Cholestane Glycosides from the Bulbs of *Ornithogalum thyrsoides* and Their Cytotoxic Activity against HL-60 Leukemia Cells

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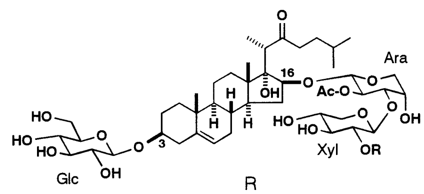
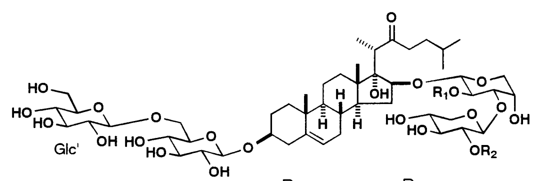
Twelve bisdesmosidic cholestane glycosides (**1**−**12**), including nine new ones (**1**−**9**), were isolated from the bulbs of *Ornithogalum thyrsoides* by monitoring the cytotoxic activity on HL-60 leukemia cells. The structural assignment of the new compounds was carried out by spectroscopic analysis and the results of hydrolytic cleavage. The 3-*O*-monoglucosides with an aromatic acyl group at the C-16 diglycoside moiety (**1**, **12**) were extremely cytotoxic, with respective IC_{50} values of 0.00016 and 0.00013 μg/mL, and the other compounds, except for **2**, **5**, and **8**, also showed cytotoxic activity as potent as etoposide (IC_{50} 0.30 μg/mL), used as a positive control. These cholestanes were concluded to contribute to the potent cytotoxicity of the crude *O. thyrsoides* bulb extract.

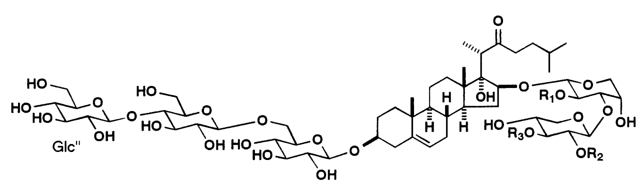
An acylated cholestane diglycoside, 17α-hydroxy-16β-[(*O*-(2-*O*-*p*-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one (**13**), isolated by us from the bulbs of *Ornithogalum saundersiae*, has received scientific attention because of its strong cytotoxicity against a variety of tumor cell culture lines and experimental animal tumors.[1] Recently, we have reported several **13**-related compounds and their cytotoxic activity against HL-60 human promyelocytic leukemia cells.[2] Furthermore, two novel cytotoxic cholestane glycosides, named galtonioside A[3] and candicanoside A,[4] along with several **13** derivatives[5] and polyoxygenated cholestane bisdesmosides,[6] have been isolated from *Galtonia candicans*, a Liliaceae plant taxonomically related to *O. saundersiae*. Compound **13**, galtonioside A, and candicanoside A were considered to have potential as new anticancer agents with a new mode of action because they displayed differential cytotoxicities in the Japanese Foundation for Cancer Reserch 38 cell line assay,[7] and their cytotoxic profiles in the mean graphs were not correlated to those shown by any of the other compounds including currently used anticancer drugs. During our ongoing project, which focused on higher-plant antineoplastic constituents, a phytochemical analysis was conducted with the MeOH extract of the bulbs of *Ornithogalum thyrsoides* since it exhibited potent cytotoxic activity against HL-60 cells. A cytotoxicity-guided fractionation procedure of the MeOH extract has resulted in the isolation of 12 cholestane glycosides (**1**−**12**), including nine new ones (**1**−**9**). This paper deals with the structural assignment of the new cholestane glycosides based on spectroscopic analysis and the results of hydrolytic cleavage and with the cytotoxicity of the isolated compounds against HL-60 cells.



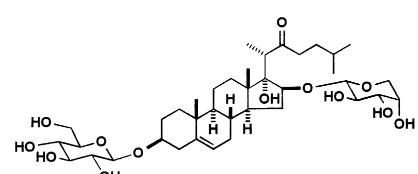
	R
1	3,4,5-trimethoxybenzoyl
11	H
12	3,4-dimethoxybenzoyl



	R_1	R_2
2	Ac	H
2a	H	H
3	Ac	3,4-dimethoxybenzoyl
4	Ac	3,4,5-trimethoxybenzoyl



	R_1	R_2	R_3
5	Ac	H	H
5a	H	H	H
6	Ac	3,4-dimethoxybenzoyl	H
7	Ac	3,4,5-trimethoxybenzoyl	H
8	Ac	4-hydroxy-3-methoxybenzoyl	H
9	Ac	H	3,4-dimethoxybenzoyl



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Results and Discussion

The fresh bulbs of *O. thyrsoides* were extracted with hot MeOH. The concentrated MeOH extract, which showed cytotoxic activity against HL-60 cells with an IC_{50} value of 0.79 μg/mL, was passed through a porous-polymer polystyrene resin (Diaion HP-20) column and successively

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eluted with 30% MeOH, 50% MeOH, and EtOH. The EtOH eluate fraction exhibited a highly cytotoxic activity against HL-60 cells (IC_{50} 0.028 μg/mL). Then, the EtOH fraction was repeatedly subjected to column chromatography on silica gel and on octadecylsilanized (ODS) silica gel and to reversed-phase HPLC to furnish compounds **1** (35.6 mg), **2** (130 mg), **3** (61.1 mg), **4** (84.5 mg), **5** (894 mg), **6** (140 mg), **7** (128 mg), **8** (90.2 mg), **9** (22.1 mg), **10** (23.6 mg), **11** (18.9 mg), and **12** (68.1 mg).

Compounds **10**−**12** were identified as 16β-[(α-L-arabinopyranosyl)oxy]-3β-[(β-D-glucopyranosyl)oxy)]-17α-hydroxycholest-5-en-22-one (**10**),[5] 3β-[(β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one (**11**),[5] and 3β-[(β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one (**12**),[5] respectively.

All the new compounds were revealed to be based upon 3β,16β,17α-trihydroxycholest-5-en-22-one by analysis of their spectral data and differed from each other with regard to the structures of the sugar moieties and the acyl groups attached at the C-16 sugar residue.

Compound **1** was obtained as an amorphous solid with a molecular formula $C_{55}H_{82}O_{22}$, as determined by the data of the positive-ion FABMS, showing an $[M + Na]^+$ ion at *m/z* 1117, the ^{13}C NMR spectrum with a total of 55 carbon signals, and elemental analysis. Analysis of the ^1H and ^{13}C NMR spectra of **1** and comparison with those of **12** implied that **1** differed from **12** only in terms of the aromatic acid constituent. Instead of the signals for a 3,4-dimethoxybenzoyl group, those assignable to a 3,4,5-trimethoxybenzoyl residue were observed at δ_H 7.71 (2H, s), 3.97 (3H, s), and 3.81 (3H × 2, s); δ_C 126.3 (C), 108.1 (CH) × 2, 153.7 (C) × 2, 143.2 (C), 165.4 (C=O), 60.7 (OMe), and 56.2 (OMe × 2). Alkaline hydrolysis of **1** with 0.4% KOH in EtOH gave 3,4,5-trimethoxybenzoic acid and **11**. The ester linkage position of the 3,4,5-trimethoxybenzoyl group at C-2 of the xylosyl group in **4** was ascertained by a long-range correlation from the xylose H-2 proton at δ 5.79 (dd, J = 8.9, 7.6 Hz) to the carbonyl resonance of the 3,4,5-trimethoxybenzoyl moiety at δ 165.4 in the HMBC spectrum of **1**. Thus, the structure of **1** was shown to be 3β-[(β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4,5-trimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

Compound **2** was obtained as an amorphous solid. The positive-ion FABMS (*m/z* 1085 $[M + Na]^+$), ^{13}C NMR data (51 carbon signals), and elemental analysis allowed the determination of the molecular formula of **2** as $C_{51}H_{82}O_{23}$, which was higher by $C_6H_{10}O_5$ than that of **11**. The ^1H NMR spectrum of **2** showed signals for four anomeric protons at δ 5.15 (d, J = 7.8 Hz), 4.96 (d, J = 7.7 Hz), 4.91 (d, J = 7.5 Hz), and 4.66 (d, J = 6.5 Hz), along with signals for five steroid methyl groups and an acetyl group. Acid hydrolysis of **2** with 1 M HCl in dioxane−H_2O (1:1) liberated L-arabinose, D-xylose, and D-glucose as the carbohydrate moieties, and several degradation products from the aglycon, whereas alkaline treatment with 3% NaOMe in MeOH yielded a deacetyl derivative (**2a**: $C_{49}H_{80}O_{22}$). The identification of the monosaccharides, including their absolute configurations, was established by direct HPLC analysis of the sugar fraction of the acid hydrolysate using a combination of RI and optical rotary (OR) detectors. Analysis of the ^1H−^1H COSY spectrum, starting from each anomeric proton, and of the HMQC spectrum of **2** led to the assignment of all the proton and carbon signals due to the sugar moieties. The assigned ^1H and ^{13}C NMR shifts were indicative of a terminal β-D-glucopyranosyl unit and a substituted β-D-glucopyranosyl residue glycosylated at C-6, as well as the *O*-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl group attached at C-16 of the aglycon as in **11**. The anomeric proton signal of the terminal glucosyl residue at δ 5.15 gave an HMBC correlation with the δ 70.1 resonance assignable to C-6 of the inner glucosyl group, whose anomeric proton at δ 4.96, in turn, was correlated with C-3 of the aglycon at δ 78.4. Thus, the structure of **2** was defined as 3β-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]-cholest-5-en-22-one.

Compound **3** was shown to have the molecular formula $C_{60}H_{90}O_{26}$ on the basis of the positive-ion FABMS (*m/z* 1249 $[M + Na]^+$), ^{13}C NMR data (60 carbon signals), and elemental analysis. The ^1H and ^{13}C NMR spectral data of **3** were essentially analogous with those of **2** and suggestive of a cholestane glycoside of the same type. The existence of a 3,4-dimethoxybenzoyl group in addition to an acetyl group in the molecule was indicated by the IR [1694 cm^{-1} (C=O), 1600, 1515 and 1456 cm^{-1} (aromatic ring)], UV [λ_{max} 292 nm (log ϵ 3.74), 263 nm (log ϵ 4.02)], ^1H NMR [δ 8.06 (1H, dd, J = 8.6, 1.9 Hz), 7.30 (1H, d, J = 1.9 Hz), 7.07 (1H, d, J = 8.6 Hz), 3.82 (3H, s), and 3.81 (3H, s)], and ^{13}C NMR [δ 123.1 (C), 113.5 (CH), 145.9 (C), 154.0 (C), 111.2 (CH), 124.4 (CH), 165.6 (C=O), 55.9 (OMe), and 55.8 (OMe)] spectra. Alkaline hydrolysis of **3** with 0.4% KOH in EtOH yielded **2**, **2a**, and 3,4-dimethoxybenzoic acid. An HMBC correlation from the xylose H-2 resonance at δ 5.71 (dd, J = 9.0, 7.8 Hz) to the conjugated carbonyl carbon signal at δ 165.6 gave evidence for the ester linkage position of the 3,4-dimethoxybenzoyl moiety at C-2 of the xylosyl residue. The structure of **3** was revealed to be 3β-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

The ^1H and ^{13}C NMR spectral data of **4** ($C_{61}H_{92}O_{27}$) were superimposable on those of **3**, except for the aromatic region signals due to the substituted benzoyl moiety. The aromatic acid linked to C-2 of the xylosyl residue was suggested to be 3,4,5-trimethoxybenzoic acid by the UV, ^1H NMR, and ^{13}C NMR spectra. Alkaline hydrolysis of **4** with 0.4% KOH in EtOH gave 3,4,5-trimethoxybenzoic acid, **2**, and **2a**. Thus, **4** was characterized as 3β-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4,5-trimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

Compound **5** was deduced as $C_{57}H_{92}O_{28}$ from the positive-ion FABMS (*m/z* 1247 $[M + Na]^+$), ^{13}C NMR spectrum (57 carbon signals), and elemental analysis data. The ^1H NMR spectrum of **5** contained signals for five anomeric protons at δ 5.17 (d, J = 7.9 Hz), 5.08 (d, J = 7.8 Hz), 4.96 (d, J = 7.7 Hz), 4.91 (d, J = 7.5 Hz), and 4.65 (d, J = 6.5 Hz), along with signals for five steroid methyl groups and an acetyl group. Acid hydrolysis of **5** with 1 M HCl in dioxane−H_2O (1:1) gave L-arabinose, D-xylose, and D-glucose, whereas alkaline treatment with 3% NaOMe in MeOH yielded a deacetyl derivative (**5a**: $C_{55}H_{90}O_{27}$). The above data and inspection of the ^{13}C NMR spectrum of **5** assumed that **5** structurally corresponded to **2** with one more β-D-glucopyranosyl unit present and that the acetyl diglycosyl group linked to C-16 of the aglycon was identical to that of **2** and **11**. The additional glucosyl unit was supposed to be located at C-4 of the terminal glucosyl part in **2** since a glycosylation shift could be detected around it when the ^{13}C NMR spectrum of **5** was compared with that of **2**. This was confirmed by a $^{3}J_{C,H}$ correlation from the anomeric proton signal of the terminal glucosyl residue at δ 5.17 to the δ 81.0 resonance assignable to C-4 of the inner glucosyl group, whose anomeric proton at δ 5.08 had an HMBC correlation with the δ 70.1 signal due to C-6 of the glucosyl unit attached at C-3 of the aglycon. Accordingly, the structure of **5** was elucidated as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

Compound **6** was analyzed for $C_{66}H_{100}O_{31}$ by combined negative-ion FABMS (*m/z* 1387 $[M - H]^-$), ^{13}C NMR spectrum (66 carbon signals), and elemental analysis. Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of **5** showed their considerable structural similarity and confirmed that **6** differed from **5** in the presence of a 3,4-dimethoxybenzoyl group. Alkaline hydrolysis of **6** with 0.4% KOH in EtOH gave 3,4-dimethoxybenzoic acid, **5**, and **5a**. The ester linkage at the xylose C-2 position in **6** was formed from 3,4-dimethoxybenzoic acid, as was evident from an HMBC correlation between the signals of the xylose H-2 proton at δ 5.71 (dd, $J = 8.4$, 7.7 Hz) and the carbonyl carbon at δ 165.6. The structure of **6** was elucidated as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

The ^{1}H and ^{13}C NMR spectra of **7** ($C_{67}H_{102}O_{32}$) and **8** ($C_{65}H_{98}O_{31}$) were identical to those of **6**, except for the aromatic region signals due to the substituted benzoyl moiety. The aromatic acid linked to C-2 of xylosyl residue was suggested to be 3,4,5-trimethoxybenzoic acid in **7** and 4-hydroxy-3-methoxybenzoic acid in **8** by the UV, ^{1}H NMR, and ^{13}C NMR spectra. On alkaline hydrolysis of **7** and **8** with 0.4% KOH, 3,4,5-trimethoxybenzoic acid, **5**, and **5a** were obtained from **7**, and 4-hydroxy-3-methoxybenzoic acid, **5**, and **5a** from **8**. The structures of **7** and **8** were assigned as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4,5-trimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one and 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(2-*O*-4-hydroxy-3-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one, respectively.

Compound **9** ($C_{66}H_{100}O_{31}$) was presumed to be an isomer of **6** with regard to the location of the 3,4-dimethoxybenzoyl group linked to the xylosyl moiety. Alkaline hydrolysis of **9** gave **5**, **5a**, and 3,4-dimethoxybenzoic acid. In the HMBC spectrum of **9**, the downfield-shifted proton signal at δ 5.95 (dd, $J = 9.0$, 8.8 Hz) attributable to H-3 of the xylosyl moiety was correlated to the ester carbonyl carbon signal at δ 166.4. The structure of **9** was characterized as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy)]-17α-hydroxy-16β-[(*O*-(3-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

The isolated compounds were evaluated for their cytotoxic activity against HL-60 cells. The cells were continuously treated with each sample for 72 h, and the cell growth was measured by an MTT reduction assay procedure (Table 2).[8] The 3-*O*-monoglucosides with an aromatic acyl group at the C-16 diglycoside moiety (**1**, **12**) were extremely cytotoxic, with respective IC_{50} values of 0.00016 and 0.00013 μg/mL, and the other compounds, except for **2**, **5**, and **8**, also showed cytotoxic activity as potent as etoposide (IC_{50} 0.30 μg/mL), used as a positive control. These cholestanes were concluded to contribute to the potent cytotoxicity of the crude *O. thyrsoides* bulb extract. Compound **11** is the corresponding deacyl derivative of **1** and **12** and was less cytotoxic compared with **1** and **12**. Compounds **2** and **5**, which are the corresponding deacyl cholestanes of **3** and **4**, and **6** and **7**, respectively, did not show any apparent cytotoxic activity even at the sample concentration of 10 μg/mL. These facts were consistent with the aromatic acid ester group attached at the C-16 glycoside moiety playing an important role for the appearance of the strong cytotoxic activity, as observed in the related cholestanes.[1,2,5] The cytotoxic activity of **3** and **4**, having an additional glucosyl unit at C-6 of the terminal glucosyl moiety of **12** and **1**, respectively, was far less potent than that of **12** and **1** by about 3 orders of magnitude. However, further glycosylation of the C-4 hydroxy group of the terminal glucosyl moiety of **3** and **4** resulted in no discernible effects on the activity.

Experimental Section

General Experimental Procedures. Optical rotation was measured by using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DPX-400 spectrometer (400 MHz for ^{1}H NMR, Karlsruhe, Germany) or on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol and dithioerythritol (3:1) matrix. Elemental analysis was carried out using an Elemental Vario EL (Hanau, Germany) elemental analyzer. Silica gel (Fuji-Silysia Chemical, Aichi, Japan), ODS silica gel (Nacalai Tesque, Kyoto, Japan), and Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP-18 F_{254}S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh), a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port with a 20 μL sample loop. A Kaseisorb NH_2-60-5 column (4.6 mm i.d. $\times$ 250 mm, 5 μm, Tokyo-Kasei, Tokyo, Japan) was employed for HPLC analysis. The following reagents were obtained from the indicated companies: RPMI 1640 medium (Gibco, Gland Island, NY); FBS (Bio-Whittaker, Walkersville, MD); MTT (Sigma, St. Louis, MO); penicillin and streptomycin (Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

Plant Material. The bulbs of *O. thyrsoides* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowered plant was identified by one of the authors (Y.S). A voucher specimen has been deposited in our laboratory (voucher No. OT-99-004, Laboratory of Medicinal Plant Science).

Extraction and Isolation. The plant material (fresh weight, 15.4 kg) was extracted with hot MeOH (twice). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (702 g) was passed through a Diaion HP-20 column, successively eluting with 30% MeOH, 50% MeOH, and EtOH. The EtOH eluate fraction exhibited potent cytotoxic activity against HL-60 cells (IC_{50} 0.028 μg/mL), while the other fractions did not show apparent cytotoxicity (30% MeOH and 50% MeOH: IC_{50} >20 μg/mL). Column chroma-

Table 1. ^{13}C NMR Spectral Data for Compounds **1**, **2**, **2a**, **3**–**5**, **5a**, and **6**–**9** in Pyridine-d_5

carbon	**1**	**2**	**2a**	**3**	**4**	**5**	**5a**	**6**	**7**	**8**	**9**
1	37.4	37.4	37.4	37.4	37.4	37.4	37.4	37.4	37.4	37.4	37.4
2	30.2	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4	30.4
3	78.1	78.4	78.4	78.4	78.4	78.5	78.4	78.4	78.4	78.4	78.4
4	39.3	39.5	39.4	39.5	39.5	39.5	39.4	39.4	39.4	39.4	39.4
5	140.8	141.0	140.9	140.9	140.9	140.9	140.8	140.9	140.9	140.9	140.9
6	121.8	121.7	121.8	121.7	121.6	121.8	121.9	121.8	121.8	121.8	121.8
7	32.2	32.2	32.2	32.2	32.1	32.1	32.2	32.1	32.1	32.1	32.1
8	32.0	32.0	32.0	31.9	31.9	32.0	32.0	31.9	32.0	31.9	32.0
9	50.0	49.9	49.9	49.9	49.9	49.9	49.9	49.9	49.9	49.9	49.9
10	36.9	36.9	36.8	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9
11	20.9	20.8	20.9	20.8	20.8	20.8	20.9	20.8	20.8	20.8	20.8
12	32.7	32.7	32.5	32.7	32.7	32.7	32.5	32.7	32.7	32.7	32.7
13	46.5	46.5	46.4	46.5	46.5	46.5	46.4	46.5	46.5	46.5	46.5
14	48.5	48.4	48.5	48.4	48.4	48.4	48.5	48.4	48.4	48.4	48.4
15	34.6	35.0	36.1	34.6	34.5	35.0	36.1	34.6	34.5	34.7	35.0
16	88.4	88.3	88.9	88.3	88.4	88.3	88.9	88.3	88.4	88.3	88.2
17	85.7	85.7	86.2	85.7	85.7	85.8	86.2	85.7	85.7	85.8	85.7
18	13.6	13.5	13.6	13.6	13.6	13.5	13.6	13.6	13.6	13.6	13.5
19	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.4	19.4	19.4	19.4
20	46.3	46.4	46.1	46.3	46.3	46.4	46.1	46.3	46.3	46.3	46.4
21	11.9	11.9	12.1	11.9	11.9	11.9	12.1	11.9	11.9	11.9	11.8
22	218.9	219.0	219.6	218.9	218.9	218.9	219.5	218.8	218.8	218.9	218.8
23	32.7	32.8	32.6	32.6	32.6	32.8	32.6	32.7	32.7	32.7	32.9
24	39.3	39.5	39.5	39.2	39.2	39.4	39.4	39.2	39.2	39.2	39.5
25	27.7	27.9	27.9	27.7	27.7	27.9	27.9	27.7	27.7	27.7	27.9
26	22.8	22.8	23.0	22.8	22.8	22.8	23.0	22.8	22.8	22.8	22.8
27	22.4	22.5	22.5	22.4	22.4	22.5	22.5	22.4	22.4	22.4	22.5
Glc 1	102.5	103.0	103.0	102.9	102.9	102.8	102.8	102.7	102.7	102.7	102.8
2	75.6	75.1	75.1	75.1	75.1	75.1	75.1	75.1	75.2	75.2	75.2
3	78.6	78.5	78.4	78.5	78.5	78.4	78.4	78.4	78.5	78.5	78.4
4	71.7	71.7	71.7	71.7	71.7	71.5	71.5	71.5	71.6	71.6	71.5
5	78.5	77.2	77.2	77.2	77.2	77.1	77.1	77.1	77.1	77.1	77.1
6	62.9	70.1	70.1	70.0	70.0	70.1	70.0	70.0	70.1	70.1	70.1
Glc′ 1		105.4	105.5	105.4	105.4	104.9	104.9	104.9	104.9	104.9	104.9
2		75.2	75.2	75.2	75.2	74.7	74.7	74.7	74.7	74.7	74.7
3		78.5	78.5	78.5	78.5	76.7	76.7	76.6	76.7	76.7	76.7
4		71.6	71.6	71.6	71.6	81.0	80.9	80.9	80.9	81.0	81.0
5		78.7	78.7	78.6	78.6	76.5	76.5	76.5	76.5	76.5	76.5
6		62.8	62.8	62.8	62.8	62.0	62.0	62.0	62.0	62.0	62.0
Glc″ 1						104.8	104.9	104.9	104.9	105.0	104.9
2						74.6	74.6	74.6	74.6	74.6	74.6
3						78.4	78.5	78.4	78.5	78.5	78.5
4						71.6	71.6	71.5	71.5	71.5	71.6
5						78.2	78.2	78.2	78.2	78.2	78.2
6						62.4	62.4	62.4	62.4	62.4	62.4
Ara 1	100.8	101.4	105.4	100.8	100.8	101.4	105.5	100.8	100.8	100.8	101.3
2	72.2	72.2	71.7	72.1	72.1	72.2	71.7	72.1	72.2	72.0	72.1
3	80.9	80.0	83.8	80.9	80.9	80.0	83.8	81.0	81.0	81.0	80.5
4	67.8	68.6	68.9	67.7	67.7	68.6	68.9	67.7	67.8	67.6	68.8
5	65.5	66.5	67.3	65.4	65.5	66.5	67.3	65.4	65.5	65.3	68.9
Xyl 1	103.6	106.7	106.9	103.7	103.5	106.7	106.9	103.7	103.5	103.8	106.4
2	75.4	74.2	75.1	75.2	75.6	74.2	75.1	75.2	75.6	75.0	71.9
3	76.3	78.2	78.3	76.3	76.2	78.2	78.2	76.3	76.2	76.4	79.3
4	70.7	70.9	71.0	70.7	70.7	70.9	71.0	70.7	70.7	70.7	66.7
5	67.1	67.2	67.1	67.0	67.0	67.2	67.1	67.0	67.0	67.0	66.7
Ar 1	126.3			123.1	126.3			123.1	126.3	122.1	123.1
2	108.1			113.5	108.1			113.5	108.1	113.9	113.2
3	153.7			149.5	153.6			149.5	153.6	148.3	149.5
4	143.2			154.0	143.2			154.0	143.2	153.2	153.8
5	153.7			111.2	153.6			111.2	153.6	116.1	111.2
6	108.1			124.4	108.1			124.4	108.1	125.0	124.2
7	165.4			165.6	165.4			165.6	165.4	165.7	166.4
OMe	60.7			55.9	60.7			55.9	60.7	55.8	55.8
	56.2 × 2			55.8	56.2 × 2			55.8	56.2 × 2		55.7
Ac	169.3	170.0		169.3	169.3	170.0		169.3	169.3	169.3	170.0
	20.9	21.5		20.9	20.9	21.5		20.9	20.9	20.9	21.5

tography of the EtOH eluate portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (90:10:0; 40:10:1; 20:10:1), and finally with MeOH alone, gave four fractions (I–IV). Fraction II was subjected to a silica gel column eluting with $CHCl_3$–MeOH–H_2O (60:10:1) to collect two additional fractions (IIa, IIb). Fraction IIa was purified by silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (70:10:1; 80:10:1) and ODS silica gel column chromatography with MeOH–H_2O (2:1) and MeCN–H_2O (1:2; 2:3) and by preparative HPLC using MeCN–H_2O (4:5) to yield **1** (35.6 mg), **10** (23.6 mg), and **12** (68.1 mg). Fraction IIb was subjected to an ODS silica gel column with MeCN–H_2O (1:2) to give **11** (18.9 mg). Fraction III was separated by silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (40:10:1) and ODS silica gel column chromatography with MeCN–H_2O (1:2) to give four fractions (IIIa–IIId). Fraction

Table 2. ^1H and ^{13}C NMR Spectral Data for the Triglucoside Moiety of **5** in Pyridine-d_5

position	^1H	J (Hz)	^{13}C
Glc 1	4.96 d	7.7	102.8
2	3.99 dd	8.3, 7.7	75.1
3	4.21 dd	8.9, 8.3	78.4
4	4.22 dd	9.2, 8.9	71.5
5	4.10 ddd	9.2, 8.1, 2.1	77.1
6	4.80 dd	11.4, 2.1	70.1
	4.34 dd	11.4, 8.1	
Glc′ 1	5.08 d	7.8	104.9
2	4.05 dd	8.5, 7.8	74.7
3	4.25 dd	8.6, 8.5	76.7
4	4.32 dd	9.3, 8.6	81.0
5	3.86 ddd	9.3, 6.6, 2.4	76.5
6	4.54 dd	11.9, 6.6	62.0
	4.44 dd	11.9, 2.4	
Glc″ 1	5.17 d	7.9	104.8
2	4.09 dd	8.8, 7.9	74.6
3	4.26 dd	8.8, 8.9	78.4
4	4.22 dd	10.9, 8.9	71.6
5	3.97 ddd	10.9, 5.8, 2.7	78.2
6	4.53 dd	11.5, 2.7	62.4
	4.29 dd	11.5, 5.8	

Table 3. Cytotoxic Acitivity of Compounds **1**−**12** and Etoposide against HL-60 Cells

compound	IC_{50} (μg/mL)
1	0.00016
2	>10
3	0.81
4	0.70
5	>10
6	0.73
7	0.77
8	6.6
9	0.46
10	0.12
11	0.013
12	0.00013
etoposide	0.30

IIIb was subjected to a silica gel column eluting with $CHCl_3$−MeOH−H_2O (60:10:1) and preparative HPLC using MeCN−H_2O (4:5) to give **3** (61.1 mg) and **4** (84.5 mg). Fraction IIIc was chromatographed on silica gel eluting with $CHCl_3$−MeOH−H_2O (30:10:1) and ODS silica gel with MeCN−H_2O (2:5) to give **2** (130 mg) and a mixture of **6** and **7**, which was separated by preparative HPLC using MeCN−H_2O (2:3) to furnish **6** (140 mg) and **7** (128 mg). Compound **9** (22.1 mg) was isolated from fraction IIId by subjecting it to preparative HPLC using MeCN−H_2O (2:3). Fraction IV was subjected to a silica gel column eluting with $CHCl_3$−MeOH−H_2O (40:10:1; 30:10:1) and an ODS silica gel column with MeCN−H_2O (2:5) and to preparative HPLC using MeCN−H_2O (2:3) to give **5** (894 mg) and **8** (90.2 mg).

Compound 1: amorphous solid; $[\alpha]_D^{25}$ −34.0° (c 0.10, MeOH); IR (film) ν_{max} 3418 (OH), 2937 and 2871 (CH), 1732, 1716, and 1696 (C=O), 1590, 1504, and 1457 (aromatic ring), 1416, 1371, 1338, 1255, 1228, 1173, 1127, 1070, 1042 cm^{-1}; UV (MeOH) λ_{max} 268 nm (log ϵ 4.07); ^1H NMR (pyridine-d_5) δ 7.71 (2H, s, H-2, H-6 of Ar), 5.79 (1H, dd, J = 8.9, 7.6 Hz, H-2 of Xyl), 5.56 (1H, dd, J = 7.4, 5.9 Hz, H-2 of Ara), 5.29 (1H, br d, J = 4.6 Hz, H-6), 5.16 (1H, d, J = 7.6 Hz, H-1 of Xyl), 5.05 (1H, d, J = 7.7 Hz, H-1 of Glc), 4.61 (1H, d, J = 5.6 Hz, H-1 of Ara), 3.97 (3H, s, OMe), 3.93 (1H, m, $W_{1/2}$ = 22.6 Hz, H-3), 3.81 (3H × 2, s, OMe × 2), 3.21 (1H, q, J = 7.4 Hz, H-20), 2.01 (3H, s, Ac), 1.31 (3H, d, J = 7.4 Hz, Me-21), 1.00 (3H, s, Me-18), 0.98 (3H, s, Me-19), 0.86 (3H, d, J = 6.0 Hz, Me-26 or Me-27), 0.88 (3H, d, J = 6.0 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) m/z 1117 [M + Na]$^+$; *anal.* C 58.59%; H 7.68% (calcd for $C_{55}H_{82}O_{22}\cdot 3/2H_2O$, C 58.86%, H 7.63%).

Alkaline Hydrolysis of 1. Compound **1** (10.4 mg) was treated with 0.4% KOH in EtOH (9 mL) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo, Tokyo, Japan) and chromatographed on silica gel eluting with $CHCl_3$−MeOH−H_2O (40:10:1) to give **11** (3.6 mg) and 3,4,5-trimethoxybenzoic acid (0.5 mg).

Compound 2: amorphous solid; $[\alpha]_D^{25}$ −64.0° (c 0.10, MeOH); IR (film) ν_{max} 3386 (OH), 2935 and 2875 (CH), 1737 and 1691 (C=O), 1646, 1455, 1407, 1373, 1244, 1166, 1048 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.82 (1H, dd, J = 8.5, 6.5 Hz, H-2 of Ara), 5.30 (1H, br d, J = 4.8 Hz, H-6), 5.15 (1H, d, J = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, J = 7.7 Hz, H-1 of Glc), 4.91 (1H, d, J = 7.5 Hz, H-1 of Xyl), 4.66 (1H, d, J = 6.5 Hz, H-1 of Ara), 3.94 (1H, m, overlapping, H-3), 3.86 (1H, dd, J = 8.2, 7.5 Hz, H-2 of Xyl), 3.33 (1H, q, J = 7.4 Hz, H-20), 2.35 (3H, s, Ac), 1.32 (3H, d, J = 7.4 Hz, Me-21), 0.96 (3H, s, Me-18), 0.95 (3H, s, Me-19), 0.95 (3H, d, J = 6.2 Hz, Me-26 or Me-27), 0.92 (3H, d, J = 6.2 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive-mode) m/z 1085 [M + Na]$^+$; *anal.* C 55.10%, H 8.13% (calcd for $C_{51}H_{82}O_{23}\cdot 3H_2O$, C 54.82%, H 7.94%).

Acid Hydrolysis of 2. A solution of **2** (5.0 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 92 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Diaion HP-20 eluting with H_2O−MeOH (3:2), followed by Me_2CO−EtOH (1:1), to give a sugar fraction (2.1 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA) and a Toyopak IC-SP M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 0.9 mL/min; detection, RI and OR. The identification of L-arabinose, D-xylose, and D-glucose present in the sugar fraction was carried out by comparison of their retention times and polarities with those of authentic samples. t_R (min): 11.42 (L-arabinose, positive polarity), 11.98 (D-xylose, positive polarity), 19.98 (D-glucose, positive polarity).

Alkaline Hydrolysis of 2. Compound **2** (39.0 mg) was treated with 3% NaOMe in MeOH (3 mL) at room temperature for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and then chromatographed eluting with $CHCl_3$−MeOH−H_2O (30:10:1) to yield **2a** (20.0 mg).

Compound 2a: amorphous powder; $[\alpha]_D^{25}$ −50.0° (c 0.10, MeOH); IR (film) ν_{max} 3376 (OH), 2934, 2902, and 2871 (CH), 1685 (C=O), 1072, 1044 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.28 (1H, br s, H-6), 5.19 (1H, d, J = 7.6 Hz, H-1 of Xyl), 5.16 (1H, d, J = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, J = 7.7 Hz, H-1 of Glc), 4.66 (1H, d, J = 7.7 Hz, H-1 of Ara), 3.92 (1H, m, overlapping, H-3), 3.86 (1H, dd, J = 8.2, 7.5 Hz, H-2 of Xyl), 3.39 (1H, q, J = 7.4 Hz, H-20), 1.32 (3H, d, J = 7.4 Hz, Me-21), 0.94 (3H, s, Me-19), 0.94 (3H, d, J = 6.4 Hz, Me-26 or Me-27), 0.89 (3H, s, Me-18), 0.87 (3H, d, J = 6.4 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FAB-MS (positive-mode) m/z 1043 [M + Na]$^+$; *anal.* C 53.14%, H 7.93% (calcd for $C_{49}H_{80}O_{22}\cdot 4H_2O$, C 53.14%, H 8.11%).

Compound 3: amorphous solid; $[\alpha]_D^{25}$ −72.0° (c 0.10, MeOH); IR (film) ν_{max} 3365 (OH), 2932 and 2871 (CH), 1715 and 1694 (C=O), 1600, 1515, and 1456 (aromatic ring), 1416, 1370, 1270, 1226, 1174, 1131, 1067, 1043 cm^{-1}; UV (MeOH) λ_{max} 292 nm (log ϵ 3.74), 263 nm (log ϵ 4.02); ^1H NMR (pyridine-d_5) δ 8.06 (1H, dd, J = 8.6, 1.9 Hz, H-6 of Ar), 7.30 (1H, d, J = 1.9 Hz, H-2 of Ar), 7.07 (1H, d, J = 8.6 Hz, H-5 of Ar), 5.71 (1H, dd, J = 9.0, 7.8 Hz, H-2 of Xyl), 5.55 (1H, dd, J = 7.4, 6.2 Hz, H-2 of Ara), 5.29 (1H, br d, J = 4.7 Hz, H-6), 5.15 (1H, d, J = 7.8 Hz, H-1 of Glc′), 5.15 (1H, d, J = 7.8 Hz, H-1 of Xyl), 4.95 (1H, d, J = 7.7 Hz, H-1 of Glc), 4.60 (1H, d, J = 5.8 Hz, H-1 of Ara), 3.94 (1H, m, $W_{1/2}$ = 20.0 Hz, H-3), 3.82 and 3.81 (each 3H, s, OMe × 2), 3.20 (1H, q, J = 7.4 Hz, H-20), 2.00 (3H, s, Ac), 1.30 (3H, d, J = 7.4 Hz, Me-21), 0.99 (3H, s, Me-18), 0.95 (3H, s, Me-19), 0.88 (3H, d, J = 6.1 Hz, Me-26 or Me-27), 0.86 (3H, d, J = 6.1 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive-mode) *m/z* 1249 [M + Na]$^+$; *anal.* C 56.55%, H 7.80% (calcd for $C_{60}H_{90}O_{26}\cdot H_2O$, C 56.64%; H 7.53%).

Compound 4: amorphous solid; $[\alpha]_D^{25}$ −58.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3363 (OH), 2936, 2904, and 2871 (CH), 1731, 1716, and 1694 (C=O), 1589, 1504, and 1457 (aromatic ring), 1416, 1370, 1337, 1228, 1172, 1126, 1067, 1043 cm^{-1}; UV (MeOH) λ_{max} 266 nm (log ϵ 4.01); ^{1}H NMR (pyridine-d_5) δ 7.56 (2H, s, H-2, H-6 of Ar), 5.71 (1H, dd, *J* = 9.1, 7.6 Hz, H-2 of Xyl), 5.55 (1H, dd, *J* = 7.5, 5.9 Hz, H-2 of Ara), 5.28 (1H, br d, *J* = 4.7 Hz, H-6), 5.16 (1H, d, *J* = 7.6 Hz, H-1 of Xyl), 5.14 (1H, d, *J* = 7.6 Hz, H-1 of Glc′), 4.95 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.60 (1H, d, *J* = 5.9 Hz, H-1 of Ara), 4.00 (3H, s, OMe), 3.94 (1H, m, overlapping, H-3), 3.81 (3H × 2, s, OMe), 3.21 (1H, q, *J* = 7.4 Hz, H-20), 2.01 (3H, s, Ac), 1.31 (3H, d, *J* = 7.4 Hz, Me-21), 0.98 (3H, s, Me-18), 0.94 (3H, s, Me-19), 0.88 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27), 0.86 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive-mode) *m/z* 1279 [M + Na]$^+$; *anal.* C 55.84%, H 7.66% (calcd for $C_{61}H_{92}O_{27}\cdot 3H_2O$, C 55.87%, H 7.53%).

Alkaline Hydrolysis of 3 and 4. Compounds **3** and **4** were treated separately (each 20.0 mg) with 0.4% KOH in EtOH (each 12 mL) at room temperature for 90 min. After neutralization by passage through an Amberlite IR-120B column, each reaction mixture was chromatographed on silica gel using $CHCl_3$−MeOH−H_2O (30:10:1). Compound **3** gave **2** (4.5 mg), **2a** (2.5 mg), and 3,4-dimethoxybenzoic acid (0.8 mg). Compound **4** gave **2** (3.8 mg), **2a** (2.2 mg), and 3,4,5-trimethoxybenzoic acid (0.4 mg).

Compound 5: amorphous solid; $[\alpha]_D^{25}$ −46.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3381 (OH), 2935 and 2904 (CH), 1737 and 1690 (C=O), 1372, 1244, 1163, 1048 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.81 (1H, dd, *J* = 8.3, 6.5 Hz, H-2 of Ara), 5.31 (1H, br d, *J* = 4.5 Hz, H-6), 5.17 (1H, d, *J* = 7.9 Hz, H-1 of Glc″), 5.08 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.91 (1H, d, *J* = 7.5 Hz, H-1 of Xyl), 4.65 (1H, d, *J* = 6.5 Hz, H-1 of Ara), 3.94 (1H, m, $W_{1/2}$ = 22.9 Hz, H-3), 3.86 (1H, dd, *J* = 8.2, 7.5 Hz, H-2 of Xyl), 3.32 (1H, q, *J* = 7.4 Hz, H-20), 2.34 (3H, s, Ac), 1.30 (3H, d, *J* = 7.4 Hz, Me-21), 0.96 (3H, s, Me-19), 0.95 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27), 0.94 (3H, s, Me-18), 0.92 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive-mode) *m/z* 1247 [M + Na]$^+$; *anal.* C 53.90%, H 8.01% (calcd for $C_{57}H_{92}O_{28}\cdot 5/2H_2O$, C 53.51%, H 7.72%).

Acid Hydrolysis of 5. Compound **5** (8.0 mg) was subjected to acid hydrolysis as described for **5** to give a sugar fraction (2.3 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2** showed the presence of L-arabinose, D-xylose, and D-glucose.

Alkaline Hydrolysis of 5. Compound **5** (50.2 mg) was treated with 3% NaOMe in MeOH at room temperature for 3 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and chromatographed on silica gel eluting with $CHCl_3$−MeOH−H_2O (20:10:1) to yield **5a** (39.7 mg).

Compound 5a: amorphous powder; $[\alpha]_D^{25}$ −66.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3376 (OH), 2934, 2902, and 2871 (CH), 1685 (C=O), 1375, 1162, 1071, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 5.29 (1H, br d, *J* = 4.2 Hz, H-6), 5.19 (1H, d, *J* = 7.6 Hz, H-1 of Xyl), 5.16 (1H, d, *J* = 7.8 Hz, H-1 of Glc″), 5.09 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.97 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.52 (1H, d, *J* = 7.7 Hz, H-1 of Ara), 3.94 (1H, m, $W_{1/2}$ = 21.1 Hz, H-3), 3.38 (1H, q, *J* = 7.4 Hz, H-20), 1.29 (3H, d, *J* = 7.4 Hz, Me-21), 0.94 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.4 Hz, Me-26 or Me-27), 0.90 (3H, s, Me-18), 0.86 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (negative-mode) *m/z* 1181 [M − H]$^-$; *anal.* C 53.18%, H 8.11% (calcd for $C_{55}H_{90}O_{27}\cdot 3H_2O$, C 53.39%, H 7.82%).

Compound 6: amorphous solid; $[\alpha]_D^{25}$ −60.0° (*c* 0.10, MeOH); FABMS (negative-mode) *m/z* 1387 [M − H]$^-$; IR (film) ν_{max} 3394 (OH), 2938 and 2907 (CH), 1736, 1714, and 1697 (C=O), 1600, 1515, and 1459 (aromatic ring), 1417, 1371, 1270, 1227, 1173, 1131, 1065, 1044 cm^{-1}; UV (MeOH) λ_{max} 293 nm (log ϵ 3.81), 262 nm (log ϵ 4.09); ^{1}H NMR (pyridine-d_5) δ 8.06 (1H, dd, *J* = 8.5, 1.6 Hz, H-6 of Ar), 7.93 (1H, d, *J* = 1.6 Hz, H-2 of Ar), 7.06 (1H, d, *J* = 8.5 Hz, H-5 of Ar), 5.71 (1H, dd, *J* = 8.4, 7.7 Hz, H-2 of Xyl), 5.54 (1H, dd, *J* = 7.2, 5.8 Hz, H-2 of Ara), 5.29 (1H, br s, H-6), 5.16 (1H, d, *J* = 8,0 Hz, H-1 of Glc″), 5.14 (1H, d, *J* = 7.7 Hz, H-1 of Xyl), 5.08 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.59 (1H, d, *J* = 5.8 Hz, H-1 of Ara), 3.94 (1H, m, overlapping, H-3), 3.82 and 3.80 (each 3H, s, OMe), 3.19 (1H, q, *J* = 7.3 Hz, H-20), 2.00 (3H, s, Ac), 1.29 (3H, d, *J* = 7.3 Hz, Me-21), 0.99 (3H, s, Me-18), 0.94 (3H, s, Me-19), 0.87 (3H, d, *J* = 6.0 Hz, Me-26 or Me-27), 0.85 (3H, d, *J* = 6.0 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; *anal.* C 51.79%, H 7.31% (calcd for $C_{66}H_{100}O_{31}\cdot 15/2H_2O$, C 51.99%, H 7.60%).

Compound 7: amorphous solid; $[\alpha]_D^{25}$ −44.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3386 (OH), 2938 and 2905 (CH), 1727 and 1695 (C=O), 1590, 1502, and 1460 (aromatic ring), 1416, 1370, 1338, 1229, 1166, 1125, 1066, 1045 cm^{-1}; UV (MeOH) λ_{max} 266 nm (log ϵ 3.98); ^{1}H NMR (pyridine-d_5) δ 7.58 (2H, s, H-2, H-6 of Ar), 5.71 (1H, dd, *J* = 9.1, 7.4 Hz, H-2 of Xyl), 5.56 (1H, dd, *J* = 7.4, 5.8 Hz, H-2 of Ara), 5.30 (1H, br d, *J* = 4.5 Hz, H-6), 5.17 (1H, d, *J* = 7.8 Hz, H-1 of Glc″), 5.16 (1H, d, *J* = 7.5 Hz, H-1 of Xyl), 5.08 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.97 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.60 (1H, d, *J* = 5.8 Hz, H-1 of Ara), 3.97 (3H, s, OMe), 3.94 (1H, m, overlapping, H-3), 3.81 (3H × 2, s, OMe × 2), 3.20 (1H, q, *J* = 7.4 Hz, H-20), 1.89 (3H, s, Ac), 1.30 (3H, d, *J* = 7.4 Hz, Me-21), 0.99 (3H, s, Me-18), 0.96 (3H, s, Me-19), 0.88 (3H, d, *J* = 6.0 Hz, Me-26 or Me-27), 0.86 (3H, d, *J* = 6.0 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (positive-mode) *m/z* 1441 [M + Na]$^+$; *anal.* C 54.28%, H 7.60% (calcd for $C_{67}H_{102}O_{32}\cdot 3H_2O$, C 54.61%, H 7.39%).

Compound 8: amorphous solid; $[\alpha]_D^{25}$ −60.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3392 (OH), 2939 and 2904 (CH), 1738 and 1695 (C=O), 1606, 1515, and 1458 (aromatic ring), 1428, 1372, 1277, 1230, 1165, 1066, 1047 cm^{-1}; UV (MeOH) λ_{max} 292 nm (log ϵ 3.77), 263 nm (log ϵ 4.09); ^{1}H NMR (pyridine-d_5) δ 8.04 (1H, dd, *J* = 8.3, 1.9 Hz, H-6 of Ar), 7.99 (1H, d, *J* = 1.9 Hz, H-2 of Ar), 7.28 (1H, d, *J* = 8.3 Hz, H-5 of Ar), 5.71 (1H, dd, *J* = 8.9, 7.6 Hz, H-2 of Xyl), 5.55 (1H, dd, *J* = 7.2, 5.8 Hz, H-2 of Ara), 5.30 (1H, br d, *J* = 4.4 Hz, H-6), 5.17 (1H, d, *J* = 7.9 Hz, H-1 of Glc″), 5.15 (1H, d, *J* = 7.6 Hz, H-1 of Xyl), 5.08 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.59 (1H, d, *J* = 5.8 Hz, H-1 of Ara), 3.94 (1H, m, $W_{1/2}$ = 20.5 Hz, H-3), 3.79 (3H, s, OMe), 3.19 (1H, q, *J* = 7.3 Hz, H-20), 2.00 (3H, s, Ac), 1.29 (3H, d, *J* = 7.3 Hz, Me-21), 0.99 (3H, s, Me-18), 0.97 (3H, s, Me-19), 0.90 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27), 0. 87 (3H, d, *J* = 6.1 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 1; FABMS (negative-mode) *m/z* 1373 [M − H]$^-$; *anal.* C 54.65%, H 7.54% (calcd for $C_{65}H_{98}O_{31}\cdot 3/2H_2O$, C 54.96%, H 7.55%).

Compound 9: amorphous solid; $[\alpha]_D^{25}$ −68.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2934 and 2872 (CH), 1716 and 1694 (C=O), 1600, 1515, 1459, 1417, 1371, 1270, 1227, 1040 cm^{-1}; UV (MeOH) λ_{max} 292 nm (log ϵ 3.77), 262 nm (log ϵ 4.09); ^{1}H NMR (pyridine-d_5) δ 7.94 (1H, dd, *J* = 8.5, 1.9 Hz, H-6 of Ar), 7.78 (1H, d, *J* = 1.9 Hz, H-2 of Ar), 6.93 (1H, d, *J* = 8.5 Hz, H-5 of Ar), 5.95 (1H, dd, *J* = 9.0, 8.8 Hz, H-3 of Xyl), 5.83 (1H, dd, *J* = 8.4, 6.7 Hz, H-2 of Ara), 5.29 (1H, br d, *J* = 4.7 Hz, H-6), 5.17 (1H, d, *J* = 7.8 Hz, H-1 of Glc″), 5.14 (1H, d, *J* = 7.7 Hz, H-1 of Xyl), 5.08 (1H, d, *J* = 7.8 Hz, H-1 of Glc′), 4.96 (1H, d, *J* = 7.7 Hz, H-1 of Glc), 4.65 (1H, d, *J* = 6.7 Hz, H-1 of Ara), 3.94 (1H, m, overlapping, H-3), 3.75 and 3.68 (each 3H, s, OMe), 3.28 (1H, q, *J* = 7.4 Hz, H-20), 2.29 (3H, s, Ac), 1.29 (3H, d, *J* = 7.4 Hz, Me-21), 0.96 (3H, s, Me-19), 0.95 (3H, d, *J* = 6.2 Hz, Me-26 or Me-27), 0.93 (3H, s, Me-18), 0.92 (3H, d, *J* = 6.2 Hz, Me-26 or Me-27); ^{13}C NMR, see Table 2; FABMS (positive-mode) *m/z* 1411 [M + Na]$^+$; *anal.* C 52.18%, H 7.56% (calcd for $C_{66}H_{100}O_{31}\cdot 7H_2O$, C 52.30%, H 7.58%).

Alkaline Hydrolysis of 9−12. Compounds **6** (25.0 mg), **7** (5.0 mg), **8** (5.1 mg), and **9** (5.3 mg) were treated with 0.4% KOH in EtOH. Compound **6** gave **5** (5.1 mg), **5a** (5.0 mg), and 3,4-dimethoxybenzoic acid (1.8 mg); **7** gave **5** (1.1 mg), **5a** (1.4 mg), and 3,4,5-trimethoxybenzoic acid (0.2 mg); **8** gave **5** (1.0 mg), **5a** (1.1 mg), and 4-hydroxy-3-methoxybenzoic acid (0.3 mg); **9** gave **5** (0.7 mg), **5a** (1.0 mg), and 3,4-dimethoxybenzoic acid (0.2 mg).

Cell Culture Assay. HL-60 cells, which were obtained from Human Science Research Resources Bank (JCRB 0085, Osaka, Japan), were maintained in RPMI 1640 medium containing heat-inactivated 10% FBS supplemented with L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The leukemia cells were washed and resuspended in the above medium to 4×10^4 cells/mL, and 196 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μL of EtOH−H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.00001−10 μg/mL; 4 μL of EtOH−H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure. At the end of incubation, 10 μL of 5 mg/mL MTT in phosphate-buffered saline was added to every well, and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* for 5 min to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from every well, and 175 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as IC_{50} value, which reduced the viable cell number by 50%.

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