Cholestane Glycosides from the Bulbs of *Galtonia candicans* and Their Cytotoxicity

Minpei KURODA, Yoshihiro MIMAKI,* Akihito YOKOSUKA, and Yutaka SASHIDA

Laboratory of Medicinal Plant Science, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1, Horinouchi, Hachioji, Tokyo 192–0392, Japan. Received March 14, 2001; accepted April 19, 2001

Further search for cytotoxic compounds contained in the bulbs of *Galtonia candicans* (Liliaceae) led to the isolation of four potent cytotoxic cholestane glycosides (1—4) based upon 3β,16β,17α-trihydroxycholest-5-en-22-one, three of which (2—4) have not been reported previously. A new cholestane bisdesmoside (5) and a new rearranged cholestane glycoside (6) were also isolated. The structural assignment of the new constituents was carried out by spectroscopic analysis and a few chemical transformations.

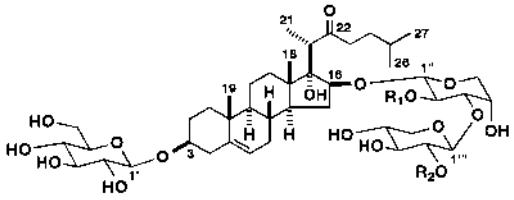
Key words *Galtonia candicans*; Liliaceae; cholestane glycoside; cytotoxic activity; HL-60 cell

An acylated cholestane diglycoside isolated by us from the bulbs of *Ornithogalum saundersiae* has garnered scientific attention because of its remarkable cytotoxicity against a variety of tumor cell culture lines and experimental animal tumors.[1] Several research groups including our own have actively studied total synthesis of the cytotoxic cholestane,[2] and the first total synthesis including its acylated diglycoside moiety was recently established by Deng and co-workers.[3] During our ongoing project focused on higher-plant antineoplastic constituents, we have found that an MeOH extract of the bulbs of *Galtonia candicans* (Bak.) Decne., which is indigenous to South Africa and taxonomically related to *O. saundersiae*, exhibited potent cell growth inhibitory activity against HL-60 human promyelocytic leukemia cells. Our previous examination in order to identify active constituents from *G. candicans* bulbs by way of a cytotoxicity-guided fractionation procedure, combined with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction assay procedure, led to the isolation of a novel polyoxygenated 5β-cholestane diglycoside, called galtonioside A.[4] It was not only cytotoxic against HL-60 cells (IC_{50} 0.057 μM) but also showed differential cytotoxicities in the Jpn. Fdn. for Cancer Res. 38 cell line assay.[5] However, the yield and cytotoxic potency of galtonioside A were not enough to explain the potent cytotoxicity of the crude extract, suggesting the presence of other, more potent principles. Our further search aimed at finding cytotoxic components from a *G. candicans* bulb extract resulted in the isolation of four cholestane glycosides (**1**—**4**) with potent cytotoxic activity against HL-60 cells. Although **1** has already been reported,[1] **2**—**4** are newly described compounds. In addition, a new cholestane bisdesmoside (**5**) and a new rearranged cholestane glycoside (**6**) were also isolated. This paper reports the structural assignment of the new cholestane glycosides, and cytotoxicity of the isolated compounds and some derivatives against HL-60 cells.

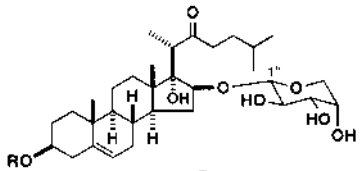
Results and Discussion

A concentrated MeOH extract of *G. candicans* bulbs showed cytotoxic activity against HL-60 cells with an IC_{50} value of 0.017 μg/ml. On fractionation of the MeOH extract into *n*-BuOH-soluble phase and H_2O phase, the cytocidal activity appeared only in the *n*-BuOH phase (IC_{50} 0.0056 μg/ml). The *n*-BuOH phase was subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, to give **1**—**6**.

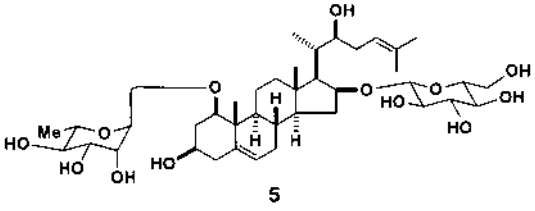
Compound **1** was identified as 16β-[(*O*-(2-*O*-(*E*)-cinna-



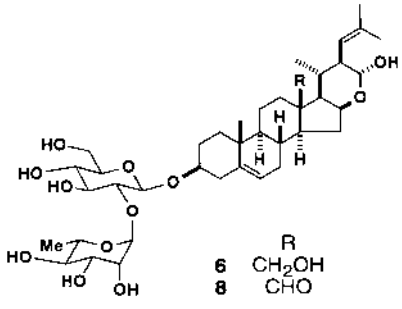
	R_1	R_2
1	Ac	(*E*)-cinnamoyl
2	Ac	3,4-dimethoxybenzoyl
2a	H	H
3	Ac	H



	R
4	β-D-glucopyranosyl
4a	H



5



	R
6	CH_2OH
8	CHO

* To whom correspondence should be addressed. e-mail: mimakiy@ps.toyaku.ac.jp

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**, **2**, **2a**, **3**, **4**, **4a**, **5**, and **6** in Pyridine-d_5

C	**1**	**2**	**2a**	**3**	**4**	**4a**	**5**	**6**
1	37.4	37.4	37.4	37.4	37.4	37.8	81.4	37.6
2	30.2	30.2	30.2	30.2	30.2	32.5	36.0	30.3
3	78.1	78.1	78.1	78.1	78.1	71.3	68.1	78.1
4	39.3	39.3	39.4	39.4	39.4	43.5	43.8	39.1
5	140.8	140.8	140.9	140.9	140.8	141.9	139.2	141.0
6	121.8	121.8	121.9	121.8	121.9	121.2	125.1	121.8
7	32.2	32.2	32.2	32.2	32.3	32.3	31.5	32.7
8	32.0	31.9	32.0	32.0	32.1	32.1	33.5	31.7
9	50.0	50.0	50.1	50.0	50.1	50.2	50.9	50.7
10	36.9	36.9	36.9	36.9	36.9	36.9	42.9	37.2
11	20.8	20.9	20.9	20.8	20.9	21.0	24.8	21.5
12	32.8	32.7	32.7	32.7	32.7	32.7	40.6	35.2
13	46.5	46.5	46.4	46.5	46.2	46.5	42.3	46.8
14	48.5	48.5	48.6	48.5	48.6	48.7	55.4	53.3
15	34.6	34.6	36.1	35.0	36.0	36.0	37.0	33.9
16	88.2	88.3	88.9	88.3	89.1	89.1	82.5	70.7
17	85.6	85.7	86.2	85.7	86.2	86.2	58.1	60.6
18	13.5	13.6	13.6	13.5	13.7	13.7	13.9	60.4
19	19.4	19.4	19.4	19.4	19.4	19.6	14.6	19.5
20	46.3	46.3	46.1	46.4	46.4	46.2	35.6	31.5
21	11.8	11.9	12.1	11.9	12.0	12.1	12.6	18.7
22	218.9	218.9	219.5	218.9	219.5	219.5	72.8	48.3
23	39.4	39.2	39.4	39.5	39.5	39.5	34.8	98.8
24	32.7	32.7	32.7	32.7	32.5	32.6	124.6	127.4
25	27.8	27.7	27.9	27.9	27.9	27.9	131.4	133.5
26	22.8	22.8	23.0	22.8	22.9	22.9	26.1	26.2
27	22.4	22.4	22.5	22.5	22.5	22.5	18.3	19.4
1′	102.5	102.5	102.6	102.5	102.6		97.8	100.5
2′	75.3	75.2	75.4	75.3	75.3		72.9	77.9
3′	78.6	78.6	78.6	78.6	78.6		73.0	79.7
4′	71.7	71.7	71.7	71.4	71.7		73.7	71.9
5′	78.5	78.5	78.5	78.5	78.5		70.7	78.3
6′	62.8	62.9	62.9	62.9	62.9		18.7	62.7
1″	101.0	100.8	105.5	101.4	105.7	105.7	106.8	102.1
2″	72.3	72.1	71.7	72.2	72.8	72.9	75.7	72.6
3″	80.7	81.0	83.8	80.0	74.4	74.4	78.2	72.9
4″	68.4	67.7	68.9	68.6	69.0	69.1	71.8	74.2
5″	66.3	65.4	67.1	66.5	66.6	66.6	78.8	69.5
6″	—	—	—	—	—	—	63.0	18.9
1‴	103.6	103.7	106.9	106.7				
2‴	74.9	75.4	75.1	74.2				
3‴	76.5	76.3	78.3	78.2				
4‴	70.8	70.7	71.0	70.9				
5‴	67.1	67.0	67.3	67.2				
1⁗	135.0	*a*)						
2⁗	128.5	133.5						
3⁗	129.3	*a*)						
4⁗	130.6	154.0						
5⁗	129.3	111.2						
6⁗	128.5	124.4						
7⁗	145.0	165.6						
8⁗	119.3	—						
9⁗	166.0	—						
OMe	—	55.9						
		55.8						
Ac	169.3	169.3		170.0				
	20.9	20.9		21.5				

a) Signals are unclear due to overlapping with solvent signals.

moyl-β-D-xylopyranosyl)-(1→2)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]-3β-[(β-D-glucopyranosyl)oxy]-17α-hydroxycholest-5-en-22-one, which has already been isolated from the bulbs of *O. saundersiae* and reported in the preliminary communication.[1)] The physical and spectral data are reported here in Table 1 and the experimental section.

Compound **2** was isolated as an amorphous solid with a molecular formula, $C_{54}H_{80}O_{21}$, as determined by data of the positive-ion high-resolution (HR)-FAB-MS exhibiting an accurate $[M+Na]^+$ ion at *m*/*z* 1087.5084 and ^{13}C-NMR spectrum. The spectral properties of **2** were essentially analogous with those of **1** and suggestive of a cholestane glycoside closely related to **1**. The presence of a 3,4-dimethoxybenzoyl group and an acetyl group in the molecule of **2** was readily

Table 2. Cytotoxic Activity of Compounds **1**, **2**, **2a**, **3**, **4**, **4a**, **5**, **6**, and Etoposide against HL-60 Cells[a)]

Compounds	IC_{50} (μM)
1	0.00012
2	0.00048
2a	0.77
3	0.0024
4	0.053
4a	0.014
5	>10
6	>10
Etoposide[b)]	0.025

a) The cells were continuously treated with each sample for 72 h, and the cell growth was evaluated using modified MTT reduction assay. Data are mean value of three experiments each done in triplicate. *b*) Clinically used anticancer agent.

identified by the ^{1}H- and ^{13}C-NMR spectra. Alkaline hydrolysis of **2** with 4% KOH in EtOH gave 3,4-dimethoxybenzoic acid and a deacylcholestane glycoside (**2a**), which was identical with the product produced by alkaline treatment of **1**. The respective linkage positions of the acetyl and 3,4-dimethoxybenzoyl groups at C-2 of the arabinosyl and at C-2 of the xylosyl in **2** were ascertained by long-range correlations from H-2 of arabinose at δ 5.55 (dd, J=8.1, 5.9 Hz) to the carbonyl carbon signal of the acetyl moiety at δ 169.3, and from H-2 of xylose at δ 5.71 (dd, J=9.0, 7.8 Hz) to the carbonyl resonance of the 3,4-dimethoxybenzoyl moiety at δ 165.6 in the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum of **2**. Thus, **2** was formulated as 3β-[(β-D-glucopyranosyl)oxy]-17α-hydroxy-16β-[(*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→2)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

Compound **3** was analyzed for $C_{45}H_{72}O_{18}$ by combined HR-FAB-MS (*m/z* 923.4623 $[M+Na]^+$) and ^{13}C-NMR analyses. Although the spectral features of **3** were similar to those of **1** and **2**, signals for an aromatic acid ester group could not be observed in **3**. Mild alkaline hydrolysis of **2** with 0.4% KOH in EtOH gave **3**, together with **2a** and 3,4-dimethoxybenzoic acid. The structure of **3** was shown to be 3β-[(β-D-glucopyranosyl)oxy]-17α-hydroxy-16β-[(*O*-β-D-xylopyranosyl-(1→2)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

Compound **4** was shown to have the molecular formula $C_{38}H_{62}O_{13}$ on the basis of HR-FAB-MS (*m/z* 749.4075 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectral data of **4** immediately allowed the identification of its aglycon as being identical with that of **1**—**3**. Acid hydrolysis of **4** with 1 M HCl in dioxane–H_2O (1 : 1) gave D-glucose and L-arabinose as the carbohydrate moieties. Signals due to a β-D-glucopyranosyl unit and an α-L-arabinopyranosyl unit were identified in the ^{1}H- and ^{13}C-NMR spectra of **4** with the help of two-dimensional (2D) NMR information. The respective linkage positions of the β-D-glucosyl and α-L-arabinosyl groups were revealed to be at C-3 and C-16 of the aglycon by the observation of HMBC correlations from the δ 5.05 (d, J=7.7 Hz, anomer of glucose) signal to the δ 78.1 resonance (C-3 of aglycone), and from δ 4.51 (d, J=6.6 Hz, anomer of arabinose) to δ 89.1 (C-16 of aglycone). The structure of **4** was assigned as 16β-[(α-L-arabinopyranosyl)oxy]-3β-[(β-D-glucopyranosyl)oxy]-17α-hydroxycholest-5-en-22-one. Compound **4** was treated with β-D-glucosidase to give the C-3 deglucosyl derivative (**4a**), which was evaluated for its cytotoxicity.

Compound **5**, obtained as an amorphous solid, was deduced as $C_{39}H_{64}O_{13}$ from HR-FAB-MS (*m/z* 763.4258 $[M+Na]^+$) and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **5** showed signals for five methyl groups characteristic of the cholestane skeleton, as well as signals for two anomeric protons at δ 5.63 (br s) and 4.75 (d, J=7.7 Hz). The ^{13}C-NMR spectrum displayed a pair of olefinic carbon signals at δ 131.4 (C) and 124.6 (CH) in addition to those assignable to C-5 [δ 139.2 (C)] and C-6 [δ 125.1 (CH)] of the cholest-5-ene derivative. Acid hydrolysis of **5** with 1 M HCl in dioxane–H_2O (1 : 1) gave D-glucose and L-rhamnose as the carbohydrate moieties. When the ^{1}H-NMR spectrum of **5** was compared with that of (22*S*)-3β,22-dihydroxy-1β-[(α-L-rhamnopyranosyl)oxy]cholest-5-en-16β-yl β-D-glucopyranoside (**7**),[6] a partial hydrolysate of the cholestane bisdesmoside isolated by us from *Allium albopilosum* bulbs, two three-proton doublet signals at δ 0.94 (J=6.2 Hz) and 0.93 (J=6.3 Hz) assignable to Me-26 and Me-27 in the reference compound (**7**) were displaced by two three-proton singlet signals at δ 1.75 and 1.68 due to methyl groups on a double bond in **5**. All other signals appeared at almost the same positions between the two compounds. Hydrogenation of **5** over PtO_2 under an H_2 atmosphere gave **7**. Accordingly, the structure of **5** was defined as (22*S*)-3β,22-dihydroxy-1β-[(α-L-rhamnopyranosyl)oxy]cholest-5,24-dien-16β-yl β-D-glucopyranoside.

Compound **6** was isolated as an amorphous solid. Its molecular formula was determined to be $C_{39}H_{62}O_{13}$ by FAB-MS (*m/z* 737 $[M-H]^-$), ^{13}C-NMR data, and elemental analysis. The ^{1}H-NMR spectrum showed signals for a tertiary methyl group at δ 1.08 (s), a secondary methyl group at δ 1.32 (J=6.3 Hz), two methyl groups on a double bond at δ 1.90 and 1.77 (each s), two olefinic protons at δ 5.39 (br d, J=4.9 Hz) and 5.11 (br d, J=9.8 Hz), and an acetalic proton at δ 5.34 (d, J=6.8 Hz). Furthermore, two anomeric proton signals of monosaccharides were observed at δ 6.43 (br s) and 5.09 (d, J=7.4 Hz). These ^{1}H-NMR features, combined with ^{13}C-NMR data were indicative of **5** being a 24(23→22)*abeo*-cholestane diglycoside.[7] When the ^{1}H- and ^{13}C-NMR spectra of **6** were compared with those of (22*S*,23*R*)-16β,23-epoxy-23-hydroxy-22-(2-methyl-1-propenyl)-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-24-norchol-5-en-18-al (**8**),[8] isolated by us from *O. saundersiae* bulbs, significant differences could be observed only in the signals due to H-18/C-18; the signals for the H-18 aldehyde proton at δ 10.14 (s) and C-18 carbon at δ 207.3 (CH) of the reference compound (**8**) were displaced by the hydroxymethyl proton signals at δ 4.33 and 4.13 (ABq, J=10.4 Hz) and hydroxymethyl carbon signal at δ 60.4 (CH_2). Treatment of **8** with $NaBH_4$ in MeOH gave **6**. The structure of **6** was determined to be (22*S*,23*R*)-16β,23-epoxy-18,23-dihydroxy-22-(2-methyl-1-propenyl)-24-norchol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The isolated compounds and some derivatives 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).[9] Compounds **1**—**4** exhibited very strong cytotoxic activity and were concluded to contribute to the potent cytotoxicity of crude *G. candicans* bulbs extract. The de-

rivative (**4a**), the most simple cholestane glycoside based upon 3β,16β,17α-trihydroxycholest-5-en-22-one, was also found to show considerable cytotoxicity.

Experimental

NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. HPLC was performed using a system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh RI-8010 detector, a Tosoh UV-800 detector, and Rheodyne injection port. Capcell Pak C_{18} columns (4.6 mm i.d.×250 mm and 1.0 mm i.d.×250 mm, 5 μm, Shiseido, Tokyo, Japan) were employed for HPLC. 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, U.S.A.); RPMI 1640 medium (GIBCO BRL, Rockville, MD, U.S.A.); and MTT (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant material The bulbs of *G. candicans* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowered plant was identified by one of the authors, Prof. Yutaka Sashida. A voucher of the plant is on file in our laboratory (97-7-GC-F).

Extraction and Isolation The plant material (fresh weight, 5.5 kg) was extracted with hot MeOH twice. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between *n*-BuOH and H_2O. Column chromatography of the *n*-BuOH phase on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (9 : 1; 4 : 1; 2 : 1), and finally with MeOH alone, gave four fractions (I—IV). Fraction II was subjected to column chromatography on silica gel using $CHCl_3$–MeOH gradients (11 : 1; 9 : 1; 7 : 1) and MeOH, and was further divided into three fractions (IIa—IIc). Fraction IIa was chromatographed on silica gel eluting with $CHCl_3$–MeOH (9 : 1) and ODS silica gel with MeOH–H_2O (3 : 1; 7 : 3) to give galtonioside A (54.3 mg),[4] **1** (44.9 mg) and **4** (106 mg), and **2** with a few impurities. Compound **2** was purified by preparative HPLC using MeCN–H_2O (9 : 11) to afford **2** (16.8 mg) as a pure compound. Fraction IIb was subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH–H_2O (40 : 10 : 1; 30 : 10 : 1) and ODS silica gel with MeOH–H_2O (3 : 1) and, finally, to preparative HPLC employing MeOH–H_2O–tetrahydrofuran (THF) (7 : 3 : 1) and MeCN–H_2O (1 : 1) to yield **3** (31.7 mg) and **5** (17.0 mg). Compound **6** (12.7 mg) was isolated from fraction IIc by subjecting it to silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (50 : 20 : 1), ODS silica gel column chromatography with MeOH–H_2O (7 : 3), and to preparative HPLC using MeCN–H_2O (2 : 3).

Compound **1**: Amorphous solid, $[\alpha]_D^{25}$ −16.0° (MeOH, *c*=0.10). FAB-MS (negative mode) *m/z*: 1029 $[M-H]^-$. *Anal.* Calcd for $C_{54}H_{78}O_{19}\cdot 3/2H_2O$: C, 61.32; H, 7.89. Found: C, 61.29; H, 7.72. IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 2970, 2935 and 2870 (CH), 1720 (C=O), 1695 (C=O), 1635, 1445, 1365, 1255, 1160, 1065, 1030, 905. ^{1}H-NMR (pyridine-d_5) δ: 7.99 (1H, d, *J*=16.0 Hz, H-7‴), 7.64 (2H, dd, *J*=7.8, 2.2 Hz, H-2‴, H-6‴), 7.39 (3H, overlapping, H-3‴, H-4‴, H-5‴), 6.87 (1H, d, *J*=16.0 Hz, H-8‴), 5.67 (1H, dd, *J*=8.9, 6.6 Hz, H-2″), 5.59 (1H, dd, *J*=9.1, 7.7 Hz, H-2‴), 5.29 (1H, br s, H-6), 5.08 (1H, d, *J*=7.7 Hz, H-1‴), 5.04 (1H, d, *J*=7.7 Hz, H-1′), 4.61 (1H, d, *J*=6.6 Hz, H-1″), 3.92 (1H, br m, $W_{1/2}$=22.1 Hz, H-3), 3.25 (1H, q, *J*=7.4 Hz, H-20), 2.29 (3H, s, Ac), 1.29 (3H, d, *J*=7.4 Hz, Me-21), 0.95 (3H, s, Me-19), 0.93 (3H, s, Me-18), 0.86 (3H, d, *J*=6.3 Hz, Me-26 or Me-27), 0.83 (3H, d, *J*=6.3 Hz, Me-26 or Me-27). ^{13}C-NMR: Table 1.

Compound **2**: Amorphous solid, $[\alpha]_D^{25}$ −50.0° (MeOH, *c*=0.10). HR-FAB-MS (positive mode) *m/z*: 1087.5084 $[M+Na]^+$ ($C_{54}H_{80}O_{21}\cdot$Na, Calcd for 1087.5090). IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2935 and 2875 (CH), 1720 (C=O), 1705 (C=O), 1690 (C=O), 1595, 1510, 1460, 1405, 1365, 1265, 1220, 1170, 1130, 1015, 750. ^{1}H-NMR (pyridine-d_5) δ: 8.06 (1H, dd, *J*=8.4, 1.9 Hz, H-6‴), 7.93 (1H, d, *J*=1.9 Hz, H-2‴), 7.07 (1H, d, *J*=8.4 Hz, H-5‴), 5.71 (1H, dd, *J*=9.0, 7.8 Hz, H-2‴), 5.55 (1H, dd, *J*=8.1, 5.9 Hz, H-2″), 5.29 (1H, br s, H-6), 5.15 (1H, d, *J*=7.6 Hz, H-1‴), 5.05 (1H, d, *J*=7.7 Hz, H-1′), 4.60 (1H, d, *J*=5.9 Hz, H-1″), 4.16 (1H, dd, *J*=7.8, 5.2 Hz, H-16), 3.93 (1H, br m, $W_{1/2}$=21.5 Hz, H-3), 3.82, 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), 1.00 (3H, s, Me-19), 0.95 (3H, s, Me-18), 0.88 (3H, d, *J*=6.1 Hz, Me-26 or Me-27), 0.85 (3H, d, *J*=6.1 Hz, Me-26 or Me-27). ^{13}C-NMR: Table 1.

Alkaline Hydrolysis of 2 Compound **2** (5.0 mg) was treated with 4% KOH in EtOH (2 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 using $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to give **2a** (3.7 mg) and 3,4-dimethoxybenzoic acid (0.7 mg). Compound **2a** was identical to the product produced on alkaline hydroysis of **1** by the same method as for **2**.

Compound **2a**: Amorphous solid, $[\alpha]_D^{25}$ −36.0° (MeOH, *c*=0.10). FAB-MS (positive mode) *m/z*: 881 $[M+Na]^+$. FAB-MS (negative mode) *m/z*: 857 $[M-H]^-$. IR ν_{max} (film) cm^{-1}: 3387 (OH), 2928 and 2870 (CH), 1685 (C=O), 1457, 1405, 1383, 1341, 1260, 1161, 1076, 1044. ^{1}H-NMR (pyridine-d_5) δ: 5.29 (1H, br d, *J*=4.3 Hz, H-6), 5.18 (1H, d, *J*=7.6 Hz, H-1‴), 5.05 (1H, d, *J*=7.7 Hz, H-1′), 4.49 (1H, d, *J*=6.5 Hz, H-1″), 3.93 (1H, br m, $W_{1/2}$=20.9 Hz, H-3), 3.40 (1H, q, *J*=7.4 Hz, H-20), 1.31 (3H, d, *J*=7.4 Hz, Me-21), 0.95 (3H, s, Me-19), 0.92 (3H, d, *J*=6.4 Hz, Me-26 or Me-27), 0.92 (3H, s, Me-18), 0.87 (3H, d, *J*=6.4 Hz, Me-26 or Me-27). ^{13}C-NMR: Table 1.

Compound **3**: Amorphous solid, $[\alpha]_D^{25}$ −50.0° (MeOH, *c*=0.10). HR-FAB-MS (positive mode) *m/z*: 923.4623 $[M+Na]^+$ ($C_{45}H_{72}O_{18}\cdot$Na, Calcd for 923.4616). IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2920 and 2870 (CH), 1730 (C=O), 1680 (C=O), 1450, 1360, 1330, 1230, 1150, 1070, 1030, 880, 775. ^{1}H-NMR (pyridine-d_5) δ: 5.82 (1H, dd, *J*=8.7, 6.5 Hz, H-2″), 5.31 (1H, br s, H-6), 5.05 (1H, d, *J*=7.7 Hz, H-1′), 4.91 (1H, d, *J*=7.6 Hz, H-1‴), 4.66 (1H, d, *J*=6.5 Hz, H-1″), 3.93 (1H, br m, $W_{1/2}$=20.3 Hz, H-3), 3.33 (1H, q, *J*=7.3 Hz, H-20), 2.35 (3H, s, Ac), 1.31 (3H, d, *J*=7.3 Hz, Me-21), 0.96 (3H×2, s, Me-18, Me-19), 0.95 (3H, d, *J*=6.0 Hz, Me-26 or Me-27), 0.92 (3H, d, *J*=6.0 Hz, Me-26 or Me-27). ^{13}C-NMR: Table 1.

Preparation of 3 from 2 Compound **2** (8.0 mg) was hydrolyzed with 0.4% KOH in EtOH at room temperature for 30 min. After neutralization of the reaction mixture by passage through an Amberlite IR120 column, it was subjected to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to result in the production of **3** (1.3 mg), together with **2a** (4.1 mg) and cinnamic acid (0.9 mg).

Compound **4**: Amorphous solid, $[\alpha]_D^{25}$ −40.0° (MeOH, *c*=0.10). HR-FAB-MS (positive mode) *m/z*: 749.4075 $[M+Na]^+$ ($C_{38}H_{62}O_{13}\cdot$Na, Calcd for 749.4088). IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2930 and 2880 (CH), 1680 (C=O), 1450, 1375, 1340, 1250, 1150, 1130, 1070, 1030, 965, 775. ^{1}H-NMR (pyridine-d_5) δ: 5.30 (1H, br d, *J*=4.5 Hz, H-6), 5.05 (1H, d, *J*=7.7 Hz, H-1′), 4.51 (1H, d, *J*=6.6 Hz, H-1″), 4.24 (1H, dd, *J*=7.8, 5.1 Hz, H-16), 3.93 (1H, br m, $W_{1/2}$=22.4 Hz, H-3), 3.39 (1H, q, *J*=7.4 Hz, H-20), 1.31 (3H, d, *J*=7.4 Hz, Me-21), 0.96 (3H, s, Me-19), 0.92 (3H, s, Me-18), 0.87 (3H×2, d, *J*=6.4 Hz, Me-26, Me-27). ^{13}C-NMR: Table 1.

Acid Hydrolysis of 4 A solution of **4** (10.0 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column and fractionated by a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) successively eluting with H_2O, H_2O–MeOH (4 : 1), and MeOH to give a sugar fraction (3.5 mg) and an aglycon fraction. TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction 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 AcOH (0.2 ml) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4 : 1; 1 : 1, each 5 ml) mixtures as eluting solvent. The H_2O–MeCN (1 : 1) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of a monosaccharide,[10] 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-glucose and L-arabinose were detected; t_R (min): 13.44 (L-arabinose derivative); 17.81 (D-glucose derivative).

Enzymatic Hydrolysis of 4 Compound **4** (37.9 mg) was dissolved in an AcOH/AcONa buffer (pH 5, 5.0 ml) with β-D-glucosidase (Sigma, EC 3. 2. 1. 21) (50.0 mg) and incubated at room temperature for 48 h. The crude mixture was chromatographed on silica gel eluting with $CHCl_3$–MeOH (9 : 1) to yield **4a** (5.4 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample. R_f 0.43 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Compound **4a**: Amorphous solid, $[\alpha]_D^{27}$ −24.0° (MeOH, *c*=0.10). FAB-MS (positive mode) *m/z*: 587 $[M+Na]^+$. FAB-MS (negative mode) *m/z*: 563 $[M-H]^-$. IR ν_{max} (film) cm^{-1}: 3395 (OH), 2932 and 2871 (CH), 1691 (C=O), 1460, 1410, 1381, 1325, 1250, 1141, 1051, 1025. ^{1}H-NMR (pyridine-d_5) δ: 5.39 (1H, br d, *J*=4.0 Hz, H-6), 4.51 (1H, d, *J*=6.6 Hz, H-1″), 4.24 (1H, dd, *J*=7.6, 5.1 Hz, H-16), 3.84 (1H, br m, $W_{1/2}$=22.5 Hz, H-3), 3.41 (1H, q, *J*=7.4 Hz, H-20), 1.33 (3H, d, *J*=7.4 Hz, Me-21), 1.09 (3H, s, Me-19), 0.95 (3H, s, Me-18), 0.88 (3H, d, *J*=6.3 Hz, Me-26 or Me-27), 0.87 (3H, d, *J*=6.3 Hz, Me-26 or Me-27). ^{13}C-NMR: Table 1.

Compound **5**: Amorphous solid, $[\alpha]_D^{25}$ −40.0° (MeOH, c=0.10). HR-FAB-MS (positive mode) m/z: 763.4258 $[M+Na]^+$ ($C_{39}H_{64}O_{13}$·Na, Calcd for 763.4245). IR ν_{max} (KBr) cm^{-1}: 3395 (OH), 2920 and 2860 (CH), 1435, 1375, 1340, 1250, 1120, 1065, 1020, 970. ^{1}H-NMR (pyridine-d_5) δ: 5.87 (1H, t-like, J=6.8 Hz, H-24), 5.63 (1H, br s, H-1″), 5.54 (1H, br d, J=4.9 Hz, H-6), 4.75 (1H, d, J=7.7 Hz, H-1′), 1.75 (3H, s, Me-27), 1.68 (3H, s, Me-26), 1.66 (3H, d, J=5.9 Hz, Me-6″), 1.21 (3H, s, Me-19), 1.19 (3H, d, J=6.9 Hz, Me-21), 1.04 (3H, s, Me-18). ^{13}C-NMR: Table 1.

Acid Hydrolysis of 5 Compound **5** (5.0 mg) was subjected to acid hydrolysis as described for **4** to give a sugar fraction (1.8 mg). The monosaccharide constituent in the fraction was converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which was then analyzed by HPLC. The derivatives of D-glucose and L-rhamnose were detected; t_R (min): 18.50 (D-glucose derivative); 20.51 (L-rhamnose derivative).

Catalytic Hydrogenation of 5 A mixture of **5** (5.0 mg) and PtO_2 (5.0 mg) was stirred under an H_2 atmosphere at room temperature for 12 h. The reaction mixture, after removal of the catalyst by filtration, was subjected to a silica gel column eluting with $CHCl_3$–MeOH (4 : 1) to give **7**.[6]

Compound **6**: Amorphous solid, $[\alpha]_D^{25}$ −18.0° (MeOH, c=0.10). FAB-MS (negative mode) m/z: 737 $[M-H]^-$. *Anal.* Calcd for $C_{39}H_{62}O_{13}$·$2H_2O$: C, 60.45; H, 8.58. Found: C, 60.56; H, 8.65. IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 2950, 2920 and 2870 (CH), 1690 (C=O), 1420, 1365, 1305, 1265, 1120, 1020, 890, 830, 805, 795, 765. ^{1}H-NMR (pyridine-d_5) δ: 6.43 (1H, br s, H-1″), 5.39 (1H, br d, J=4.9 Hz, H-6), 5.34 (1H, d, J=6.8 Hz, H-23), 5.11 (1H, br d, J=9.8 Hz, H-24), 5.09 (1H, d, J=7.4 Hz, H-1′), 4.91 (1H, q-like, J=7.8 Hz, H-16), 4.33 (1H, d, J=10.4 Hz, H-18a), 4.13 (1H, d, J=10.4 Hz, H-18b), 4.00 (1H, br m, $W_{1/2}$=21.2 Hz, H-3), 1.90 (3H, s, Me-27), 1.81 (3H, d, J=6.2 Hz, Me-6″), 1.77 (3H, s, Me-26), 1.32 (3H, d, J=6.3 Hz, Me-21), 1.08 (3H, s, Me-19). ^{13}C-NMR: Table 1.

Preparation 6 from 8[8] A mixture of **8** (10.0 mg) dissolved in MeOH (4 ml) with $NaBH_4$ (4.0 mg) was allowed to stand at room temperature for 30 min. The reaction mixture was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to afford **6** (8.2 mg).

Cell Culture Assay HL-60 cells were maintained in 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 ml 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 ml 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 to 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.[9] The assay was carried out according to a modification of the method of Sargent and Taylor as follows. After termination of the cell culture, 10 μl of 5 mg/ml MTT in phosphate buffered saline was added to each well and the plate was 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 formazan. An aliquot of 150 μl of the supernatant was removed from each well, and 175 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min, and then read on a microplate reader at 550 nm. The concentration giving 50% inhibition (IC_{50}) was calculated from a dose response curve.

Acknowledgments We are grateful to Dr. Y. Shida and Mr. H. Fukaya, Tokyo University of Pharmacy and Life Science, for the measurements of the mass spectra and elemental analysis.

References

1) Mimaki Y., Kuroda M., Kameyama A., Sashida Y., Hirano T., Oka K., Maekawa R., Wada T., Sugita K., Beutler J. A., *Bioorg. Med. Chem. Lett.*, **7**, 633—636 (1997).
2) Guo C., Fuchs P. L., *Tetrahedron Lett.*, **39**, 1099—1102 (1998); Morzycki J. W., Gryszkiewicz A., Jastrzebska I., *ibid.*, **41**, 3751—3754 (2000).
3) Deng S., Yu B., Lou Y., Hui Y., *J. Org. Chem.*, **64**, 202—208 (1999).
4) Kuroda M., Mimaki Y., Sashida Y., Yamori T., Tsuruo T., *Tetrahedron Lett.*, **41**, 251—255 (2000).
5) Yamori T., Matsunaga A., Sato S., Yamazaki K., Komi A., Ishizu K., Mita I., Edatsugi H., Matsuba Y., Takezawa K., Nakanishi O., Kohno H., Nakajima Y., Komatsu H., Andoh T., Tsuruo T., *Cancer Res.*, **59**, 4042—4049 (1999).
6) Mimaki Y., Kawashima K., Kanmoto T., Sashida Y., *Phytochemistry*, **34**, 799—805 (1993).
7) Mimaki Y., Kuroda M., Sashida Y., Hirano T., Oka K., Dobashi A., Koshino H., Uzawa J., *Tetrahedron Lett.*, **37**, 1245—1248 (1996).
8) Kuroda M., Mimaki Y., Sashida Y., *Phytochemistry*, **52**, 435—443 (1999).
9) Sargent J. M., Taylor C. G., *Br. J. Cancer*, **60**, 206—210 (1989).
10) Oshima R., Yamauchi Y., Kumanotani J., *Carbohydr. Res.*, **107**, 169—176 (1982).