Cytotoxic Cholestane Glycosides from the Bulbs of *Ornithogalum saundersiae*

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Further phytochemical analysis of the bulbs of *Ornithogalum saundersiae* has yielded two new cytotoxic cholestane triglycosides (**1** and **2**). The structures of these compounds were determined by spectroscopic analysis, including 2D NMR spectroscopic data, and the results of hydrolytic cleavage. Compounds **1** and **2** and several analogues were evaluated for their cytotoxicity against HL-60 cells.

An acylated cholestane glycoside (**3**), isolated previously from the bulbs of *Ornithogalum saundersiae* L. (Liliaceae) in good yield, has been found to show potent cytotoxicity against a variety of tumor cell culture lines and experimental animal tumors.[1,2] Recently, we have isolated **3** and several related compounds and evaluated their cytotoxic activity against HL-60 human promyelocytic leukemia cells.[2] Further phytochemical work has been carried out on *O. saundersiae* bulbs, with particular inference to the cholestane glycoside constituents, and has resulted in the isolation of two new compounds (**1** and **2**). In this paper, we report the structure determination and cytotoxicity of **1** and **2**.

The concentrated MeOH extract of the bulbs of *O. saundersiae* (16.2 kg, fresh wt) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was passed through a porous-polymer resin (Diaion HP-20), and the MeOH eluate fraction was subjected to column chromatography on Si gel and octadecylsilanized (ODS) Si gel, as well as preparative HPLC to give compounds **1** (11.2 mg) and **2** (18.5 mg).

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ −56.0° (MeOH). The HRFABMS (positive mode) showed an accurate $[M + Na]^+$ ion peak at *m*/*z* 1087.5108 in accordance to the empirical molecular formula $C_{54}H_{80}O_{21}$ (Δ +1.8 mmu), which was supported by the ^{13}C NMR spectrum combined with DEPT data. The ^1H NMR spectrum of **1** (C_5D_5N) was very similar to that of **4**,[1] showing signals for five typical steroid methyl groups at δ 1.32 (3H, d, *J* = 7.4 Hz), 1.08 (3H, s), 1.01 (3H, s), 0.89 (3H, d, *J* = 6.1 Hz), and 0.86 (3H, d, *J* = 6.1 Hz); a trisubstituted olefinic group at δ 5.39 (1H, br d, *J* = 4.2 Hz); a 3,4-dimethoxybenzoyl group at δ 8.05 (1H, dd, *J* = 8.5, 1.7 Hz), 7.91 (1H, d, *J* = 1.7 Hz), 7.04 (1H, d, *J* = 8.5 Hz), and 3.81 and 3.79 (each 3H, s); and an acetyl group at δ 1.99 (3H, s). Furthermore, three anomeric proton signals were recognized at δ 5.13 (1H, d, *J* = 7.1 Hz), 5.04 (1H, d, *J* = 7.8 Hz), and 4.58 (1H, d, *J* = 6.0 Hz). Acid hydrolysis of **1** with 1 M HCl in dioxane−H_2O (1:1) gave L-arabinose, D-xylose, and D-glucose, and alkaline hydrolysis with 4% KOH in EtOH resulted in the production of 3,4-dimethoxybenzoic acid and a deacyl cholestane glycoside (**1a**). The ^{13}C NMR spectrum of **1** also showed a close similarity to that of **4**. However, a set of additional signals, corresponding to a terminal β-D-glucopyranosyl group, appeared at δ 103.7 (CH), 74.5 (CH), 78.4 (CH), 71.6 (CH), 78.9 (CH), and 62.5 (CH_2) in the ^{13}C NMR spectrum of **1**. The glucosyl group was involved in a glycosidic linkage at C-4 of the xylosyl group, because the signal due to C-4 of the xylosyl residue was markedly displaced downfield at δ 77.7 (+7.0 ppm), while the signals due to C-3 and C-5 were shifted upfield at δ 73.6 (−1.7 ppm) and 64.1 (−2.9 ppm), respectively, when comparing the ^{13}C NMR spectrum of **1** with that of **4**. This was confirmed by the observation of a long-range correlation from the anomeric proton signal of the glucosyl group at δ 5.04 to C-4 of the xylosyl moiety in the HMBC spectrum. The xylosyl-(1→3)-arabinosyl structure and its linkage to C-16 of the aglycon, and 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 were ascertained by additional HMBC correlations as shown in Figure 1. All of these data were consistent with the structure 3β,17α-dihydroxy-16β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one, which was assigned to **1**.

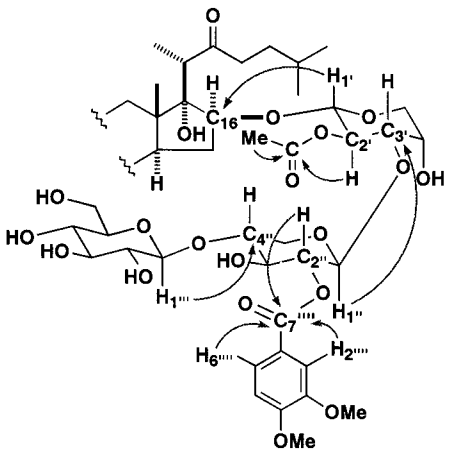

Figure 1. HMBC correlations of the acylated triglycoside moiety of **1**.

Compound **2** was isolated as an amorphous solid with a molecular formula $C_{55}H_{82}O_{22}$, as determined by the data of the HRFABMS (positive mode), which showed an $[M + Na]^+$ peak at *m*/*z* 1117.5170 (Δ −2.6 mmu), in conjunction with the ^{13}C NMR and DEPT spectra. The ^1H and ^{13}C NMR spectra of **2** were almost superimposable on those of **1**, except for the aromatic region signals due to the substi-

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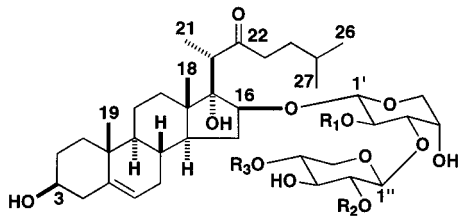
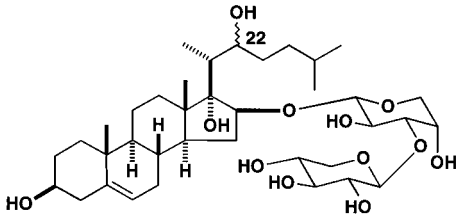


	R_1	R_2	R_3
1	Ac	3,4-dimethoxybenzoyl	β-D-Glc*p*
1a	H	H	β-D-Glc*p*
2	Ac	3,4,5-trimethoxybenzoyl	β-D-Glc*p*
3	Ac	*p*-methoxybenzoyl	H
3a	H	H	H
4	Ac	3,4-dimethoxybenzoyl	H



3b

tuted benzoyl moiety. The aromatic acid was assigned as 3,4,5-trimethoxybenzoic acid from the UV [λ_{max} 260 nm (log ϵ 4.02)], ^{1}H NMR [δ 7.69 (2H, s)], and ^{13}C NMR [δ 126.1 (C), 108.2 (CH) × 2, 153.6 (CH) × 2, 143.3 (C), 165.4 (C=O), 60.7 (Me), and 56.2 (Me) × 2] spectra. Alkaline hydrolysis of **2** furnished 3,4,5-trimethoxybenzoic acid and **1a**. An HMBC correlation from the resonance at δ 5.68 (dd, J = 8.9, 7.1 Hz, H-2 of xylose) to the carbonyl carbon signal at δ 165.4 gave evidence for the ester linkage position of the 3,4,5-trimethoxybenzoyl moiety at C-2 of the xylosyl residue. Thus, the structure of **2** was established as 3*β*,-17α-dihydroxy-16*β*-[(*O*-*β*-D-glucopyranosyl-(1→4)-*O*-(2-*O*-3,4,5-trimethoxybenzoyl-*β*-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl)oxy]cholest-5-en-22-one.

The IC_{50} values of **1**−**3**, when evaluated against HL-60 cells, are shown in Table 2. The ester groups attached to the glycoside moiety were found to be essential for the exhibition of potent cytotoxic activity because the deacyl derivatives (**1a** and **3a**) were far less cytotoxic compared with the original compounds (**1** and **3**). Slight differences in the aromatic acid structures and glucosylation of the C-3 hydroxyl group of the aglycon resulted in no discernible effects on activity. However, the cytotoxicities of the new compounds (**1** and **2**), having a glucosyl unit at C-4 of the terminal xylosyl moiety, were less potent than that of **3** by about 2 orders of magnitude. Recently, Fuchs and co-workers have provided some insight regarding the common and important role of C-22 oxocarbenium ions in the bioactivity of both **3** and cephalostatin[3] as a result of the synthesis of the aglycon of **3** and several cephalostatin derivatives followed by evaluation for their cytotoxic activity.[4,5] In fact, the activity of **3a** was about 40-fold more potent than that of the corresponding C-22 hydroxyl derivative (**3b**) prepared by treatment of **3a** with $NaBH_4$ in MeOH. Although the role of the acylated diglycoside moiety remains to be determined, a structural requirement for the significant cytotoxic activity of **3** may be concluded to be a combination of the acylated diglycoside moiety and the C-22 carbonyl group. This suggests that the mechanism of action of **3** may be somewhat different from that of cephalostatin, despite their similar cytotoxicity profiles in the National Cancer Institute 60 cell line assay (see Monks *et al.*[6] and Supporting Information).

Table 1. ^{13}C NMR Spectral Data for Compounds **1**, **1a**, **2**, and **3b**[a]

carbon	**1**	**1a**	**2**	**3b**
1	37.8	37.8	37.8	37.8
2	32.6	32.5	32.6	32.6
3	71.3	71.3	71.3	71.3
4	43.5	43.5	43.5	43.5
5	141.9	141.9	141.9	141.9
6	121.1	121.2	121.2	121.2
7	32.3	32.3	32.2	32.4
8	32.1	32.1	32.1	32.3
9	50.2	50.2	50.2	50.4
10	36.9	36.9	36.9	36.9
11	20.9	21.0	20.9	21.1
12	32.7	32.7	32.7	32.9
13	46.6	46.5	46.6	47.3
14	48.6	48.7	48.5	49.1
15	34.6	36.2	34.5	36.0
16	88.4	88.9	88.4	90.1
17	85.7	86.2	85.7	88.0
18	13.6	13.7	13.6	13.6
19	19.6	19.6	19.6	19.6
20	46.3	46.1	46.3	43.0
21	11.9	12.2	11.9	11.2
22	218.9	219.6	218.8	74.5
23	39.3	39.4	39.3	37.2
24	32.7	32.6	32.7	32.4
25	27.7	27.9	27.7	28.6
26	22.8	23.0	22.8	23.2
27	22.4	22.6	22.4	22.8
1′	100.8	105.5	100.8	106.9
2′	71.8	71.7	71.9	72.2
3′	80.4	84.0	80.4	84.0
4′	67.8	68.9	68.0	69.4
5′	65.6	67.2	65.7	67.2
1″	102.9	106.7	102.8	107.0
2″	74.6	74.8	74.9	75.4
3″	73.6	76.3	73.6	78.2
4″	77.7	78.1	77.7	71.0
5″	64.1	64.8	64.2	67.5
1‴	103.7	103.8	103.7	
2‴	74.5	74.4	74.6	
3‴	78.4	78.2	78.3	
4‴	71.6	71.7	71.6	
5‴	78.9	78.9	78.9	
6‴	62.5	62.7	62.5	
1⁗	*b*		126.1	
2⁗	113.6		108.2	
3⁗	*b*		153.6	
4⁗	154.1		143.3	
5⁗	111.2		153.6	
6⁗	124.5		108.2	
7⁗	165.5		165.4	
OMe	55.9 × 2		60.7	
			56.2 × 2	
Ac	169.3	169.3		
	20.9	20.9		

[a] Spectra were measured in C_5D_5N. [b] Signals were overlapped with the residual solvent signals and could not be assigned unambiguously.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol−dithioerythritol (3:1) matrix. 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. HPLC was performed using a system composed of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh

Table 2. Cytotoxic Activities of **1**, **1a**, **2**, **3**, **3a**, **3b**, and Controls against HL-60 Cells

compound	IC_{50} (μM)
1	0.016
1a	*a*
2	0.014
3	0.00025
3a	0.19
3b	8.3
etoposide	0.025
adriamycin	0.0072
methotrexate	0.012

[a] $IC_{50} > 10$ μM.

CCP PX-8010 controller, a Tosoh UV-8000, or a Tosoh RI-8010 detector, and a Rheodyne injection port with a 2-mL sample loop for preparative HPLC and a 20-μL sample loop for analytical HPLC. A Capcell Pak C_{18} column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC, and a Capcell Pak C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Shiseido) was employed for analytical HPLC. The following materials and reagents were used for bioassays: Inter Med Immuno-Mini NJ-2300 microplate reader (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).

Plant Material. The bulbs of *O. saundersiae* were purchased from a nursery in Heiwaen, Nara, Japan, in October 1996. The bulbs were cultivated, and the flowered plant was identified by Y. Sashida. A voucher of the plant is on file in our laboratory (96/96-OS-2).

Extraction and Isolation. The crude saponin fraction prepared from *O. saundersiae* bulbs (16.2 kg) was subjected to Si gel column chromatography, eluting with stepwise gradients of $CHCl_3$−MeOH (9:1; 4:1; 2:1) and finally with MeOH alone, to give six fractions (I−VI).[7] Fraction III was further separated by an ODS Si gel column eluting with MeOH−H_2O (4:1) into four fractions (IIIa−IIId). Fraction IIId was chromatographed on Si gel eluting with $CHCl_3$−MeOH (9:1) and ODS Si gel with MeCN−H_2O (3:2) to give **1** and **2** in impure form. Final purification was carried out by preparative HPLC using MeCN−H_2O (7:3) to yield **1** (11.2 mg) and **2** (18.5 mg) in pure form.

Compound 1: amorphous solid; $[\alpha]^{25}_D$ −56.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 257 nm (log ϵ 4.10); IR (KBr) ν_{max} 3420 (OH), 2925 (CH), 1720 (C═O), 1705 (C═O), 1690 (C═O), 1595 and 1515 (aromatic ring), 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 8.05 (1H, dd, J = 8.5, 1.7 Hz, H-6‴″), 7.91 (1H, d, J = 1.7 Hz, H-2‴″), 7.04 (1H, d, J = 8.5 Hz, H-5‴″), 5.68 (1H, dd, J = 8.9, 7.1 Hz, H-2″), 5.56 (1H, dd, J = 8.0, 6.0 Hz, H-2′), 5.39 (1H, br d, J = 4.2 Hz, H-6), 5.13 (1H, d, J = 7.1 Hz, H-1″), 5.04 (1H, d, J = 7.8 Hz, H-1‴), 4.58 (1H, d, J = 6.0 Hz, H-1′), 4.29 (1H, m, H-4″), 4.18 (1H, m, H-16), 4.16 (1H, m, H-3′), 3.81 (3H, s, OMe), 3.80 (1H, m, H-3), 3.79 (3H, s, OMe), 3.23 (1H, q, J = 7.4 Hz, H-20), 1.99 (3H, s, Ac), 1.32 (3H, d, J = 7.4 Hz, Me-21), 1.08 (3H, s, Me-19), 1.01 (3H, s, Me-18), 0.89 (3H, d, J = 6.1 Hz, Me-26), 0.86 (3H, d, J = 6.1 Hz, Me-27); ^{13}C NMR (C_5D_5N), see Table 1; FABMS (negative mode) m/z 1063 $[M - H]^-$; HRFABMS (positive mode) m/z 1087.5108 $[M + Na]^+$ (calcd for $C_{54}H_{80}O_{21}Na$, 1087.5090).

Acid Hydrolysis of 1. A solution of **1** (3 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized using an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), eluting with H_2O−MeCN (4:1, 10 mL) followed by MeOH (10 mL), to give a sugar fraction (1 mg). 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 HOAc (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) 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] 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 L-arabinose, D-xylose, and D-glucose were detected as follows: t_R (min) 13.09 (derivative of L-arabinose), 13.90 (derivative of D-xylose), 17.42 (derivative of D-glucose).

Alkaline Hydrolysis of 1. Compound **1** (6 mg) was treated with 4% KOH in EtOH (2 mL) at room temperature for 30 min. The reaction mixture was neutralized by an Amberlite IR-120B (Organo, Tokyo, Japan) column and passed through a Sephadex LH-20 (Pharmacia, Uppsala, Sweden) column to give a crude deacyl triglycoside and 3,4-dimethoxybenzoic acid (0.8 mg). Purification of the glycoside was carried out by Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (40:10:1) to afford **1a** (4.7 mg) as a pure compound.

Compound 1a: amorphous solid; $[\alpha]^{28}_D$ −18.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3360 (OH), 2960, 2925, 2870, and 2850 (CH), 1740 (C═O), 1077, 1045 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.39 (1H, br d, J = 4.3 Hz, H-6), 5.12 (1H, d, J = 7.7 Hz, H-1″), 5.08 (1H, d, J = 7.9 Hz, H-1‴), 4.46 (1H, d, J = 5.6 Hz, H-1′), 4.29 (1H, m, H-4″), 4.23 (1H, m, H-16), 4.03 (1H, m, H-3′), 3.84 (1H, m, $W_{1/2}$ = 21.0 Hz, H-3), 3.41 (1H, q, J = 7.4 Hz, H-20), 1.33 (3H, d, J = 7.4 Hz, Me-21), 1.08 (3H, s, Me-19), 0.94 (3H, s, Me-18), 0.93 (3H, d, J = 6.5 Hz, Me-26), 0.88 (3H, d, J = 6.4 Hz, Me-27); ^{13}C NMR (C_5D_5N), see Table 1; FABMS (negative mode) m/z 857 $[M - H]^-$; FABMS (positive mode) m/z 881 $[M + Na]^+$.

Compound 2: amorphous solid; $[\alpha]^{25}_D$ −54.4° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 260 nm (log ϵ 4.02); IR (KBr) ν_{max} 3410 (OH), 2930 and 2880 (CH), 1720 (C═O), 1705 (C═O), 1690 (C═O), 1585 and 1500 (aromatic ring), 1055, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 7.69 (2H, s, H-2‴″ and H-6‴″), 5.68 (1H, dd, J = 8.9, 7.1 Hz, H-2″), 5.56 (1H, dd, J = 8.1, 6.1 Hz, H-2′), 5.39 (1H, br d, J = 4.0 Hz, H-6), 5.15 (1H, d, J = 7.1 Hz, H-1″), 5.04 (1H, d, J = 7.8 Hz, H-1‴), 4.58 (1H, d, J = 6.1 Hz, H-1′), 4.30 (1H, m, H-4″), 4.17 (1H, m, H-16), 4.16 (1H, m, H-3′), 3.96 (3H, s, OMe), 3.82 (3H × 2, s, OMe × 2), 3.80 (1H, m, H-3), 3.23 (1H, q, J = 7.4 Hz, H-20), 1.99 (3H, s, Ac), 1.33 (3H, d, J = 7.4 Hz, Me-21), 1.07 (3H, s, Me-19), 1.01 (3H, s, Me-18), 0.89 (3H, d, J = 6.1 Hz, Me-26), 0.87 (3H, d, J = 6.1 Hz, Me-27); ^{13}C NMR (C_5D_5N), see Table 1; FABMS (negative mode) m/z 1093 $[M - H]^-$; HRFABMS (positive mode) m/z 1117.5170 $[M + Na]^+$ (calcd for $C_{55}H_{82}O_{22}Na$, 1117.5196).

Alkaline Hydrolysis of 2. Compound **2** (6.5 mg) was subjected to alkaline hydrolysis as described for **1** to give **1a** (5.0 mg) and 3,4,5-trimethoxybenzoic acid (1.1 mg).

Reduction of 3a. A mixture of **3a**[1] (60 mg) and $NaBH_4$ (90 mg) in MeOH (12 mL) was stirred for 30 min at room temperature. The reaction mixture was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (60:10:1) and ODS Si gel with MeCN−H_2O (1:1) to yield **3b** (12.1 mg).

Compound 3b: amorphous solid; $[\alpha]^{25}_D$ −6.7° (*c* 0.10, MeOH); IR (film) ν_{max} 3420 (OH), 2948 (CH), 1076 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.39 (1H, br d, J = 4.1 Hz, H-6), 5.15 (1H, d, J = 7.6 Hz, H-1″), 4.83 (1H, d, J = 7.5 Hz, H-1′), 4.49 (1H, dd, J = 7.9, 4.5 Hz, H-16), 4.18 (1H, br d, J = 9.1 Hz, H-22), 4.14 (1H, dd, J = 9.2, 3.6 Hz, H-3′), 3.80 (1H, m, H-3), 1.32 (3H, d, J = 7.2 Hz, Me-21), 1.10 (3H, s, Me-19), 1.06 (3H, s, Me-18), 0.89 (3H, d, J = 6.6 Hz, Me-26), 0.87 (3H, d, J = 6.5 Hz, Me-27); ^{13}C NMR (C_5D_5N), see Table 1; FABMS (negative mode) m/z 697 $[M - H]^-$; FABMS (positive mode) m/z 721 $[M + Na]^+$.

HL-60 Cell Culture Assay. HL-60 leukemia 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 cells (3×10^4 cells/mL) were continuously treated with each compound for 72 h, and

the cell growth was measured with an MTT reduction assay procedure.[9] A dose−response curve was plotted for each compound, and the concentration giving 50% inhibition (IC_{50}) was calculated.

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Supporting Information Available: GI_{50}, TGI, and LC_{50} values of **3** against the NCI 60 cell-line tumor panel. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969−3973.
(2) 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.* **1997**, *7*, 633−636.
(3) Pettit, G. R.; Tan, R.; Xu, J. P.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955−958, and references therein.
(4) Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099−1102.
(5) Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419−424.
(6) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(7) Kuroda, M.; Mimaki, Y.; Sashida, Y. *Phytochemistry* **1999**, *52*, 435−443.
(8) Oshima, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.* **1982**, *107*, 169−176.
(9) Sargent, J. M.; Taylor, C. G. *Br. J. Cancer* **1989**, *60*, 206−210.

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