Steroidal Saponins from *Dracaena surculosa*

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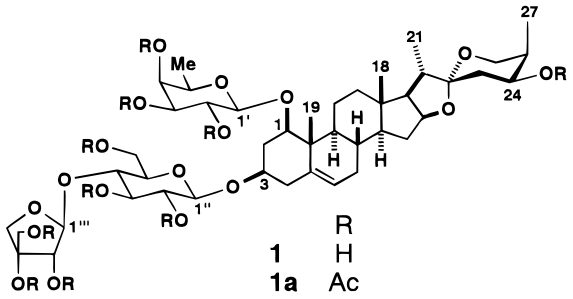
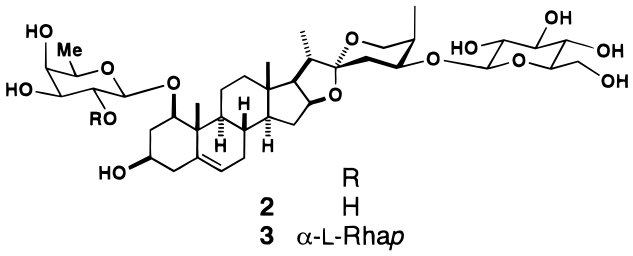
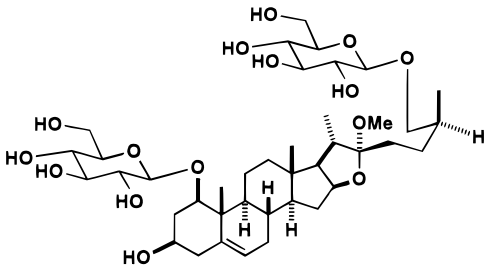
A phytochemical investigation of the whole plant of *Dracaena surculosa* resulted in the isolation of nine steroidal saponins, including three new bisdesmosidic spirostanol saponins, named surculosides A (**1**), B (**2**), and C (**3**), and a new bisdesmosidic furostanol saponin (**4**), which are based on (25*S*)-spirost-5-ene-1β,3β-diol [(25*S*)-ruscogenin] as the aglycon. The structures of **1**−**4** were determined by spectroscopic analysis, including 2D NMR spectroscopic data, and the results of hydrolytic cleavage. The isolated saponins were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

The family Agavaceae, with more than 480 species, is distributed in tropical and subtropical regions of the world. The occurrence of steroidal saponins in several Agavaceae species, especially those belonging to such representative genera as *Agave*, *Dracaena*, and *Yucca*, is well documented.[1,2] The genus *Dracaena* consists of about 50 species, and some of them have been used in medicine. *Dracaena draco* has been used as an antidiarrheal and a hemostatic drug in traditional Chinese medicine.[3] Previously, we studied the chemical components of the aerial parts of *D. draco* and isolated five new steroidal saponins, together with four known saponins.[4] We have also characterized the structures of a variety of steroidal saponins and of a cholestane glycoside isolated from the foliage plant *D. concinna*.[5,6]

As part of our chemical investigation of plants of the genus *Dracaena*, we have now examined the fresh whole plant of *D. surculosa* Lindle, on whose constituents no previous reports have appeared in the literature. This has resulted in the isolation of a total of nine steroidal saponins, of which three are new spirostanol saponins, surculosides A−C (**1**−**3**), and one a new furostanol saponin (**4**). We describe herein the identification and structure determination of saponins **1**−**4** based on spectroscopic data interpretation and the results of hydrolytic cleavage. The cytotoxic activity of all nine saponins on HL-60 human promyelocytic leukemia cells is also reported.







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Results and Discussion

The whole plant of *D. surculosa* (fresh wt of 4.3 kg) was extracted with hot MeOH. After removal of saccharides contained in the extract by passage through a porous-polymer resin (Diaion HP-20) column, the eluent was repeatedly chromatographed on Si gel and octadecylsilanized (ODS) Si gel to afford nine saponins in pure form. Compounds **5**−**9** were known constituents and identified as (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**5**);[7] (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**6**);[7,8] (25*S*)-3β-hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (**7**);[9] (25*S*)-1β-[(β-D-fucopyranosyl)oxy]-3β-hydroxy-22α-methoxyfurost-5-en-26-yl β-D-glucopyranoside (**8**);[10] and (25*S*)-3β-hydroxy-22α-methoxy-1β-[(2-*O*-α-L-rhamnopyranosyl-β-D-fucopyranosyl)oxy]furost-5-en-26-yl β-D-glucopyranoside (**9**).[11]

Surculoside A (**1**), isolated as an amorphous solid, showed an accurate $[M + Na]^+$ ion at *m/z* 909.4465 in the HRFABMS, corresponding to the empirical molecular formula $C_{44}H_{70}O_{18}$ (Δ +0.5 mmu of calcd), which was also deduced on the basis of the ^{13}C NMR spectrum combined with DEPT data. The glycosidic nature of **1** was shown by strong IR absorptions at 3385 and 1060 cm^{-1}. The ^1H NMR spectrum in C_5D_5N displayed the following representative signals: four steroid methyl protons at δ 1.30 (d, J = 6.9 Hz), 1.10 (d, J = 7.0 Hz), 1.09 (s), and 0.83 (s); an olefinic proton at δ 5.53 (br d, J = 5.5 Hz); three anomeric protons at δ 6.05 (d, J = 3.4 Hz), 5.04 (d, J = 7.7 Hz), and 4.56 (d, J = 7.6 Hz); and the methyl protons of a 6-deoxyhexopy-

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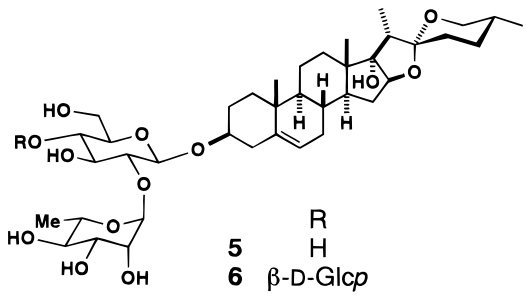

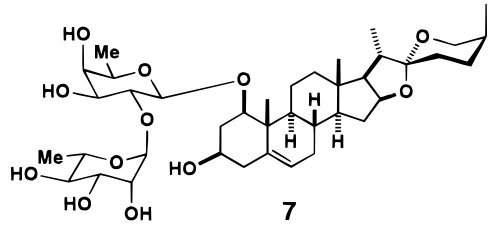

ranosyl unit at δ 1.55 (d, J = 6.4 Hz). Acid hydrolysis of **1** with 0.2 M HCl in dioxane−H_2O (1:1) resulted in the production of D-glucose, D-fucose, and D-apiose as the carbohydrate components, while the labile genuine sapogenin decomposed under acid conditions. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded Si gel column using MeCN−H_2O (3:1) as solvent system, with detection being carried out by using a combination of refractive index (RI) and optical rotation (OR) detectors. The above data, along with the three anomeric carbon signals at δ 111.0 (CH), 102.7 (CH), and 102.1 (CH) and one distinctive quaternary carbon signal at 111.4[12] led to the hypothesis that **1** was a spirostanol saponin with three monosaccharides. Comparison of the ^{1}H and ^{13}C NMR assignments of the aglycon moiety of **1**, which were established by analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra (Table 1), with those of (25*S*)-spirost-5-ene-1β,3β-diol [(25*S*)-ruscogenin] 1,3-di-*O*-glycoside (lirioproliside A), isolated from *Liriope spicata* var. *prolifera*,[13] revealed that the structure of the ring A−E portion of the molecule (C-1−C-21) was identical to that of the reference compound, including the orientations of the C-1 and C-3 oxygen atoms (1β-equatorial, 3β-equatorial) and ring junctions (B/C *trans*, C/D *trans*, D/E *cis*). However, significant differences were recognized in the signals from the ring F portion (C-22−C-27). The ^{1}H−^{1}H COSY spectrum was carefully inspected to assign the structure of the ring F residue, with the three-proton doublet signal at δ 1.30 (J = 6.9 Hz), attributable to Me-27, being used as the starting point for analysis. The Me-27 protons showed a spin-coupling correlation with the broad multiplet signal centered at δ 2.05, which was assigned unambiguously to H-25, and exhibited correlations with a pair of oxymethylene protons at δ 4.05 and 3.55, and with an oxymethine proton at δ 4.63. The oxymethine proton, in turn, displayed correlations with the terminal methylene protons at δ 2.13 and 2.04. These subsequent correlations led us to propose the ring F fragment of **1** as −CH_2−CH(O)−CH(Me)−CH_2−O−. Evidence for the connectivities of the terminal free bonds of the fragment to C-22 was obtained by the observation of ^{1}H/^{13}C long-range correlations from the methylene protons at δ 2.13 and 2.04 and one of the oxymethylene protons at δ 3.55 to C-22 at δ 111.4. Thus, the presence of an oxygen atom at C-24 was evident. The proton multiplicity of H-24, with the J values of 11.6 Hz (H-24/H-23ax), 5.0 Hz (H-24/H-23eq), and 5.0 Hz (H-24/H-25), and NOE correlations from H-23ax to H-20 and Me-27 and from H-26ax to H-16 and H-24 in the phase-sensitive NOESY spectrum were consistent with the C-22α, C-24*S*, and C-25*R* configurations. Treatment of **1** with Ac_2O in pyridine gave the corresponding decaacetate (**1a**). When the ^{1}H NMR spectrum of **1a** was compared with that of **1**, the H-24 proton was shifted downfield by 0.88 ppm, whereas the shifts of H-1 and H-3 were almost unaffected. This finding indicated that C-24 had a free hydroxyl group and that C-1 and C-3 were substituted.

We next turned our attention to the structures of the glycoside moieties. The ^{1}H−^{1}H COSY experiment allowed the sequential assignments from H-1 to H_2-6 and Me-6 of two monosaccharides. Their signal multiplet patterns and coupling constants identified a β-D-glucopyranosyl ($^4C_1$) unit and a β-D-fucopyranosyl ($^4C_1$) unit (Table 2). The HMQC spectrum correlated the proton resonances with those of the corresponding one-bond coupled carbons. The fucose residue was considered to be a terminal unit, as shown by the absence of any glycosylation shift for its carbon resonances, whereas C-4 of the glucose unit was shifted downfield in comparison with that of methyl β-D-glucopyranoside and was suggested to be substituted. The remaining monosaccharide was concluded to be a terminal D-apiofuranose as a result of acid hydrolysis and by the observation of three pairs of ABq signals at δ 6.05 and 4.82 (J = 3.4 Hz), 4.19 and 4.16 (J = 11.2 Hz), and 4.78 and 4.33 (J = 9.3 Hz). The quaternary carbon signal at δ 80.1 was typical of C-3 of apiofuranose. The ^{13}C NMR chemical shift of the anomeric carbon of the apiose at δ 111.0[14] indicated a β-orientation of the anomeric center. Finally, a $^3J_{C,H}$ correlation from each anomeric proton across the glycosidic linkage to another monosaccharide or aglycon revealed the structures of the glycoside moieties. In the HMBC spectrum, the anomeric proton signals at δ 6.05 (apiose), 5.04 (glucose), and 4.56 (fucose) exhibited correlations with the carbon signals at δ 79.2 (C-4 of glucose), 74.5 (C-3 of aglycon), and 84.1 (C-1 of aglycon), respectively. Accordingly, the structure of **1** was elucidated as (24*S*,25*R*)-1β-[(β-D-fucopyranosyl)oxy]-24-hydroxyspirost-5-en-3β-yl *O*-β-D-apiofuranosyl-(1→4)-β-D-glucopyranoside.

Surculoside B (**2**) was obtained as an amorphous solid. The HRFABMS of **2** showed an accurate $[M + Na]^+$ ion at m/z 777.4054, consistent with the molecular formula $C_{39}H_{62}O_{14}$ (Δ +1.7 mmu of calcd). The ^{1}H NMR spectrum contained two anomeric proton signals at δ 5.04 (d, J = 7.7 Hz) and 4.74 (d, J = 7.7 Hz), as well as four steroid methyl proton signals at δ 1.32 (d, J = 7.0 Hz), 1.24 (s), 1.05 (d, J = 6.9 Hz), and 0.82 (s). Acid hydrolysis of **2** with 1 M HCl in dioxane−H_2O (1:1) gave D-glucose, D-fucose, and several secondarily produced sapogenols. The ^{1}H and ^{13}C NMR assignments of **2**, which were carried out by analysis of the ^{1}H−^{1}H COSY spectrum followed by HMQC data, confirmed the identity of the aglycon of **2** with that of **1**. The NMR signals due to a β-D-glucopyranosyl unit [δ_H 5.04; δ_C 101.2 (CH), 75.3 (CH), 78.6 (CH), 71.5 (CH), 78.4 (CH), and 62.5 (CH_2)] and a β-D-fucopyranosyl unit [δ_H 4.74; δ_C 102.5 (CH), 72.1 (CH), 75.4 (CH), 72.5 (CH), 71.2 (CH), and 17.4 (Me)] could be also assigned, and each monosaccharide was considered to be directly attached to the aglycon because no glycosylation shift was observed at the assigned ^{13}C NMR shifts. The respective linkage positions of the β-D-glucopyranosyl and β-D-fucopyranosyl groups were revealed to be at C-24 and C-1 of the aglycon by the observation of three-bond-coupled ^{1}H/^{13}C correlations from the δ 5.04 signal (anomer of glucose) to the δ

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments and HMBC and NOESY Data for the Aglycon Moiety of **1**[a]

position	^{1}H	*J* (Hz)	^{13}C	HMBC (^{1}H)	NOESY
1	3.63 dd	12.0, 4.3	84.1	2ax, 19	3, 9
2eq	2.81 br d	12.0	36.0		
ax	2.10				19
3	3.99 br m	22.7[b]	74.5	2ax	1
4eq	2.71 dd	12.8, 3.4	39.5	6	
ax	2.51 dd	12.8, 12.3			19
5			138.3	19	
6	5.53 br d	5.5	125.6		
7eq	1.83		31.9		
ax	1.47				
8	1.50		32.9	6	11ax, 18, 19
9	1.41		50.5	1, 19	1, 12ax, 14
10			42.8	1, 6, 19	
11eq	2.94 br d	9.5	23.7		
ax	1.44				8, 18, 19
12eq	1.61 br d	12.0	40.3		
ax	1.32				9, 17
13			40.1	15α, 16, 17, 18	
14	1.15		57.0	18	9, 16, 17
15α	2.00		32.3		
β	1.42				
16	4.51 q-like	6.6	81.5	15*β*	14, 26ax
17	1.81 dd	8.3, 6.6	62.5	15α, 18, 21	12ax, 14, 21
18	0.83 s		16.7	17	8, 11ax, 20
19	1.09 s		14.5	1	2ax, 4ax, 8, 11ax
20	1.97		42.5	21	18, 23ax
21	1.10 d	7.0	14.7	17, 20	17
22			111.4	20, 21, 23ax, 23eq, 26eq	
23eq	2.04 dd	12.6, 5.0	35.9	25	
ax	2.13 dd	12.6, 11.6			20, 27
24	4.63 ddd	11.6, 5.0, 5.0	66.5	23ax, 23eq, 26eq, 27	25, 26ax
25	2.05		35.8	23eq, 26ax, 27	24, 26ax, 26eq, 27
26eq	3.55 br d	10.3	64.7	27	25, 27
ax	4.05				16, 24, 25
27	1.30 d	6.9	9.7	26ax, 26eq	23ax, 25, 26eq

[a] Spectra were measured in pyridine-d_5. [b] $W_{1/2}$.

Table 2. ^{1}H and ^{13}C NMR Chemical Shift Assignments for the Glycoside Moieties of **1**−**3**[a]

1					**2**					**3**				
position		^{1}H	*J* (Hz)	^{13}C	position		^{1}H	*J* (Hz)	^{13}C	position		^{1}H	*J* (Hz)	^{13}C
Fuc	1′	4.56 d	7.6	102.7	Fuc	1′	4.74 d	7.7	102.5	Fuc	1′	4.68 d	7.8	100.3
	2′	4.28 dd	8.9, 7.6	72.0		2′	4.32 dd	9.3, 7.7	72.1		2′	4.54 dd	9.2, 7.8	74.5
	3′	4.05 dd	8.9, 2.4	75.3		3′	4.06		75.4		3′	4.11 dd	9.2, 2.9	76.8
	4′	4.03 br d	2.4	72.4		4′	3.89		72.5		4′	3.90 br d	2.9	73.2
	5′	3.68 br q	6.4	71.1		5′	3.75 br q	6.3	71.2		5′	3.67 br q	6.3	71.1
	6′	1.55 d	6.4	17.3		6′	1.57 d	6.3	17.4		6′	1.51 d	6.3	17.2
Glc	1″	5.04 d	7.7	102.1	Glc	1″	5.04 d	7.7	101.2	Rha	1″	6.39 br s		101.6
	2″	4.03 dd	8.6, 7.7	75.0		2″	4.06 dd	8.8, 7.7	75.3		2″	4.76 br d	3.5	72.6
	3″	4.29 dd	8.6, 9.3	76.6		3″	4.26 dd	9.1, 8.8	78.6		3″	4.66 dd	8.9, 3.5	72.7
	4″	4.34 dd	9.3, 9.3	79.2		4″	4.31 dd	9.1, 9.1	71.5		4″	4.32 dd	9.3, 8.9	74.3
	5″	3.87 m		76.9		5″	3.92 m		78.4		5″	4.91 dq	9.3, 6.1	69.3
	6″a	4.41 br d	10.7	61.5		6″a	4.48 br d	11.6	62.5		6″	1.75 d	6.1	19.0
	b	4.29				b	4.38			Glc	1‴	5.04 d	7.7	101.2
Api	1‴	6.05 d	3.4	111.0							2‴	4.06 dd	8.9, 7.7	75.3
	2‴	4.82 d	3.4	77.4							3‴	4.26 dd	9.3, 8.9	78.7
	3‴			80.1							4‴	4.32 dd	9.3, 9.3	71.5
	4‴a	4.19 d	11.2	64.8							5‴	3.92 ddd	9.3, 4.5, 1.9	78.4
	b	4.16 d	11.2								6‴a	4.48 dd	11.6, 1.9	62.5
	5‴α	4.33 d	9.3	75.0							b	4.39 dd	11.6, 4.5	
	β	4.78 d	9.3											

[a] Spectra were measured in pyridine-d_5.

72.9 resonance (C-24 of aglycon) and from *δ* 4.74 (anomer of fucose) to *δ* 83.9 (C-1 of aglycon). All of these data were consistent with the structure (24*S*,25*R*)-1*β*-[(*β*-D-fucopyranosyl)oxy]-3*β*-hydroxyspirost-5-en-24-yl *β*-D-glucopyranoside, which was assigned to **2**.

Surculoside C (**3**), obtained as an amorphous solid, exhibited a molecular formula of $C_{45}H_{72}O_{18}$ based on the HRFABMS, in which an accurate $[M + Na]^+$ ion was observed at *m*/*z* 923.4642 (Δ +2.6 mmu of calcd). The deduced molecular formula was higher by $C_6H_{10}O_4$ than that of **2**, and the ^{1}H NMR spectrum showed signals for three anomeric protons at *δ* 6.39 (br s), 5.04 (d, *J* = 7.7 Hz), and 4.68 (d, *J* = 7.8 Hz), along with signals for four steroid methyl protons at *δ* 1.44 (s), 1.31 (d, *J* = 6.9 Hz), 1.04 (d, *J* = 6.9 Hz), and 0.83 (s). Acid hydrolysis of **3** with 1 M HCl in dioxane−H_2O (1:1) gave D-glucose, D-fucose, and L-rhamnose. On comparison of the whole ^{13}C NMR spectrum of **3** with that of **2**, a set of additional six signals corresponding to a terminal α-L-rhamnopyranosyl moiety appeared at *δ* 101.6 (CH), 72.6 (CH), 72.7 (CH), 74.3 (CH),

69.3 (CH), and 19.0 (Me), and the signals due to C-2 of the fucose moiety and its neighboring carbons varied, although all other signals remained almost unaffected. In the HMBC spectrum, correlation peaks from the anomeric proton of the rhamnose at δ 6.39 to C-2 of the fucose at δ 74.5, and from the anomeric proton of the fucose at δ 4.68 to C-1 of the aglycon at δ 84.1 confirmed that the additional rhamnose was linked to C-2 of the fucose, which, in turn, was attached to C-1 of the aglycon. An HMBC correlation between the signals of the anomeric proton of the glucose at δ 5.04 and the C-24 carbon of aglycon at δ 72.9 was also noted. The structure of **3** was thus formulated as (24*S*,25*R*)-3β-hydroxy-1β-[(2-*O*-α-L-rhamnopyranosyl-β-D-fucopyranosyl)oxy]spirost-5-en-24-yl β-D-glucopyranoside.

Compound **4** gave a positive Ehrlich's reaction, suggestive of a furostanol saponin.[7,15] Its molecular formula, $C_{40}H_{66}O_{15}$, was deduced from the negative-ion FABMS (*m*/*z* 785 $[M - H]^-$), ^{13}C NMR data, and elemental analysis. The ^1H NMR spectrum showed signals for two anomeric protons at δ 4.97 (d, $J = 7.7$ Hz) and 4.84 (d, $J = 7.8$ Hz), together with signals for four steroid methyls at δ 1.25 (s), 1.13 (d, $J = 6.9$ Hz), 1.04 (d, $J = 6.7$ Hz), and 0.88 (s). The ^{13}C NMR assignments of **4** indicated that it was a 1,26-bisdesmosidic furostanol saponin whose structure was closely related to that of **8** and **9** and that it had two terminal β-D-glucopyranosyl units [δ 101.6 (CH), 75.3 (CH), 78.6 (CH), 72.4 (CH), 78.1 (CH), and 63.6 (CH_2); δ 105.1 (CH), 75.2 (CH), 78.6 (CH), 71.7 (CH), 78.5 (CH), and 62.9 (CH_2)]. HMBC correlations were observed from δ 4.97 to δ 83.3 (C-1 of aglycon) and from δ 4.84 to δ 74.9 (C-26 of aglycon). The structure of **4** was shown to be (25*S*)-1β-[(β-D-glucopyranosyl)oxy]-3β-hydroxy-22α-methoxyfurost-5-en-26-yl β-D-glucopyranoside.

The cytotoxic activity of the isolated compounds on HL-60 leukemia cells was evaluated. Only the three known compounds (**5**−**7**) showed weak cytotoxic activity with the respective IC_{50} values of 5.2, 4.2, and 8.7 μg/mL, whereas that of etoposide used as a positive control was 0.22 μg/mL. Previously, we evaluated the cytotoxic activity of ruscogenin 1-*O*-glycosides, neoruscogenin 1-*O*-glycosides, and their corresponding furostanol saponins, and found that they exhibited significant cytotoxic activity only when their glycoside moiety was modified with an acetic acid unit or other aliphatic substituent.[16,17]

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO A-100 or a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DPX-400 (400 MHz for ^1H NMR) spectrometer or 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 (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 using a system composed 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 Rheodyne injection port with a 20-μL sample loop. A Kaseisorb NH_2-60−5 column (4.6 mm i.d. × 250 mm, 5 μm, Tokyo-Kasei, Tokyo, Japan) was employed for HPLC analysis. The following materials and reagents were used for cell culture and assay of cytostatic activity: microplate reader, Inter Med Immuno-Mini NJ-2300 (Tokyo, Japan); 96-well flat-bottom plate, Iwaki Glass (Chiba, Japan); HL-60 cells, Human Science Research Resources Bank (JCRB 0085) (Osaka, Japan); RPMI 1640 medium, GIBCO BRL (Rockville, MD); MTT, Sigma (St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Plant Material. *D. surculosa* was purchased from a nursery in Exotic Plants (Chiba, Japan) in October 1997. It was identified by Y. Sashida. A voucher of the plant is on file in our laboratory (voucher no. DS-97-007, Laboratory of Medicinal Plant Science).

Extraction and Isolation. The plant material (fresh wt, 4.3 kg) was extracted with hot MeOH twice (each 10 L, 3 h). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was passed through a Diaion HP-20 column, successively eluting with 30% MeOH, MeOH, and EtOAc. Column chromatography of the MeOH eluate portion on Si gel and elution with a stepwise gradient mixture of $CHCl_3$−MeOH (9:1; 4:1; 3:1; 2:1; 1:1), and finally with MeOH alone, gave five fractions (I−V). Fraction III was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (60:10:1; 50:10:1; 40:10:1) and ODS Si gel with MeOH−H_2O (2:1; 8:5) and MeCN−H_2O (5:8; 1:3) to give **1** (30 mg), **2** (33 mg), **4** (37 mg), **5** (23 mg), **6** (40 mg), **7** (30 mg), and **8** (38 mg). Fraction IV was subjected to column chromatography on ODS Si gel with MeCN−H_2O (3:8) to give **3** (15 mg) and **9** (570 mg). The furostanol saponins (**4**, **8**, **9**) were obtained as a mixture of the C-22 hydroxyl and C-22 methoxyl forms. The C-22 hydroxyl form present in the mixture was completely converted to the C-22 methoxyl form by treatment with hot MeOH, and the structure elucidation of **4** was carried out with the C-22 methoxyl form.

Surculoside A (1): amorphous solid; $[\alpha]^{25}_D$ −106.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3385 (OH), 2930 (CH), 1450, 1375, 1255, 1210, 1060, 995, 960, 900, 860, 835 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS (positive mode) *m*/*z* 909.4465 $[M + Na]^+$ (calcd for $C_{44}H_{70}O_{18}Na$, 909.4460).

Acid Hydrolysis of 1. A solution of **1** (9.2 mg) in 0.2 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, Tokyo, Japan) column and chromatographed over Si gel using a discontinuous gradient of $CHCl_3$−MeOH (9:1 to 1:1) to give a mixture of several decomposed sapogenols and a sugar fraction (2.3 mg). The sugar fraction was dissolved in H_2O and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), which was then analyzed by HPLC under the following conditions: solvent, MeCN−H_2O (3:1); flow rate, 0.6 mL/min; detection, RI and OR. The identification of D-glucose, D-fucose, and D-apiose present in the sugar fraction was carried out by comparing their retention times and optical rotations with those of authentic samples[18]: t_R (min) 10.6 (D-apiose, positive optical rotation); 12.1 (D-fucose, positive optical rotation); 16.5 (D-glucose, positive optical rotation).

Acetylation of 1. Compound **1** (10 mg) was treated with Ac_2O (1 mL) and pyridine (1 mL) in the presence of 4-(dimethylamino)pyridine (5 mg) catalyst at room temperature for 12 h. After addition of H_2O (2 mL) into the reaction mixture followed by evaporation to dryness, it was chromatographed on Si gel eluting with hexane−Me_2CO (2:1) to give the corresponding decaacetate (**1a**, 11 mg).

Compound 1a: amorphous solid; $[\alpha]^{25}_D$ −58.0° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 2950 (CH), 1740 (C=O), 1430, 1360, 1210, 1160, 1100, 1040, 950, 920, 910, 890, 860, 850, 830, 790 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.72 (1H, br s, H-1‴), 5.71 (dd, J = 9.7, 9.5 Hz, H-3″), 5.66 (1H, br d, $J = 5.7$ Hz, H-6), 5.61 (1H, dd, J = 10.5, 7.8 Hz, H-2′), 5.60 (1H, br d, $J = 3.2$ Hz, H-4′), 5.52 (1H, dd, J = 10.5, 3.2 Hz, H-3′), 5.51 (1H, ddd, J = 12.3, 4.9, 4.9 Hz, H-24), 5.50 (1H, br s, H-2‴), 5.41 (1H, dd, $J = 9.7$, 8.1 Hz, H-2″), 5.07 (1H, d, $J = 8.1$ Hz, H-1″), 5.02 and 4.98

(each 1H, ABq, J = 12.3 Hz, H_2-4‴), 4.84 (1H, dd, J = 12.4, 1.9 Hz, H-6″a), 4.78 (1H, d, J = 7.8 Hz, H-1′), 4.60 (1H, dd, J = 12.4, 3.7 Hz, H-6′b), 4.57 and 4.41 (each 1H, ABq, J = 10.2 Hz, H_2-5‴), 4.45 (1H, q-like J = 7.6 Hz, H-16), 4.19 (1H, dd, J = 9.5, 9.5 Hz, H-4″), 3.97 (1H, br d, J = 10.5 Hz, H-26ax), 3.97 (1H, br q, J = 6.5 Hz, H-5′), 3.95 (1H, ddd, J = 9.5, 3.7, 1.9 Hz, H-5″), 3.81 (1H, br m, $W_{1/2}$ = 23.0 Hz), 3.65 (1H, dd, J = 11.8, 4.3 Hz, H-1), 3.44 (1H, br d, J = 10.5 Hz, H-26eq), 2.18, 2.15, 2.11, 2.09, 2.07 × 2, 2.06 × 3, and 2.01 (each 3H, s, Ac × 10), 1.95 (1H, dd, J = 12.3, 12.3 Hz, H-23ax), 1.84 (1H, dd, J = 12.3, 4.9 Hz, H-23eq), 1.27 (3H, d, J = 6.5 Hz, Me-6′), 1.12 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, s, Me-19), 1.08 (3H, d, J = 6.7 Hz, Me-27), 0.83 (3H, s, Me-18).

Surculoside B (2): amorphous solid; $[\alpha]^{25}_D$ −114.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3380 (OH), 2905 (CH), 1450, 1375, 1160, 1070, 995, 955, 900, 865, 835 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.60 (1H, br d, J = 5.7 Hz, H-6), 4.81 (1H, td-like, J = 8.5, 5.0 Hz, H-24), 3.91 (1H, br m, overlapping, H-3), 3.83 (1H, dd, J = 11.5, 3.9 Hz, H-1), 2.10 (2H, d-like, J = 8.8 Hz, H_2-23), 1.32 (3H, d, J = 7.0 Hz, Me-27), 1.24 (3H, s, Me-19), 1.05 (3H, d, J = 6.9 Hz, Me-21), 0.82 (3H, s, Me-18); signals for the sugar moieties, see Table 2; ^{13}C NMR (C_5D_5N) δ 83.9, 38.1, 68.1, 43.8, 139.6, 124.7, 32.0, 33.0, 50.4, 42.8, 23.8, 40.4, 40.1, 57.0, 32.2, 81.5, 62.4, 16.8, 14.8, 42.4, 14.6, 111.2, 34.1, 72.9, 31.7, 64.2, 9.9 (C-1−C-27); signals for the sugar moieties, see Table 2; HRFABMS (positive mode) *m/z* 777.4054 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{14}Na$, 777.4037).

Acid Hydrolysis of 2. Compound **2** (6.8 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (2.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose and D-fucose; t_R (min) 12.1 (D-fucose, positive optical rotation); 16.5 (D-glucose, positive optical rotation).

Surculoside C (3): amorphous solid; $[\alpha]^{25}_D$ −136.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3375 (OH), 2905 (CH), 1455, 1375, 1065, 995, 955, 900, 860, 835 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.59 (1H, br d, J = 5.6 Hz, H-6), 4.81 (1H, td-like, J = 9.3, 4.9 Hz, H-24), 3.88 (1H, br m, $W_{1/2}$ = 21.8 Hz, H-3), 3.81 (1H, dd, J = 11.9, 4.1 Hz, H-1), 2.09 (2H, d-like, J = 9.3 Hz, H_2-23), 1.44 (3H, s, Me-19), 1.31 (3H, d, J = 6.9 Hz, Me-27), 1.04 (3H, d, J = 6.9 Hz, Me-21), 0.82 (3H, s, Me-18); signals for the sugar moieties, see Table 2; ^{13}C NMR (C_5D_5N) δ 84.1, 38.0, 68.2, 43.9, 139.6, 124.7, 32.0, 33.1, 50.6, 42.8, 24.0, 40.5, 40.1, 57.1, 32.2, 81.5, 62.4, 16.8, 15.0, 42.4, 14.6, 111.3, 34.1, 72.9, 31.7, 64.2, 9.9 (C-1−C-27); signals for the sugar moieties, see Table 2; HRFABMS (positive mode) *m/z* 923.4642 $[M + Na]^+$ (calcd for $C_{45}H_{72}O_{18}Na$, 923.4616).

Acid Hydrolysis of 3. Compound **3** (5.5 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (1.8 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-fucose, and L-rhamnose; t_R (min) 10.7 (L-rhamnose, negative optical rotation); 12.1 (D-fucose, positive optical rotation); 16.5 (D-glucose, positive optical rotation).

Compound 4: amorphous solid; $[\alpha]^{25}_D$ −104.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3400 (OH), 2920 (CH), 1450, 1370, 1150, 1090, 1060, 1020, 960, 940, 880, 830 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.58 (1H, br d, J = 5.6 Hz, H-6), 4.97 (1H, d, J = 7.7 Hz, H-1′), 4.84 (1H, d, J = 7.8 Hz, H-1″), 3.84 (1H, br m, $W_{1/2}$ = 21.7 Hz, H-3), 3.94 (1H, dd, J = 11.7, 3.9 Hz, H-1), 3.25 (3H, s OMe), 1.25 (3H, s, Me-19), 1.13 (3H, d, J = 6.9 Hz, Me-21), 1.04 (3H, d, J = 6.7 Hz, Me-27), 0.88 (3H, s, Me-18); ^{13}C NMR (C_5D_5N) δ 83.3, 37.8, 68.0, 43.7, 139.5, 124.7, 31.9, 32.9, 50.3, 42.8, 23.8, 40.3, 40.5, 56.8, 32.3, 81.3, 64.3, 16.7, 14.8, 40.5, 16.2, 112.7, 31.0, 28.1, 34.5, 74.9, 17.5 (C-1−C-27), 47.3 (OMe), 101.6, 75.3, 78.6, 72.4, 78.1, 63.6 (C-1′−C-6′), 105.1, 75.2, 78.6, 71.7, 78.5, 62.9 (C-1″−C-6″); FABMS (negative mode) *m/z* 785 $[M - H]^-$; *anal.* C 60.03%, H 8.62%, calcd for $C_{40}H_{66}O_{15}\cdot H_2O$, C 60.17%, H 8.69%).

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 2 × 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 modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay.[19] 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 **5**, **6**, and **7**, 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 in each case.

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