Steroidal Glycosides from the Aerial Parts of *Polianthes tuberosa*

Yoshihiro Mimaki,* Akihito Yokosuka, and Yutaka Sashida

School of Pharmacy, Tokyo University of Pharmacy and Life Science,1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan

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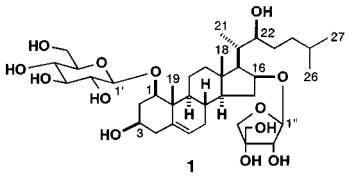
A chemical investigation of the aerial parts of *Polianthes tuberosa* resulted in the isolation of a new bisdesmosidic cholestane glycoside (**1**) and three new spirostanol saponins (**2**−**4**), along with a known cholestane glycoside. The structures of new glycosides were determined by spectroscopic analysis, including 2D NMR spectroscopic data, and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activity on HL-60 human promyelocytic leukemia cells.

The family Agavaceae has a wide distribution in tropical and subtropical regions throughout the world. Hutchinson placed 580 species in the Agavaceae and proposed a further division of this family into the six tribes: Nolineae, Phormieae, Yucceae, Dracaeneae, Agaveae, and Polianthеae.[1] Plants of the genera *Yucca* (Yucceae); *Cordyline*, *Dracaena*, and *Sansevieria* (Dracaeneae); and *Agave* (Agaveae) in the Agavaceae are known to be rich sources for steroidal sapogenins and saponins.[2,3] Previously, we showed that *Nolina recurvata*, a representative species belonging to the tribe Nolineae, also contained a variety of steroidal glycosides in high quantity.[4−6] The flowers of the Agaveae and Poliantheae have inferior ovaries, and this diagnostic feature distinguishes the Agaveae and Poliantheae from the other tribes in the Agavaceae. The secondary metabolites of some Agaveae plants were extensively investigated,[2,3] but nothing has been reported thus far on those of the Poliantheae. As part of a series of phytochemical studies on plants of the family Agavaceae, we investigated the steroidal constituents of *Polianthes tuberosa* L., a well-known ornamental plant belonging to the Poliantheae. This study resulted in the isolation of a new bisdesmosidic cholestane glycoside (**1**) and three new spirostanol saponins, each with up to five monosaccharides (**2**−**4**), along with a known cholestane glycoside. We describe herein the structure determination and cytotoxic activity of **1**−**4**.



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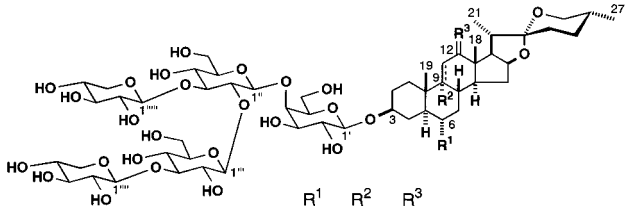

	R^1	R^2	R^3
2	OH	H	H,H
3	H	H	=O
4	H	—	=O (Δ^9)

Results and Discussion

The concentrated MeOH extract of the aerial parts of *P. tuberosa* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was passed through a porous-polymer resin (Diaion HP-20) and further divided into a 20% MeOH eluate fraction and an EtOH eluate fraction. The EtOH eluate fraction was repeatedly chromatographed on Si gel and octadecylsilanized (ODS) Si gel to give compounds **1**−**4**, along with a known cholestane glycoside. The known cholestane glycoside was identified as (22*S*)-1β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-16β-yl α-L-rhamnopyranoside.[5]

Compound **1** was isolated as an amorphous solid, $[\alpha]_D$ −30.0° (MeOH), with a molecular formula, $C_{38}H_{64}O_{13}$, as determined by elemental analysis combined with the data of the negative-ion FABMS exhibiting an $[M - H]^-$ peak at *m*/*z* 727. A broad IR absorption near 3385 cm^{-1} was attributable to hydroxyl groups. The ^1H NMR spectrum of **1** showed signals for two tertiary methyl protons at δ 1.29 and 1.05, three secondary methyl protons at δ 1.20 and 0.90 × 2, and an olefinic proton at δ 5.57. Two anomeric proton signals were also identified at δ 5.48 and 4.99. The ^{13}C NMR spectrum showed 38 resonance lines supporting the molecular formula deduced from elemental analysis and the FABMS data; 27 of them were due to the aglycon part and 11 to two monosaccharides. Acid hydrolysis of **1** with 0.2 M HCl in dioxane−H_2O (1:1) resulted in the production of an aglycon (**1a**), as well as D-glucose and D-apiose as the carbohydrate components. The physical and NMR data allowed the identification of **1a** as (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol.[5,7] The monosaccharides, including their absolute configurations, were identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.[8,9] Thus, **1** was shown to be a polyhydroxylated cholestane diglycoside. Analysis of the $^1H-^1H$ COSY spectrum allowed the sequential assignments of the protons from H-1 to H_2-6 of the glucosyl moiety. The three pairs of ABq signals at δ 5.48 and 4.52 (J = 2.0 Hz), 4.57 and 4.35 (J = 9.3 Hz), and 4.24 and 4.20 (J = 11.1 Hz) were assignable to the protons of the apiofuranosyl residue, and the quaternary carbon signal at δ 80.3 was typical of C-3 of apiofuranose. The proton signals were correlated to the corresponding one-bond-coupled carbon signals using HMQC experiments. The β-furanoid anomeric form of the apiofuranosyl residue was indicated by the downfield-shifted anomeric carbon signal at δ 112.6,[10] while the β-anomeric orientation of the glucose moiety was ascertained by the large J value of the anomeric proton (J = 7.6 Hz). Each monosaccharide was considered to be directly attached to the aglycon and was not substituted because no glycosylation shifts could be observed among the assigned ^{13}C NMR shifts. In the HMBC spectrum, the signal at δ 3.96 (dd, J = 11.5, 3.9

* To whom correspondence should be addressed. Tel.: +81-426-76-4577. Fax: +81-426-76-4579. E-mail: mimakiy@ps.toyaku.ac.jp.

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Hz) showed long-range correlations with C-9 (δ 50.3), C-10 (δ 42.8), and C-19 (δ 14.8) and was assigned to H-1. The resonance at δ 4.37 (ddd, J = 7.7, 7.7, 4.4 Hz) was attributable to H-16, which had a long-range coupling with C-13 (δ 42.0). By the HMQC spectrum, the resonances thus assigned to H-1 and H-16 were associated with the downfield-shifted signals for the oxygen-bearing carbons at δ 82.8 (C-1) and 81.1 (C-16), respectively, to which the sugars were linked. The anomeric proton of the glucose moiety (H-1′) exhibited an HMBC correlation with C-1, whereas that of the apiose residue (H-1″) was correlated to C-16. Reversed HMBC correlations from H-1 to C-1′ and from H-16 to C-1″ could be also noted. All of these data were consistent with the structure (22*S*)-1β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-16β-yl β-D-apiofuranoside, which was given to **1**.

Compound **2** was obtained as an amorphous solid, $[\alpha]_D$ −42.0° (MeOH). Its molecular formula ($C_{55}H_{90}O_{27}$) was deduced from the negative-ion FABMS, which showed an $[M - H]^-$ ion at *m*/*z* 1181, and from elemental analysis. The ^{1}H NMR spectrum showed two three-proton singlet signals at δ 0.80 and 0.74, indicating the presence of two angular methyl groups, as well as two three-proton doublet signals at δ 1.09 and 0.73 assignable to secondary methyl groups. In addition, five anomeric proton signals were observed at δ 5.42, 5.00, 4.96, 4.91, and 4.76. These ^{1}H NMR spectral features and a diagnostic acetal carbon signal at δ 109.6 were indicative of **2** being a spirostanol saponin with a sugar chain made up of five monosaccharides.[11] Acid hydrolysis of **2** with 1 M HCl gave a steroidal sapogenin (**2a**), identified as (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin),[12] with D-glucose, D-galactose, and D-xylose as the carbohydrate moieties. The determination of the sequence of the carbohydrate chain and its binding site at the aglycon were carried out by the following NMR experiments. From the preliminary inspection of the ^{1}H NMR spectra recorded in various solvent systems, it was found that measurement in a mixed solvent of C_5D_5N and CD_3OD in a ratio of 2:1 gave good spectral dispersion and minimized signal overlap. All the proton signals of the carbohydrate groups could be assigned using a combination of the ^{1}H−^{1}H COSY and TOCSY spectra. The latter spectrum provided valuable information for the assignments because only the proton resonances of the same carbohydrate unit were observed clearly and undisturbed, and no signal of the other residues appeared. The easily distinguished anomeric protons, whose J values (7.5−7.9 Hz) indicated the β-orientation at the anomeric center of all the D-pyranoses, served as the starting point in analysis. Multiplet patterns and coupling constants, as well as the proton chemical shifts of the glycoside moiety revealed that it was composed of two β-D-glucopyranosyl units, two β-D-xylopyranosyl units, and one β-D-galactopyranosyl unit (Table 1). The HMQC spectrum allowed the correlations of the proton resonances with the corresponding one-bond-coupled carbon signals, leading to the unambiguous assignments of the ^{13}C NMR shifts (Table 2). The absence of any glycosylation shift for two xylosyl moieties implied that these monosaccharides were present at the terminal position. Glycosylation shifts were detected at C-4 of galactose (δ 79.9), C-2 and C-3 of glucose (I) (δ 80.8 and 87.2, respectively), and C-3 of glucose (II) (δ 87.1). In the HMBC spectrum, correlation peaks were observed from δ 4.91 [anomer of xylose (I)] to 87.1 [C-3 of glucose (II)], δ 5.00 [anomer of xylose (II)] to 87.2 [C-3 of glucose (I)], δ 5.42 [anomer of glucose (II)] to 80.8 [C-2 of glucose (I)], δ 4.96 [anomer of glucose (I)] to 79.9 [C-4 of galactose], and δ 4.76 [anomer of galactose] to 78.2 [C-3 of aglycon], which confirmed the sugar sequence and its linkage position to the aglycon. Thus, the structure of **2** was established as (25*R*)-6α-hydroxy-5α-spirostan-3β-yl *O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Table 1. ^{1}H NMR Assignment of the Glycoside Moiety of Compound **2**[a]

position	^{1}H (ppm)	*J* (Hz)
Gal 1′	4.76 d	7.7
2′	4.16 dd	8.5, 7.7
3′	3.89 dd	8.5, 2.5
4′	4.39 br d	2.5
5′	3.79 dd	8.9, 5.4
6′a	4.41 dd	10.7, 8.9
6′b	4.01 dd	10.7, 5.4
Glc 1″	4.96 d	7.9
(I) 2″	4.15 dd	8.8, 7.9
3″	3.99 dd	8.8, 8.8
4″	3.62 dd	8.8, 8.8
5″	3.68 m	
6″a	4.31 dd	10.2, 1.8
6″b	3.90 *[b]	
Glc 1‴	5.42 d	7.6
(II) 2‴	3.88 dd	9.0, 7.6
3‴	3.91 dd	9.0, 9.0
4‴	3.85 dd	9.0, 9.0
5‴	3.71 m	
6‴a	4.29 br d	10.2
6‴b	4.14 *	
Xyl 1⁗	4.91 d	7.5
(I) 2⁗	3.74 dd	8.7, 7.5
3⁗	3.84 dd	8.7, 8.7
4⁗	3.93 ddd	11.3, 8.7, 5.3
5⁗a	4.11 dd	11.3, 5.3
5⁗b	3.46 dd	11.3, 11.3
Xyl 1⁗′	5.00 d	7.7
(II) 2⁗′	3.73 dd	8.8, 7.7
3⁗′	3.83 dd	8.8, 8.8
4⁗′	3.92 ddd	11.4, 8.8, 5.3
5⁗′a	4.12 dd	11.4, 5.3
5⁗′b	3.53 dd	11.4, 11.4

[a] Spectrum was measured in C_5D_5N−CD_3OD (2:1). [b] Multiplicities are unclear due to overlapping with other signals.

The ^{1}H NMR spectrum of compound **3** ($C_{55}H_{88}O_{27}$) showed signals for four steroid methyl groups at δ 1.25, 1.07, 0.76, and 0.73, as well as five anomeric protons at δ 5.43, 5.00, 4.98, 4.91, and 4.73. Analysis of the ^{13}C NMR spectrum of **3** and comparison with that of **2** revealed that **3** possessed a pentaglycoside sequence identical to that of **2**, but slightly differed from it in terms of the aglycon structure. The IR (1707 cm^{-1}) and ^{13}C NMR (δ 213.7) spectra demonstrated the presence of a carbonyl group in the aglycon, and it was suggested to be located at C-12 by HMBC correlations from the δ 213.7 resonance to H_2-11 at δ 2.41 (dd, J = 13.8, 13.8 Hz) and 2.20 (dd, J = 13.8, 4.9 Hz), H-17 at δ 2.65 (dd, J = 8.6, 6.7 Hz), and to Me-18 at δ 1.07 (s). This was confirmed by acid hydrolysis of **3**, giving (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin),[13] D-glucose, D-galactose, and D-xylose. The structure of **3** was thus formulated as (25*R*)-3β-[{*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl}oxy]-5α-spirostan-12-one.

The spectral features of compound **4** were quite similar to those of **3**, and the ^{1}H and ^{13}C NMR spectra immediately identified the pentaglycoside moiety of **4** as being identical to that of **2** and **3**. The presence of a conjugated carbonyl group in the aglycon moiety of **4** was shown by its molecular formula ($C_{55}H_{86}O_{27}$), which contained two fewer

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**4**[a]

C	1	2	3	4
1	82.8	38.0	37.0	35.3
2	37.5	30.0	29.9	29.9
3	68.0	78.2	77.9	77.4
4	43.7	29.5	34.8	34.8
5	139.6	52.3	44.9	43.0
6	124.7	68.9	28.9	28.2
7	31.8	42.6	32.0	33.0
8	33.1	34.6	34.7	37.3
9	50.3	54.5	56.0	172.3
10	42.8	36.9	36.6	39.9
11	23.8	21.5	38.2	120.1
12	40.5	40.4	213.7	205.3
13	42.0	41.1	55.7	51.7
14	55.1	56.6	56.3	53.1
15	36.6	32.3	31.6	32.0
16	81.1	81.4	80.0	80.5
17	57.9	63.3	54.5	54.8
18	11.9	16.7	16.2	15.4
19	14.8	13.6	11.9	18.5
20	35.6	42.3	42.9	43.2
21	13.6	15.0	13.9	13.7
22	72.5	109.6	109.7	109.8
23	34.4	32.0	32.0	32.0
24	36.5	29.4	29.4	29.4
25	28.8	30.9	30.8	30.8
26	22.9	67.1	67.1	67.1
27	22.9	17.4	17.4	17.4
1′	101.3	102.4	102.6	102.7
2′	75.4	73.1	73.0	73.1
3′	78.6	75.1	75.1	75.1
4′	72.4	79.9	79.9	79.9
5′	78.1	75.4	75.4	75.5
6′	63.6	60.7	60.8	60.9
1″	112.6	104.8	104.8	104.8
2″	78.1	80.8	80.8	80.8
3″	80.3	87.2	87.2	87.2
4″	66.1	70.3	70.3	70.3
5″	75.1	77.5	77.5	77.5
6″		62.9	62.9	62.9
1‴		103.9	103.9	103.9
2‴		75.0	75.0	75.0
3‴		87.1	87.1	87.1
4‴		69.3	69.3	69.3
5‴		78.1	78.2	78.2
6‴		62.2	62.2	62.3
1⁗		106.0	106.0	106.1
2⁗		75.2	75.3	75.3
3⁗		77.6	77.6	77.6
4⁗		70.8	70.8	70.8
5⁗		67.2	67.2	67.3
1⁗′		104.9	104.9	104.9
2⁗′		75.2	75.4	75.4
3⁗′		78.3	78.2	78.2
4⁗′		70.8	70.8	70.8
5⁗′		67.2	67.2	67.2

[a] Compound **1** was measured in C_5D_5N, and **2**−**4** in C_5D_5N−CD_3OD (2:1).

hydrogen atoms than that of **3**, the IR (1671 cm^{-1}), UV [λ_{max} 239.6 nm (log ϵ 3.96)], and ^{13}C NMR [δ 205.3 (C═O), 172.3 (C), and 120.1 (CH)] spectra. In the HMBC spectrum, cross-peaks between δ 205.3 and each H-17 at δ 2.53 (dd, J = 8.6, 7.2 Hz) and Me-18 at δ 0.98 (s) and between δ 172.3 and Me-19 at δ 0.92 (s) accounted for a 9-en-12-one structure. This was further supported by the comparison of the ^{13}C NMR spectrum of **4** with that of a steroidal saponin previously isolated by us from *Hosta longipes*.[13] The structure of **4** was revealed to be (25*R*)-3*β*-[{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl}oxy]-5α-spirost-9-en-12-one.

Compound **1** is a new bisdesmosidic cholestane glycoside and interesting structurally because it has been found to contain D-apiofuranose for the first time as a sugar component among the higher plant-originated cholestane glycosides reported up to present. Compounds **2**−**4** are new spirostanol saponins with up to five monosaccharides.

The cytotoxic activity of the isolated compounds on HL-60 leukemia cells was evaluated. Although the cholestane glycosides were not cytotoxic (IC_{50} >10 μg/mL), the new spirostanol saponins (**2**−**4**) showed moderate cytotoxic activity with respective IC_{50} values of 2.6, 3.2, and 6.1 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, and UV spectrum on a JASCO V-520 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^1H NMR) spectrometer 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 VG AutoSpec E mass spectrometer. Elemental analysis was carried out using an Elementar Vario EL elemental analyzer. Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan), Si gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS Si gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP_{18} F_{254} S plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), a UV-8000 detector (Tosoh), and Rheodyne injection port with a 20-μL sample loop. A Capcell Pak C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for HPLC analysis. The following materials and reagents were used for cell culture and assay of cytotoxic activity: microplate reader, Inter Med Immuno-Mini NJ-2300 (Tokyo, Japan); 96-well flat-bottom plate, Iwaki Glass (Chiba, Japan); HL-60 cells, ICN Biomedicals (Costa Mesa, CA); RPMI 1640 medium, GIBCO BRL (Rockville, MD); MTT Sigma (St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Plant Material. *P. tuberosa* was purchased from a nursery at Daiichi Seed (Tokyo, Japan). It was identified by one of the authors, Prof. Y. Sashida. A voucher of the plant is on file in our laboratory.

Extraction and Isolation. The plant material (fresh wt, 15.4 kg) was extracted with hot MeOH twice (each 18 L, 3 h). The MeOH extract was concentrated under reduced pressure and partitioned between *n*-BuOH and H_2O. The *n*-BuOH soluble phase was passed through a Diaion HP-20 column, eluting with 20% MeOH followed by 100% EtOH. Column chromatography of the EtOH eluate portion on Si gel and elution with $CHCl_3$−MeOH (4:1) gave three fractions (I−III). Fraction I was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (40:10:1) and ODS Si gel with MeOH−H_2O (8:5) to give a mixture of two cholestane glycosides. Separation of the mixture was carried out by ODS Si gel column chromatography eluting with MeCN−H_2O (5:12) to yield (22*S*)-1*β*-[(*β*-D-glucopyranosyl)oxy]-3*β*,22-dihydroxycholest-5-en-16*β*-yl α-L-rhamnopyranoside (79.5 mg) and **1** (109 mg). Fraction II was subjected to Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (40:10:1) and ODS Si gel with MeCN−H_2O (5:8) to furnish **3** (115 mg) and **4** (17.6 mg). Fraction III was separated by a Si gel column eluting with $CHCl_3$−MeOH−H_2O (30:10:1) and an ODS Si gel column with MeOH−H_2O (4:1; 2:1) to result in the isolation of **2** (166 mg).

Compound 1: amorphous solid; $[\alpha]^{27}_D$ −30.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3385 (OH), 2949 and 2883 (CH), 1466, 1447, 1408, 1382, 1365, 1264, 1226, 1199, 1158, 1077, 1016, 989, 955, 940 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.57 (1H, br d, J = 5.5 Hz, H-6), 5.48 and 4.52 (each 1H, ABq, J = 2.0 Hz, H-1″, H-2″),

4.99 (1H, d, J = 7.6 Hz, H-1′), 4.57 and 4.35 (each 1H, ABq, J = 9.3 Hz, H-5″β, H-5″α), 4.53 (1H, dd, J = 11.7, 3.0 Hz, H-6′a), 4.37 (1H, ddd, J = 7.7, 7.7, 4.4 Hz, H-16), 4.32 (1H, dd, J = 11.7, 5.8 Hz, H-6′b), 4.24 and 4.20 (each 1H, ABq, J = 11.1 Hz, H_2-4″), 4.22 (1H, dd, J = 9.2, 9.2 Hz, H-3′), 4.16 (1H, br dd, J = 7.7, 4.3 Hz, H-22), 4.12 (1H, dd, J = 9.2, 9.2 Hz, H-4′), 4.05 (1H, dd, J = 9.2, 7.6 Hz, H-2′), 3.96 (1H, dd, J = 11.5, 3.9 Hz, H-1), 3.93 (1H, ddd, J = 9.2, 5.8, 3.0 Hz, H-5′), 3.84 (1H, br m, $W_{1/2}$ = 21.8 Hz, H-3), 1.29 (3H, s, Me-19), 1.20 (3H, d, J = 6.9 Hz, Me-21), 1.05 (3H, s, Me-18), 0.90 (3H × 2, d, J = 6.4 Hz, Me-26, Me-27); ^{13}C NMR, see Table 2; FABMS (negative mode) m/z 727 [M − H]$^-$; *Anal.* C 59.90%, H 9.12%, calcd for $C_{38}H_{64}O_{13}$·2H_2O, C 59.67%, H 8.96%.

Acid Hydrolysis of 1. A solution of **1** (15.8 mg) in 0.2 M HCl (dioxane-H_2O, 1:1, 5 mL) was heated at 98 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA) eluting with MeOH−H_2O (1:1, 10 mL) followed by MeOH (10 mL) to give a sugar fraction and an aglycon fraction. The sugar fraction (1 mg) was dissolved in H_2O (1 mL), to which (−)-α-methylbenzylamine (5 mg) and Na-[BH_3CN] (8 mg) in EtOH (1 mL) were added. After being set aside at 40 °C for 4 h followed by addition of HOAc (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) and pyridine (0.3 mL) in the presence of 4-(dimethylamino)pyridine (5 mg) catalyst at 40 °C for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O−MeCN (4:1; 1:1, each 10 mL) mixtures as solvents. The H_2O−MeCN (1:1) eluate was further passed through a Toyopak IC−SP M cartridge (Tosoh, Tokyo, Japan) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[8,9] which was then analyzed by HPLC under the following conditions: solvent, MeCN−H_2O (2:3); flow rate, 0.8 mL/min; detection, UV 230 nm. The derivatives of D-apiose[14] and D-glucose were detected as follows: t_R (min) 13.89 (derivative of D-apiose), 17.60 (derivative of D-glucose). The aglycon fraction was purified by Si gel column chromatography using $CHCl_3$−MeOH (19:1) to give the aglycon (**1a**) (4.9 mg).

Compound 2: amorphous solid; $[\alpha]^{27}_D$ −42.0° (c 0.10, MeOH); IR (film) ν_{max} 3387 (OH), 2927 and 2872 (CH), 1454, 1418, 1375, 1260, 1242, 1209, 1158, 1073, 1039, 982, 920, 898, 866 cm^{-1}; ^{1}H NMR (C_5D_5N−CD_3OD, 2:1) δ 5.42 (1H, d, J = 7.6 Hz, H-1‴), 5.00 (1H, d, J = 7.7 Hz, H-1⁗), 4.96 (1H, d, J = 7.9 Hz, H-1″), 4.91 (1H, d, J = 7.5 Hz, H-1⁗), 4.76 (1H, d, J = 7.7 Hz, H-1′), 4.48 (1H, q-like, J = 7.4 Hz, H-16), 3.89 (1H, m, H-3), 3.53 (1H, br d, J = 10.8 Hz, H-26eq), 3.49 (1H, ddd, J = 11.9, 11.9, 4.0 Hz, H-6), 3.43 (1H, dd, J = 10.8, 10.8 Hz, H-26ax), 1.09 (3H, d, J = 6.9 Hz, Me-21), 0.80 (3H, s, Me-18), 0.74 (3H, s, Me-19), 0.73 (3H, d, J = 6.1 Hz, Me-27); ^{13}C NMR, see Table 2; FABMS (negative mode) m/z 1181 [M − H]$^-$; *anal.* C 53.79%, H 8.00%, calcd for $C_{55}H_{90}O_{27}$·5/2H_2O, C 53.78%, H 7.80%.

Acid Hydrolysis of 2. Compound **2** (10.4 mg) was subjected to acid hydrolysis with 1 M HCl in dioxane−H_2O (1:1, 5 mL) at 98 °C for 2 h under an Ar atmosphere, and a workup similar to that of **1** gave a sugar fraction and an aglycon fraction. The monosaccharides in the sugar fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of D-glucose, D-galactose, and D-xylose were detected: t_R (min) 13.54 (derivative of D-xylose), 14.63 (derivative of d-galactose), 17.08 (derivative of D-glucose). The aglycon fraction was purified by Si gel column chromatography using $CHCl_3$−MeOH (19:1) to give the aglycon (**2a**) (3.1 mg).

Compound 3: amorphous solid; $[\alpha]^{26}_D$ −20.0° (c 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2928 and 2873 (CH), 1707 (C=O), 1454, 1428, 1375, 1260, 1242, 1208, 1158, 1071, 1041, 982, 921, 899, 867 cm^{-1}; ^{1}H NMR (C_5D_5N−CD_3OD, 2:1) δ 5.43 (1H, d, J = 7.6 Hz, H-1‴), 5.00 (1H, d, J = 7.8 Hz, H-1⁗), 4.98 (1H, d, J = 7.9 Hz, H-1″), 4.91 (1H, d, J = 7.5 Hz, H-1⁗), 4.73 (1H, d, J = 7.7 Hz, H-1′), 4.43 (1H, overlapping, H-16), 3.81 (1H, m, H-3), 3.54 (1H, br d, J = 10.7 Hz, H-26eq), 3.43 (1H, dd, J = 10.7, 10.7 Hz, H-26ax), 2.65 (1H, dd, J = 8.6, 6.7 Hz, H-17), 2.41 (1H, dd, J = 13.8, 13.8 Hz, H-11ax), 2.20 (1H, dd, J = 13.8, 4.9 Hz, H-11eq), 1.25 (3H, d, J = 6.9 Hz, Me-21), 1.07 (3H, s, Me-18), 0.76 (3H, s, Me-19), 0.73 (3H, d, J = 6.1 Hz, Me-27); ^{13}C NMR, see Table 2; FABMS (negative mode) m/z 1179 [M − H]$^-$; *anal.* C 53.10%, H 8.07%, calcd for $C_{55}H_{88}O_{27}$·7/2H_2O, C 53.09%, H 7.70%.

Acid Hydrolysis of 3. Compound **3** (12.1 mg) was subjected to acid hydrolysis as described for **2** to give a sugar fraction and an aglycon fraction. The monosaccharides in the sugar fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of D-glucose, D-glucose, and d-glucose were detected. The aglycon fraction was purified by Si gel column chromatography using $CHCl_3$−MeOH (19:1) to give the aglycon (**3a**) (3.4 mg).

Compound 4: amorphous solid; $[\alpha]^{27}_D$ −35.5° (c 0.22, MeOH); IR (film) ν_{max} 3407 (OH), 2928 and 2873 (CH), 1671 (C=O), 1452, 1429, 1373, 1305, 1243, 1208, 1158, 1073, 1048, 982, 921, 899, 866 cm^{-1}; UV (MeOH) λ_{max} 239.6 nm (log ϵ 3.96); ^{1}H NMR (C_5D_5N−CD_3OD, 2:1) δ 5.73 (1H, d, J = 1.4 Hz, H-11), 5.44 (1H, d, J = 7.6 Hz, H-1‴), 5.01 (1H, d, J = 7.8 Hz, H-1⁗), 4.98 (1H, d, J = 7.9 Hz, H-1″), 4.92 (1H, d, J = 7.5 Hz, H-1⁗), 4.74 (1H, d, J = 7.7 Hz, H-1′), 4.48 (1H, q-like, J = 7.6 Hz, H-16), 3.81 (1H, m, H-3), 3.55 (1H, dd, J = 10.7, 1.6 Hz, H-26eq), 3.43 (1H, dd, J = 10.7, 10.7 Hz, H-26ax), 2.53 (1H, dd, J = 8.6, 7.2 Hz, H-17), 1.30 (3H, d, J = 6.9 Hz, Me-21), 0.98 (3H, s, Me-18), 0.92 (3H, s, Me-19), 0.74 (3H, d, J = 6.1 Hz, Me-27); ^{13}C NMR, see Table 2; FABMS (negative mode) m/z 1177 [M − H]$^-$; *anal.* C 54.35%, H 7.92%, calcd for $C_{55}H_{86}O_{27}$·2H_2O, C 54.36%, H 7.46%.

Cell Culture Assay. HL-60 cells were maintained in the RPMI 1640 medium containing 10% fetal bovine serum 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 3 × 10^4 cells/mL, and 196 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. 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 0.1−10 μg/mL, and 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 using a modified MTT reduction assay.[15] Briefly, after termination of the cell culture, 10 μL of 5 mg/mL MTT in phosphate buffered saline was added to every well, and the plate was further reincubated 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 MTT 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 at 550 nm. A dose−response curve was plotted for **2**, **3**, and **4**, which showed more than 50% of cell growth inhibition at the sample concentration of 10 μg/mL, and the concentration giving 50% inhibition (IC_{50}) was calculated. The IC_{50} value of etoposide used as a positive control was 0.3 μg/mL.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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