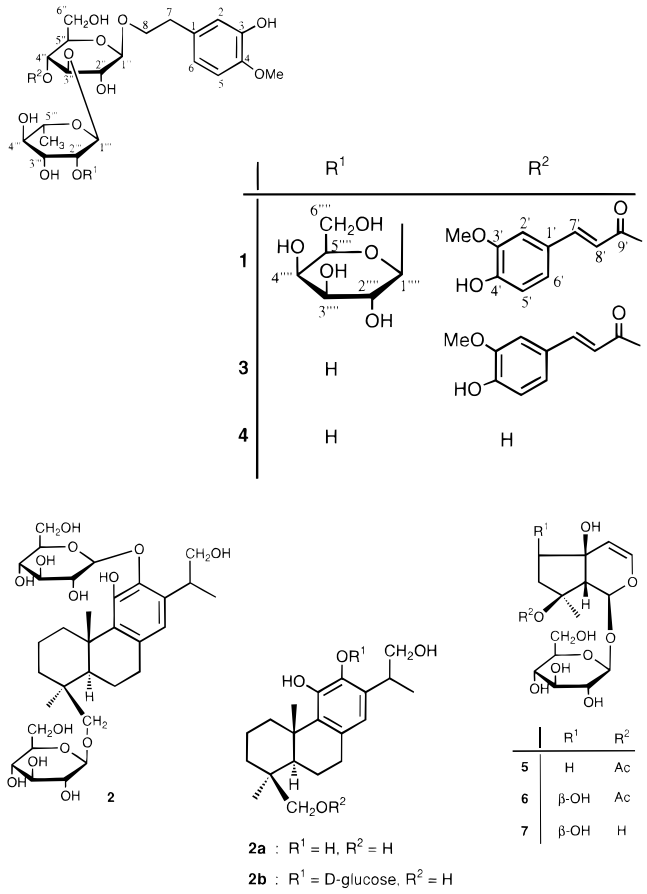

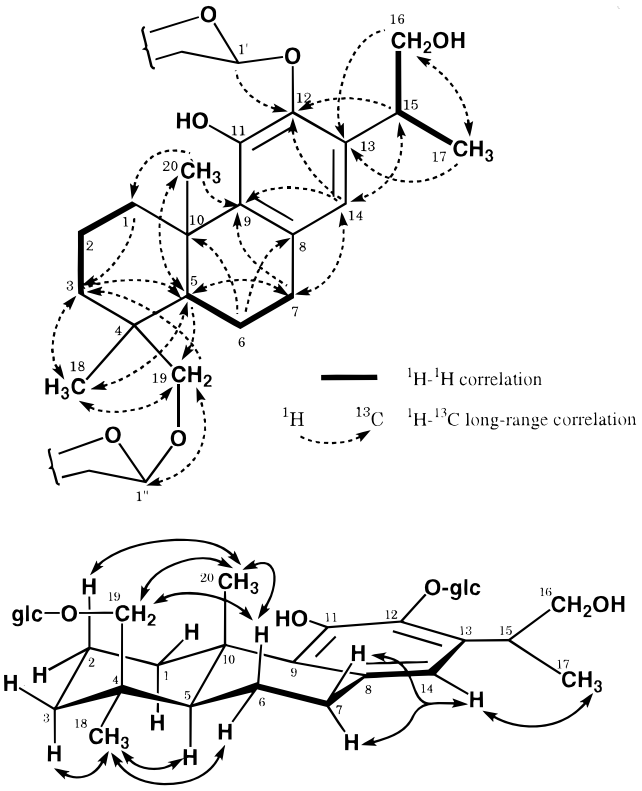

Figure 1. $^1H-^1H$ correlation and $^1H-^{13}C$ long-range correlation of ajugaside A (**2**). NOEs in difference NOE experiments of ajugaside A (**2**).

In the HMBC spectrum, both the methyl protons at δ 1.12 and the oxygenated methylene protons at δ 3.43 and 3.59 showed long-range correlations with the phenyl carbon at δ 136.36, and the methine proton at δ 3.68 was correlated with the phenyl carbons at δ 143.14 and 118.23, which was also correlated with methylene protons at δ 2.73. Further, the methyl protons at δ 1.30 showed long-range correlations with the phenyl carbon at δ 134.95, the methine carbon at δ 55.89, and the methylene carbon at δ 37.79; and each anomeric proton at δ 4.38 and 4.22 showed correlations with the phenyl carbon at δ 143.14 and the oxygenated methylene carbon at δ 74.34, respectively. Other $^1H-^{13}C$ long-range correlations, indicated by arrows in Figure 1a, were observed in the HMBC spectrum of **2**. From results of $^1H-^1H$ COSY, homonuclear Hartmann−Hahn (HOHAHA), HSQC, and HMBC spectra, all proton and carbon signals could be assigned as shown in Table 2, and consequently, compound **2** was deduced to be a diglycoside of 11,12,16,19-tetrahydroxyabietatriene.

On acid hydrolysis of **2** with 5% H_2SO_4 in EtOH, a novel diterpene, 11,12,16,19-tetrahydroxyabieta-8,11,13-triene (**2a**), the monoglucoside of **2a** (**2b**), and D-glucose were obtained. In the ^{13}C NMR spectrum of **2b**, the signal of C-19 was shifted to higher field (−8.7 ppm), and the signal of C-4 was shifted to lower field (+0.7 ppm), whereas other signals were superimposable on those of **2** within ± 0.1 ppm. Further, in the ^{13}C NMR spectrum of **2a**, the signals of the aromatic C-ring were shifted to lower or higher field compared with those of **2b** and **2**. These facts and the HMBC spectrum of **2** suggested that the glycosylation linkages of **2** were at C-12 and C-19, respectively.

In the NOE difference experiments of **2**, irradiation of the methyl signal at δ 1.30 enhanced signal intensities of the oxygenated methylene protons at δ 3.69 and 3.89, one of methylene protons at δ 1.56 (H-6β) and 1.74 (H-2β), and irradiation of the methyl signal at δ 1.07 enhanced signal intensities of the methine proton at δ 1.33 and one of the methylene protons at δ 1.02 (H-3α). Further, irradiation of the phenyl signal at δ 6.34 enhanced signal intensities of the methyl protons at δ 1.12 and methylene protons at δ 2.73, as shown in Figure 1b. Therefore, the structure of ajugaside A was characterized as **2**, including the relative configuration at C-4, C-5, and C-10.

Although several kinds of oxygenated abieta-8,11,13-trienes had been isolated from *Salvia* species[21−23] so far, the isolation of **2** is the first example of an abieta-8,11,13-triene derivative in *Ajuga* species.

The inhibitory effects of these glycosides (**1**−**7**) on EBV activation induced by TPA were examined via a primary screening for antitumor-promoter activity,[16,17] and the results are shown in Table 3. Of these compounds, 8-acetylharpagide (**6**) exhibited the most significant inhibitory effects (more than 80% and 35% inhibition of activation at 5×10^2 and 1×10^2 mol ratio to TPA, respectively) on EBV activation. Investigations of the absolute configuration of **2** and the inhibitory effects of **6** on two-stage carcinogenesis in vivo are in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto melting point apparatus and were uncorrected. IR spectra were recorded in KBr on a Shimadzu FT IR-8100 A spectrom-

Table 1. ^{13}C NMR Chemical Shift Values (δ) of **1**, **3**, and **4** (in CD_3OD, 150 MHz)

		1	**3**	**4**
aglycon	1	133.01	132.90	132.93
2-(3-hydroxy-4-	2	117.13	117.08	117.07
methoxyphenyl)ethanol	3	147.39	147.41	147.37
	4	147.57	147.57	147.53
	5	112.90	112.83	112.83
	6	121.20	121.16	121.14
	7	36.56	36.59	36.58
	8	72.10	72.15	71.99
	OMe	56.52	56.48	56.48
feruloyl	1′	127.65	127.66	
	2′	111.79	111.75	
	3′	149.44	149.40	
	4′	150.90	150.86	
	5′	116.55	116.51	
	6′	124.37	124.16	
	7′	147.90	147.91	
	8′	115.08	115.12	
	9′	168.21	168.25	
	OMe	56.48	56.45	
D-glucose	1″	104.17	104.25	104.24
	2″	76.15	76.24	75.65
	3″	82.87	81.53	84.47
	4″	70.51	70.63	70.07
	5″	76.03	76.09	77.88
	6″	62.38	62.41	62.68
L-rhamnose	1‴	102.38	103.02	102.78
	2‴	83.07	72.38	72.37
	3‴	71.87	72.08	72.24
	4‴	74.16	73.79	73.99
	5‴	70.39	70.44	70.20
	6‴	18.45	18.47	17.91
D-galactose	1⁗	107.63		
	2⁗	72.86		
	3⁗	74.92		
	4⁗	70.45		
	5⁗	76.99		
	6⁗	62.85		

Table 2. ^{13}C NMR Chemical Shift Values (δ) of **2**, **2a**, and **2b** (in CD_3OD)

carbon no.	**2**[a]	**2a**[b]	**2b**[b]
1	37.79 (t)[c]	37.87 (t)[c]	37.78
2	20.23 (t)	20.15 (t)	20.11
3	37.00 (t)	36.64 (t)	36.64
4	39.61 (s)	40.32 (s)	40.34
5	55.89 (d)	55.68 (d)	55.69
6	20.64 (t)	20.76 (t)	20.68
7	34.44 (t)	34.16 (t)	34.46
8	135.17 (s)	134.30 (s)	135.15
9	134.95 (s)	129.79 (s)	134.90
10	40.61 (s)	40.35 (s)	40.61
11	149.10 (s)	146.08 (s)	149.13
12	143.14 (s)	141.23 (s)	143.18
13	136.36 (s)	130.56 (s)	136.42
14	118.23 (d)	119.32 (d)	118.24
15	34.87 (d)	38.05 (d)	34.89
16	69.14 (t)	69.11 (t)	69.15
17	18.40 (q)	16.71 (q)	18.39
18	28.59 (q)	28.05 (q)	28.05
19	74.34 (t)	65.62 (t)	65.64
20	20.70 (q)	21.00 (q)	20.68
G-1′	107.77 (d)		107.82
-2′	75.61 (d)		75.63
-3′	78.98 (d)		79.03
-4′	71.52 (d)		71.54
-5′	77.98 (d)		77.99
-6′	63.01 (t)		63.04
G-1″	105.48 (d)		
-2″	75.38 (d)		
-3″	78.22 (d)		
-4″	71.72 (d)		
-5″	77.82 (d)		
-6″	62.81 (t)		

[a] Data recorded at 150 MHz. [b] Data recorded at 75 MHz. [c] Carbon type as determined by DEPT spectra.

Table 3. Percentages of EBV−EA Induction in Presence of Glycosides (**1**−**7**) with Respect to Positive Control (100%)

	concentration[a]			
compound	1000	500	100	10
1	0.0[b] (60)[c]	31.8 (>80)	76.0 (>80)	98.5 (>80)
2	0.0 (60)	24.9 (>80)	68.3 (>80)	91.0 (>80)
3	0.0 (60)	31.7 (>80)	66.4 (>80)	89.4 (>80)
4	22.6 (70)	53.8 (>80)	81.6 (>80)	100.0 (>80)
5	0.0 (60)	22.4 (>80)	71.3 (>80)	90.3 (>80)
6	0.0 (60)	19.9 (>80)	62.6 (>80)	89.5 (>80)
7	0.0 (60)	52.7 (>80)	80.1 (>80)	100.0 (>80)

[a] Mol ratio/TPA (20 ng = 32 pmol/mL). [b] Values represent percentages to the positive control values (100%). [c] Values in parentheses represent viability percentages of Raji cells.

eter, and UV spectra were recorded in MeOH on a Shimadzu UV-1600 spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter at 24 °C. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer and JEOL JNM-A 600 spectrometer with tetramethylsilane as internal standard. Positive FABMS were obtained on a JEOL JMS−SX 102 mass spectrometer. Recycle preparative HPLC was carried out on a Japan Analytical Industry LC-908 using a JAIGEL 310 column (Ø 20 mm × 500 mm × 2, solvent; MeOH) with RI and UV detector.

Plant Material. The whole plant of *Ajuga decumbens* Thunb. was purchased in January 1996, from Tochimoto-tenkai-do Co. Ltd., Osaka, Japan. A voucher specimen (#970101) is deposited in the herbarium of Kyoto Pharmaceutical University.

Extraction and Isolation. Chopped, dried, whole plants of *A. decumbens* (4.9 kg) were extracted with MeOH at room temperature. After the solvent was removed in vacuo, a dark green−brown residue (549 g) remained. The residue was suspended in H_2O and extracted several times with *n*-hexane. The aqueous layer was then extracted with $CHCl_3$, EtOAc, and *n*-BuOH saturated with H_2O, successively. Each organic layer was evaporated in vacuo to give residue (*n*-hexane 93.1 g, $CHCl_3$ 9.6 g, EtOAc 12.5 g, and *n*-BuOH 111.0 g). The EtOAc extract (12.5 g) was subjected to column chromatography on Si gel eluted with $CHCl_3$ containing increasing amounts of MeOH to provide fractions 1−9. Fractions 5 (eluted with 5% MeOH in $CHCl_3$, 2.0 g), 6 (eluted with 6% MeOH in $CHCl_3$, 427 mg), and 7 (eluted with 7.5% MeOH in $CHCl_3$, 1.5 g) were subjected to column chromatography on Si gel [solvent system: $CHCl_3$−MeOH−H_2O (8:3:1)] repeatedly, followed by recycle preparative HPLC. Consequently, reptoside (**5**, 217.2 mg) was obtained from fraction 5, martynoside A (**3**, 5.0 mg) was obtained from fraction 6, and 8-acetylharpagide (**6**, 246.7 mg) was obtained from fraction 7. The *n*-BuOH extract (27.3 g) was also subjected to column chromatography on Si gel eluted with a solution of MeOH in $CHCl_3$ to provide fractions 1−11. Fractions 3−8 eluted with 7.5 and 10% MeOH in $CHCl_3$ were rechromatographed on Si gel [solvent system: $CHCl_3$−MeOH−H_2O (9:1:0.1, 8:3:1, and 13:7:2)] repeatedly, followed by recycle preparative HPLC. Compounds **5** (1.05 g), **6** (4.64 g), and darendoside A (**4**, 8.8 mg) were isolated. Further, fractions 9−11, eluted with 15, 25, and 30% MeOH in $CHCl_3$,

were subjected to column chromatography on Si gel [solvent system: $CHCl_3$−MeOH−H_2O (8:3:1 and 13:7:2) and ODS [solvent system: MeOH−H_2O (10:90, 20:80, and 40:60)] repeatedly, followed by recycle preparative HPLC. Consequently, galactosylmartynoside (**1**, 18.1 mg), ajugaside A (**2**, 7.0 mg), and harpagide (**7**, 473.2 mg) were isolated, together with compounds **5** (50 mg) and **6** (3.16 g). Compounds **3**−**7** were identified by comparison with reported data.[10,18−20]

Galactosylmartynoside (1): amorphous powder; $[\alpha]^{25}_D$ −37.4° (*c* 0.34, MeOH); UV (EtOH) λ_{max} (log ϵ) 329 (4.42), 289 (4.21), 220 (4.39) nm; IR (KBr) ν_{max} 3400, 1710, 1635, 1595, 1515, 1275, 1160, 1130 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 2.82 (2H, m, H_2−7), 3.27 (1H, t like, H-4‴), 3.38 (1H, t like, H-2″), 3.45 (1H, dd, *J* = 9.6, 3.6 Hz, H-3‴), 3.57 (1H, t like, H-2‴), 3.65 (1H, dd, *J* = 9.6, 3.6 Hz, H-3″), 3.75 (1H, m, H-8a), 3.77 (1H, m, H-3″), 3.81 (3H, s, 4-OMe), 3.89 (3H, s, 3′-OMe), 3.98 (1H, br s, H-2‴), 4.05 (1H, m, H-8b), 4.36 (1H, d, *J* = 7.5 Hz, H-1‴), 4.38 (1H, d, *J* = 7.5 Hz, H-1″), 4.92 (1H, t like, H-4″), 5.58 (1H, br s, H-1‴), 6.37 (1H, d, *J* = 16.5 Hz, H-8′), 6.69 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.75 (1H, d, *J* = 2.2 Hz, H-2), 6.81 (1H, d, *J* = 8.0 Hz, H-5′), 6.83 (1H, d, *J* = 8.0 Hz, H-5), 7.08 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 7.20 (1H, d, *J* = 2.0 Hz, H-2′), 7.66 (1H, d, *J* = 16.5 Hz, H-7′);^{13}C NMR, see Table 1; FABMS (positive) *m/z* 837 $[M + Na]^+$, 815 $[M + H]^+$, 653 [M + H − galactosyl]$^+$, 507 [653 − rhamnosyl]$^+$; HRFABMS *m/z* 815.2972 (calcd for $C_{37}H_{51}O_{20}$, 815.2973).

Acid Hydrolysis of 1 and Identification of Monosaccharides. Compound **1** (10 mg) was refluxed in 10% H_2SO_4 for 1 h. The reaction mixture was neutralized with Amberlite IR-45 and evaporated in vacuo. The residue was dissolved in dry pyridine (0.2 mL) and added to a solution of L-cysteine methyl ester hydrochloride in pyridine (0.05 mol/L, 0.3 mL). After the mixture was heated at 60 °C for 1 h under N_2 flow, solvent was removed and trimethylsilylimidazole (0.2 mL) was added. The reaction mixture was heated at 60 °C for 1 h and applied to the GLC analysis (column: ULBON-HR-1701, 0.25 mm × 25 m; column temperature 240 °C; carrier gas He, 1.0 kg/cm^2; FID detector). D-Glucose (t_R 18.5 min), D-galactose (t_R 20.3 min), and L-rhamnose (t_R 13.4 min) were identified with authentic samples.[24]

Ajugaside A (2): colorless crystals (MeOH); mp 195−197 °C, $[\alpha]^{22}_D$ + 2.8° (*c* 0.66, MeOH); IR (KBr) ν_{max} 3450, 1545 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 1.02 (1H, ddd, *J* = 13.8, 13.2, 4.2 Hz, H-3α), 1.07 (3H, s, Me-18), 1.12 (3H, d, *J* = 7.2 Hz, Me-17), 1.16 (1H, ddd, *J* = 13.2, 3.6 Hz, H-1α), 1.30 (3H, s, Me-20), 1.33 (1H, d, *J* = 12.0 Hz, H-5), 1.47 (1H, m, H-2α), 1.56 (1H, m, H-6*β*), 1.74 (1H, m, H-2*β*), 1.94 (1H, br dd, *J* = 13.2, 4.8 Hz, H-6α), 2.00 (1H, br d, *J* = 13.2 Hz, H-3*β*), 2.73 (2H, m, H_2-7), 3.30 (1H, m, H-1*β*), 3.68 (1H, m, H-15), 3.59 (1H, dd, *J* = 10.5, 6.3 Hz, H-16a), 3.69, 3.89 (each 1H, ABd, *J* = 12.6 Hz, H_2-19), 4.22 (1H, d, *J* = 8.4 Hz, H-1″), 4.38 (1H, d, *J* = 7.8 Hz, H-1′), 6.34 (1H, s, H-14); ^{13}C NMR, see Table 2; FABMS (positive) *m/z* 681 $[M + Na]^+$, 659 $[M + H]^+$, 497 [M + H − glucosyl]$^+$, 335 [497 − glucosyl]$^+$; HRFABMS *m/z* 659.3287 (calcd for $C_{32}H_{50}O_{14}$: 659.3279).

Acid Hydrolysis of 2. Compound **2** (12.4 mg) was dissolved in 5% H_2SO_4 in EtOH and refluxed for 3 h. The reaction mixture was concentrated in vacuo to half volume, and extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ extract was purified by column chromatography on Si gel to afford 1.5 mg of **2a** as amorphous powder. The EtOAc extract was purified by column chromatography on Sephadex LH-20 to afford 2.0 mg of **2b**. The aqueous layer was neutralized with Amberlite IR-45 and treated with in same manner as **1** to identify D-glucose.

11,12,16,19-Tetrahydroxyabieta-8,11,13-triene (2a): amorphous powder; $[\alpha]^{25}_D$ −7.2° (*c* 0.25, MeOH); IR (KBr) ν_{max} 3450, 1545 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.02 (3H, s, Me-18), 1.23 (3H, d, *J* = 7.2 Hz, Me-17), 1.29 (3H, s, Me-20), 2.71 (2H, m, H_2-7), 3.07 (1H, m, H-1*β*), 3.43, 3.83 (ABd, *J* = 11.2 Hz, H_2-19), 3.62 (1H, dd, *J* = 10.3, 6.0 Hz, H-16a), 3.67 (1H, dd, *J* = 10.3, 5.4 Hz, H-16b), 6.27 (1H, s, H-14); ^{13}C NMR, see Table 2; FABMS (positive) *m/z* 335 $[M + H]^+$, 334 $[M]^+$; HR-FABMS *m/z* 334.2150 (calcd for $C_{20}H_{30}O_4$, 334.2144).

Monoglucoside (2b): amorphous powder; $[\alpha]^{22}_D$ −29.8 ° (*c* 0.18, MeOH); IR (KBr) ν_{max} 3450, 1545 cm^{-1}, ^1H NMR (CD_3OD, 300 MHz) δ 1.02 (3H, s, Me-18), 1.12 (3H, d, *J* = 7.2 Hz, Me-17), 1.29 (3H, s, Me-20), 2.74 (2H, m, H_2-7), 3.42, 3.82 (ABd, *J* = 11.3 Hz, H_2-19), 4.38 (1H, d, *J* = 7.5 Hz, anomeric H), 6.35 (1H, s, H-14); ^{13}C NMR, see Table 2; FABMS (positive) *m/z* 519 $[M + Na]^+$, 497 $[M + H]^+$, 335 [M + H − hexosyl]$^+$; HRFABMS *m/z* 519.2565 (calcd for $C_{26}H_{40}O_9Na$, 519.2570).

EBV Activation Experiment. The EBV genome-carrying lymphoblastoid cells, Raji cells, derived from Barkitt's lymphoma, were cultivated in RPMI-1640 medium. The Raji cells were incubated for 48 h at 37 °C in a medium containing butyric acid (4 mmol), TPA (32 pmol), and various amounts of the test compounds. Smears were made from the cell suspension, and the EBV−early antigen-inducing cells were stained by means of an indirect immunofluorescence technique. The details of the in vitro assay on EBV activation have been reported previously.[16,17]

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