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Novel Cholestane Glycosides from the Bulbs of *Ornithogalum saundersiae* and Their Cytostatic Activity on Leukemia HL-60 and MOLT-4 Cells

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Abstract: Two novel 24(23→22)*abeo*-cholestane glycosides with a six-membered hemiacetal ring system and a five-membered acetal ring system (**2**, **3**), designated as saundersioside A and B, respectively, together with a new 16,23-epoxy-5β-cholestane glycoside (**1**) were isolated from the bulbs of *Ornithogalum saundersiae*. Their structures were determined by detailed analysis of the ^1H and ^{13}C NMR spectra, including various two-dimensional spectroscopy, and acid-catalyzed hydrolysis. The conformations of the 16,23-epoxy moiety of **1** and the six-membered hemiacetal moiety of **2** and **3** were studied through molecular mechanics and molecular dynamics calculation methods. Compound **3** bearing a *p*-methoxybenzoyl group at the saccharide part showed potent cytostatic activity on leukemia HL-60 and MOLT-4 cells, and the effect to HL-60 cells was revealed to be mediated partially through induction of apoptosis by cell morphology and DNA fragmentation. © 1997 Elsevier Science Ltd.

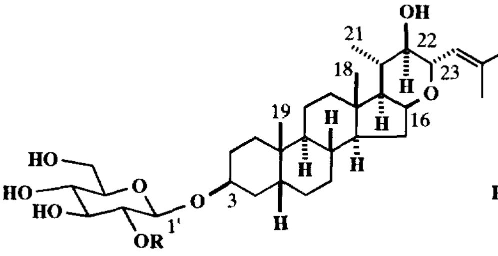
Introduction

The genus *Ornithogalum* with about 150 species belongs to the subfamily Schilloideae in the Liliaceae. Several cardenolide glycosides have been found in some species of *Ornithogalum*.1) *Ornithogalum saundersiae* is a perennial plant which is native to Natal, Swaziland, and the eastern Transvaal, and widely cultivated as an excellent garden plant. Previously, we have shown the bulbs of *O. saundersiae* to be devoid of cardenolide glycosides and to contain cholestane glycosides as the characteristic constituents.2) Further phytochemical screening of the plant material resulted in the isolation of two novel 24(23→22)*abeo*-cholestane glycosides with a six-membered hemiacetal ring system and a five-membered acetal ring system (**2**, **3**),3) designated as saundersioside A and B, respectively, together with a new 16,23-epoxy-5β-cholestane glycoside (**1**). The present paper provides a full account of the structural elucidation of the new cholestanes based on the ^1H and

^{13}C NMR spectra, including various two-dimensional spectroscopy, and acid-catalyzed hydrolysis, and also refers to the conformations of the 16,23-epoxy moiety of **1** and the six-membered hemiacetal moiety of **2** and **3** revealed through molecular mechanics and molecular dynamics calculation methods. The cytostatic activity exhibited by the cholestanes on human promyelocytic leukemia HL-60 cells and human T-lymphocytic leukemia MOLT-4 cells is described.

Results and Discussion

The concentrated 1-butanol-soluble phase of the methanolic extract of *O. saundersiae* bulbs was repeatedly subjected to Diaion HP-20, silica-gel, and octadecylsilanized (ODS) silica-gel column chromatography, as well as preparative reversed-phase HPLC to yield compounds **1** (0.016 %), **2** (0.00056 %), and **3** (0.000080 %), respectively.



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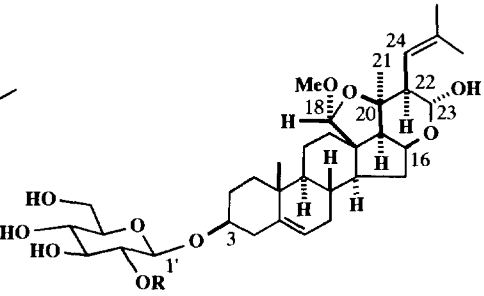
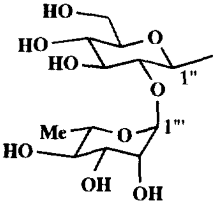
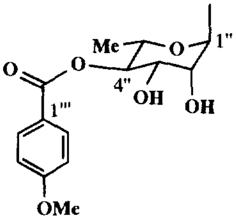


2: R = S$_1$
3: R = S$_2$

S$_1$ =



S$_2$ =



Compound **1**, $[\alpha]_D$ -64.0° (methanol), was obtained as an amorphous solid. The molecular formula was analyzed as being $C_{45}H_{74}O_{17}$ by negative-ion FABMS showing an $[M - H]^-$ ion at *m/z* 885 and elemental analysis (Calcd: C, 59.72; H, 8.46. Found: C, 59.78; H, 8.33). The ^1H NMR spectrum in pyridine-d_5 showed signals for two tertiary methyl groups at δ 1.03 and 1.02 (each s), two secondary methyl groups at δ 1.81 (d, J = 6.2 Hz) and 1.38 (d, J = 6.4 Hz), two methyl groups on a double bond at δ 1.82 and 1.72 coupled to an olefinic ptoton at δ 5.82 (br d, J = 8.7 Hz) with small J values less than 0.5 Hz, and three anomeric protons at δ 6.30 (d, J = 1.2 Hz), 5.86 (d, J = 7.2 Hz), and 5.00 (d, J = 7.7 Hz). The signal at δ 1.81 was assignable to the methyl group of 6-deoxyhexose. Acid hydrolysis of **1** with 1 M hydrochloric acid gave D-glucose and L-rhamnose in a ratio of 2 : 1. The ^{13}C NMR spectrum of **1** showed a total of 45 resonance lines, 18 of which could be assigned to two glucose units and a rhamnose unit, and three anomeric carbons were observed at δ 102.6, 101.9, and 101.2. This implied a $C_{27}H_{44}O_3$ composition for the aglycone moiety, possessing 6 degrees of unsaturation. The presence of a trisubstituted double bond was shown by a pair of the

^{13}C signals at δ 135.6 (C) and 126.1 (CH). Consequently, the aglycone of **1** was assumed to have the C_{27} steroid skeleton with a five-ring system.

Detailed interpretation of the ^{1}H-^{1}H COSY, HOHAHA, and HMQC spectra of **1**, which were measured in a mixed solvent of pyridine-d_5 and methanol-d_4 (11 : 1) to remove exchangeable protons and minimize signal overlap, gave confirmative evidence for sequential assignment of the ^{1}H NMR signals and the corresponding one-bond coupled ^{13}C signals (Table 1), giving rise to some fundamental structural features of **1**; oxygen atoms were located at the C-3, C-16, C-22, and C-23 positions, and a double bond at C-24 (Δ^{24}) on the steroidal skeleton. On comparison of the ^{1}H NMR spectrum of the corresponding decaacetate (**1a**) of **1** with that of **1**, the signal due to 22-H was shifted downfield by 1.47 ppm through *O*-acetylation; however, those due to 16-H and 23-H were almost unaffected, suggesting the formation of a six-membered ring between C-16 and C-23 in the aglycone of **1**. This was well supported by the detection of a $^{3}J_{C,H}$ coupling from 16-H (δ 4.28) to C-23 (δ 78.0) in the HMBC spectrum. Catalytic hydrogenation of **1** over platinum oxide gave the corresponding saturated derivative (**1b**), the ^{1}H NMR of which showed two additional three-proton doublet signals at δ 1.03

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignment of the Aglycone Moiety of Compounds **1** and **2** in Pyridine-d_5 - Methanol-d_4 (11 : 1)

	1				**2**		
position	^{1}H	J (Hz)	^{13}C	position	^{1}H	J (Hz)	^{13}C
1 ax	1.87		30.8	1 ax	0.98 ddd	13.5, 13.5, 4.0	37.5
eq	1.51 br d	13.3		eq	1.69		
2 ax	1.59 br dd	13.3, 13.3	27.2	2 ax	1.76		30.0
eq	1.83			eq	2.09		
3	4.30 br s	9.0 [a]	75.0	3	3.90 m	22.0 [a]	79.1
4 a	1.87		30.4	4 ax	2.55 br dd	12.5, 12.5	39.4
b	1.83			eq	2.81 br d	12.5	
5	2.42 br d	11.5	36.1	5	-		141.4
6 a	1.86		27.0	6	5.40 br d	4.1	121.4
b	1.45						
7 a	1.24		27.0	7 a	1.90		32.3
b	1.04			b	1.52		
8	1.39		35.5	8	1.49		32.4
9	1.36		40.4	9	0.94		50.1
10	-		35.3	10	-		37.2
11 a	1.34		21.2	11 ax	1.73		22.9
b	1.26			eq	1.47		
12 ax	1.85		41.1	12 ax	1.40 ddd	12.5, 12.5, 4.0	34.5
eq	1.19 dd	12.7, 3.2		eq	2.43 br d	12.5	
13	-		42.4	13	-		57.6
14	0.94 ddd	13.0, 10.0, 6.4	53.6	14	1.13		51.1
15 α	2.11		34.4	15 α	1.71		29.5
β	1.30 ddd	13.0, 13.0, 5.8		β	1.68		
16	4.28		72.6	16	4.51 ddd	9.2, 9.2, 6.6	77.2
17	1.14 dd	9.8, 7.9	60.3	17	1.99 d	9.2	53.2
18	1.01 s		15.4	18	4.72 s		108.6
19	1.00 s		24.1	19	0.87 s		19.4
20	2.14 m		34.1	20	-		85.8
21	1.35 d	6.5	17.5	21	1.38 s		27.6
22	3.55 dd	11.5, 7.5	76.6	22	2.59 dd	10.3, 8.4	50.6
23	4.53 dd	8.5, 7.5	78.0	23	5.39 d	8.4	90.7
24	5.79 br dd	8.5, 1.1	125.9	24	5.59 br d	10.3	122.6
25	-		136.2	25	-		134.7
26	1.73 br s		26.2	26	1.78 br s		26.4
27	1.81 d	1.1	18.7	27	1.75 br s		18.9
				OMe	3.36 s		55.4

[a] $W_{1/2}$

and 0.99 (each 3H, d, $J = 6.2$ Hz) instead of the two methyl groups on a double bond in comaprison with that of **1**, supporting the presence of the 24-ene group.

Analysis of the phase-sensitive NOESY spectrum made the relative stereochemistry assignable. The NOE correlations from 5-H to 19-Me, and 9-H to 2α(ax)-H and 14-H were consistent with A/B *cis* and B/C *trans* ring junctions. The 14-H in turn showed NOEs with 16-H and 17-H, indicating 16α-H and 17α-H orientations. Further NOEs from 20-H to 18-Me and 23-H, 21-Me to 12β(eq)-H, and 22-H to 21-Me and 16-H, confirmed C/D *trans* and D/E *cis* junctions, and 20*S**, 22*R**, and 23*S** configurations. The orientation of the C-3 oxygen atom was determined to be a β-form from an NOE between 3-H and 2α(ax)-H and by $W_{1/2}$ value (9.0 Hz) of 3-H. The absolute stereostructure of the aglycone of **1** was ascertained by application of an advanced Mosher's method to the aglycone (**1c**) of **1b**.[4] Compound **1c** obtained by acid hydrolysis of **1b** was treated with (*S*)- and (*R*)-α-methoxy-(trifluoromethyl)phenylacetic acid (MTPA) to yield 3β,22-bis-MTPA esters. A comparison of the ^{1}H NMR spectrum of the (*S*)-MTPA ester (**1d**) with that of the (*R*)-MTPA ester (**1e**) revealed that 23-H, 26-Me, and 27-Me of **1d** appeared at higher fields than those of **1e**, and that 21-Me as well as 18-Me and 19-Me of **1d** resonated at lower fields than those of **1e** (Fig. 2). Thus, it was shown that the aglycone of **1** had a usual steroidal absolute stereostructure.

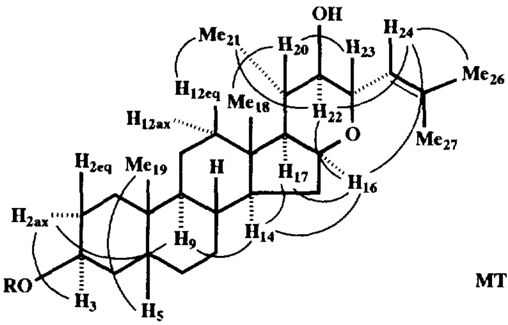


Fig. 1. NOE Correlations of the Aglycone Moiety of 1

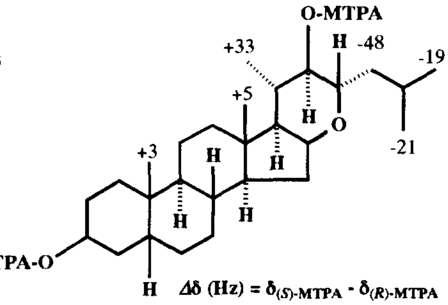


Fig. 2. Chemical Shift Differences between (*S*)-MTPA Ester (1d) of 1b and (*R*)-MTPA Ester (1e) of 1b

The proton spin coupling system and assignment of the corresponding ^{13}C signals for two glucose units and a rhamnose unit were established by a combined use of the ^{1}H-^{1}H COSY and HOHAHA spectra, followed by interpretation of the HMQC data, as shown in Table 2. The β-pyranoid anomeric form of the glucose moieties was shown by the large J value of the anomeric proton in the ^{1}H NMR spectrum. Although the anomeric orientation of rhamnose could not be deduced from the J value of the anomeric proton, the ^{13}C shifts of the C-3 and C-5 positions showed remarkable difference between methyl α- and β-rhamnopyranosides.[5] The ^{1}H-^{13}C long-range correaltion from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide or the aglycone confirmed the sugar sequence. In the HMBC spectrum, the anomeric proton signals at δ 6.14 (rhamnose), 5.72 (glucose), and 4.93 (glucose) showed correlations with the ^{13}C signals at δ 80.0 (glucose C-2), 78.1 (glucose C-2), and 75.0 (aglycone C-3), respectively.

From the evidence presented above, the full structure of **1** was revealed.

The spectral data and the result of acid hydrolysis of **2** ($C_{46}H_{72}O_{19}$) immediately allowed assignment of the triglycoside structure of **2** to be identical to that of **1**, but the ^{1}H and ^{13}C NMR features of the aglycone part of **2** were significantly different from those of **1** and also usual C_{27} steroids with cholestane skeleton, suggesting that **2** might possess an unusual aglycone structure. Analysis of the ^1H-^1H COSY spectrum combined with the TOCSY data, followed by inspection of the HMQC spectrum deduced some main fragments constituting the aglycone, along with five quaternary carbons, two angular methyls [δ_H 1.38 (3H, s), δ_C 27.6; δ_H 0.87 (3H, s), δ_C 19.4], and two methyls on a double bond [δ_H 1.78 (3H, br s), δ_C 26.4; δ_H 1.75 (3H, br s), δ_C 18.9] (Fig. 3). Furthermore, the presence of a hemiacetal group and an acetal group in **2** was shown by the following data. In the ^1H NMR spectrum of **2**, the doublet of doublets signal at δ 5.50 (J = 8.3, 6.3 Hz) in pyridine-d_5 changed to the doublet signal at δ 5.39 (J = 8.4 Hz) upon addition of a small amount of methanol-d_4 into pyridine-d_5, which was correlated to the ^{13}C signal at δ 90.7 (CH) in the HMQC spectrum. On the other hand, δ 4.72 (1H, s) showed a $^1J_{C,H}$ correlation with δ 108.6 (CH). In the HMBC spectrum, δ 4.72 and 3.36 (3H, s, OCH_3) showed long-range correlation peaks with δ 55.4 (OCH_3) and 108.6, respectively.

Table 2. ^1H and ^{13}C NMR Chemical Shift Assignment of the Saccharide Moiety of Compounds **1** and **3** in Pyridine-d_5 - Methanol-d_4 (11 : 1)

	1			**3**		
position	^1H	J (Hz)	^{13}C	^1H	J (Hz)	^{13}C
1'	4.93 d	7.4	101.0	5.10 d	7.6	100.5
2'	4.34 dd	9.0, 7.4	78.1	4.26 dd	9.2, 7.6	77.6
3'	4.39 dd	9.0, 9.0	79.2	4.32 dd	9.2, 9.2	79.7
4'	3.97 dd	9.0, 9.0	71.8	4.17 dd	9.2, 9.2	71.8
5'	3.84 ddd	9.0, 5.3, 2.2	78.2	3.94 ddd	9.2, 5.2, 1.4	78.5
6'a	4.43 dd	11.9, 2.2	63.5	4.52 dd	11.9, 1.4	62.7
b	4.26 dd	11.9, 5.3		4.36 dd	11.9, 5.2	
1"	5.72 d	7.5	101.8	6.43 br s		101.8
2"	4.06 dd	8.9, 7.5	80.0	4.82 br s		72.6
3"	4.12 dd	8.9, 8.9	79.1	4.84 br d	9.5	70.5
4"	3.93 dd	8.9, 8.9	72.9	6.13 dd	9.5, 9.5	76.8
5"	3.82 ddd	8.9, 6.7, 2.7	77.7	5.21 dq	9.5, 6.1	67.2
6"a	4.48 dd	11.6, 2.7	62.6	1.60 d	6.1	18.3
b	4.24 dd	11.6, 6.7				
1'''	6.14 d	1.2	102.6	-		123.8
2'''	4.70 dd	3.3, 1.2	72.3	8.28 d	8.9	132.5
3'''	4.55 dd	9.3, 3.3	72.6	7.03 d	8.9	114.3
4'''	4.26 dd	9.3, 9.3	74.3	-		164.0
5'''	4.90 dd	9.3, 6.2	69.4	7.03 d	8.9	114.3
6'''	1.75 d	6.2	19.0	8.28 d	8.9	132.5
7'''				-		166.6
OMe				3.76 s		55.6

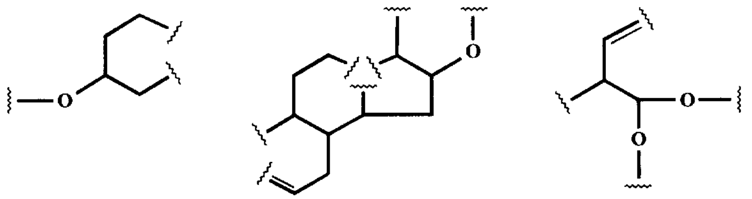


Fig. 3. Main Structural Fragments Constituting the Aglycone Moiety of 2

The molecular formula of **2** required 11 degrees of unsaturation. The three rings of the triglycoside moiety and two double bonds accounted for 5 degrees, and therefore, the aglycone moiety contained a six-ring system. The connectivities of the main fragments and four steroid methyls through quaternary carbons (δ

141.4, 134.7, 85.8, 57.6, and 37.2) were revealed by interpretation of the HMBC spectrum, as shown in Fig. 4. Cross peaks from δ 4.51 (ddd, J = 9.2, 9.2, 6.6 Hz, 16-H) to δ 90.7 (C-23), and from δ 4.72 (18-H) to δ 57.6 (C-13) and 85.8 (C-20) resulted in formation of a six-membered hemiacetal ring between C-16 and C-23, and a five-membered acetal ring between C-18 and C-20.

The configuration of the C-3 hydroxyl group bearing saccharide moiety was determined to be β from the multiplicity of the 3-H proton ($W_{1/2}$ = 22.0 Hz). The following ROE correlations, 9-H/1α(ax)-H and 14-H, 16-H/14-H, 15α-H and 17-H, 15β-H/23-H, 17-H/21-Me and 22-H, 18-H/8-H and 15β-H, and 21-Me/22-H, in the phase-sensitive ROESY spectrum provided evidence for the usual B/C *trans* and C/D *trans* steroid ring fusions, and 16S^*, 17R^*, 18R^*, 20R^*, 22R^*, and 23R^* configurations.[6)]

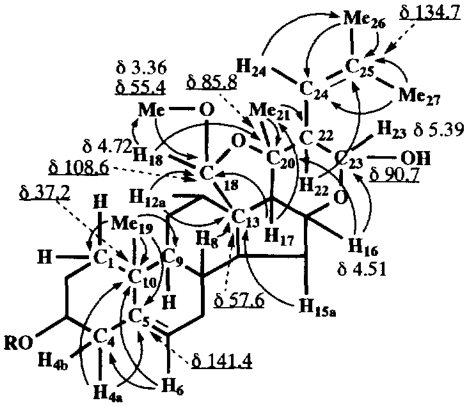


Fig. 4. HMBC Correlations of the Aglycone Moiety of 2

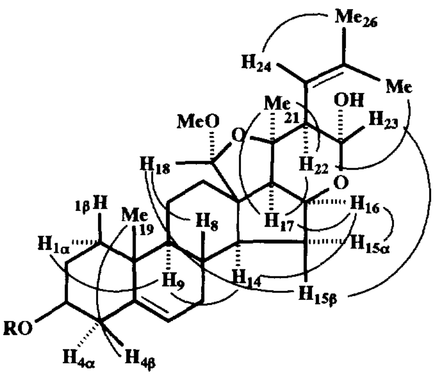


Fig. 5. ROE Correlations of the Aglycone Moiety of 2

The data presented above led to the construction of the structure of **2**, which is a novel 24(23→22)*abeo*-cholestane triglycoside.

The ^{1}H NMR spectrum of **3** ($C_{48}H_{68}O_{16}$) showed signals for four methyls at δ 1.83, 1.80, 1.46, and 0.98 (each s), an acetal proton at δ 4.63 (s), a hemiacetal proton at δ 5.55 (dd, J = 8.6, 6.9 Hz), and two olefinic protons at δ 5.73 (d, J = 10.3 Hz) and 5.51 (br d, J = 5.0 Hz) arising from the aglycone moiety. The above functional group data and the ^{13}C NMR spectrum indicated that the structure of the aglycone of **3** was identical to that of **2**. The structure of the saccharide part of **3**, which was different from that of **1** and **2**, was established by the following data. The presence of a β-D-glucopyranosyl unit [$^4C_1$: δ_H 5.10 (d, J = 7.6 Hz); δ_C 100.5 (CH)] and an α-L-rhamnopyranosyl unit [$^1C_4$: δ_H 6.43 (br s); δ_C 101.8 (CH)] was revealed by acid hydrolysis, giving D-glucose and L-rhamnose in a ratio of 1 : 1, and spin coupling information provided from the ^{1}H-^{1}H COSY and HOHAHA spectra. Furthermore, a *p*-methoxybenzoyl ester group was shown to constitute the saccharide moiety by the IR (ν_{max} 1705 cm^{-1}), UV [λ_{max} 257 nm (log ε = 4.02)], ^{1}H NMR [δ 8.28 and 7.03 (each d, J = 8.9 Hz), and 3.76 (3H, s)], and ^{13}C NMR [δ 123.8 (C), 132.5 (CH x 2), 114.3 (CH x 2), 164.0 (C), 166.6 (C=O), and 55.6 (OMe)] spectra, and by alkaline methanolysis of **3** with 3% sodium methoxide in methanol, giving methyl *p*-methoxybenzoate. In the HMBC spectrum, the anomeric proton of the rhamnose showed a $^3J_{C,H}$ correlation with C-2 of the glucose (δ 77.6), and that of the glucose with C-3 (δ 78.0) of the aglycone, leading rhamnosyl-(1→2)-glucosyl structure attached to C-3 of the aglycone. The ester linkage in the rhamnose C-4 hydroxy position of **3** was concluded to be formed from *p*-

methoxybenzoic acid, as was evident in the ^{1}H NMR paramagnetic chemical shift due to acylation; the 4-H proton of the rhamnose was deshielded to appear at δ 6.13 (dd, J = 9.5, 9.5 Hz).[7)]

Thus, the structure of **3** was established.

Compound **1** is a new 16,23-epoxy-5β-cholestane triglycoside. Compounds **2** and **3** are novel 24(23→22)*abeo*-cholestane glycosides with a six-membered hemiacetal ring between C-16 and C-23 and a five-membered acetal ring between C-18 and C-20, and proposed to be named saundersioside A and B, respectively.

The structural peculiarities of the aglycone part of **1** - **3** prompted us to examine the conformations of the E-ring part of **1** and the six-membered hemiacetal ring of **2** and **3**. The conformations were examined through molecular mechanics (MM) calculations using the MM2 force field as implemented in Macro-model. The starting geometries were generated by a systematic Monte Carlo conformational search. The most stable conformer thus found was taken as starting structures for molecular dynamics (MD) calculations *in vacuo* at 296 K with a path length of 100 ps and followed by minimizing random structures sampled after multiple 1 ps interval. In this run, three conformers were obtained for the aglycone part of **1**; the most stable conformer (227.2 kJ mol^{-1}), whose boltzmann population was 99.8% at 296 K, showed 176.8° for the H_{20}-C_{20}-C_{22}-H_{22} torsion angle and 150.9° for the H_{22}-C_{22}-C_{23}-H_{23} torsion angle. The observed proton coupling constants, $^{3}J_{20\text{-H},22\text{-H}}$ = 11.5 Hz and $^{3}J_{22\text{-H},23\text{-H}}$ = 7.5 Hz, almost corresponded to those (10.8 Hz and 7.1 Hz, respectively) calculated through the application of the given dihedral angles to the advanced Karplus-type equation proposed by Altona *et al.*[8)] The above data were consistent with the E-ring conformation being a boat-form. On the other hand, the results of MM and MD calculations of the aglycone part of **2** showed that two stable conformers (A: 139.0 kJ mol^{-1}; B: 139.7 kJ mol^{-1}) were obtained at 296 K, whose boltzmann populations were 77% and 23%, respectively. In the two conformers, the torsion angle of H_{22}-C_{22}-C_{23}-H_{23} was almost identical to each other; -179.5° for the conformer A and -178.0° for B. The difference was recognized at the torsion angle of H_{22}-C_{22}-C_{24}-H_{24} between the two conformers; -163.8° for A and +157.1° for B. The experimental coupling constants, $^{3}J_{22\text{-H},23\text{-H}}$ = 8.4 Hz and $^{3}J_{22\text{-H},24\text{-H}}$ = 10.3 Hz, corresponded to the theoretical values (9.5 Hz and 10.7 Hz, respectively), which were calculated through the application of the given dihedral angles, taking into account the boltzmann populations, to the advanced Karplus-type equation. The above findings proved the six-membered hemiacetal ring to be a half-chair form, which was well supported by an ROE correlation between 15β-H and 23-H. In conclusion, the E-ring part of **1** exists in a boat-form, while the six-membered hemiacetal ring of **2** and **3** in a half-chair form. This difference is considered to be caused by the five-membered acetal ring formed between C-18 and C-20, which leaves the six-membered hemiacetal ring for the half-chair form.

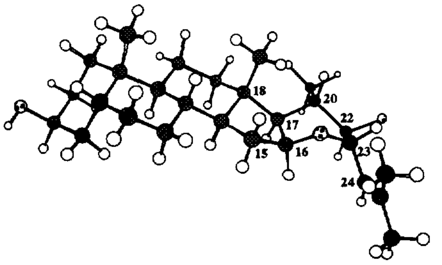


Fig. 6. The Lowest Energy Conformer of the Aglycone of 1

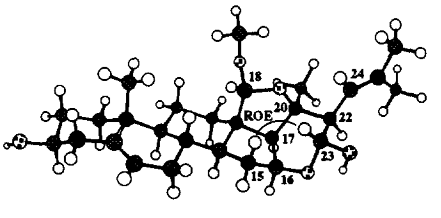


Fig. 7. The Lowest Energy Conformer A of the Aglycone of 2

The effects of **1** - **3** on growth of HL-60 and MOLT-4 cells was evaluated. The cells were continuously treated with the agents for 96 h, and the cell growth was measured with an MTT assay procedure. Compound **3** suppressed the growth of HL-60 cells in a dose-dependent manner with an IC_{50} 9.2 nM, which is almost equal or more potent in comparison of the clinically applied anticancer agents, etoposide (IC_{50} 25 nM), methotrexate (12 nM), and adriamycin (7.2 nM). It is also cytostatic against MOLT-4 cells with an IC_{50} of 3.2 nM, which is about ten times more potent than those of etoposide (38 nM), methotrexate (48 nM), and adriamycin (35 nM) (Table 3). The growth inhibiting activity of **3** on HL-60 cells appeared to be mediated partially through induction of apoptosis by cell morphology and DNA fragmentation. Untreated HL-60 cells exhibited their typical non-adherent, fairly round morphology until 96 h of culture. After 24 h or more of incubation with 11.1 nM of **3**, some of the cells still appeared normal, whereas others exhibited dramatic morphological alternations characteristic of apoptosis.[9)] Numerous apoptotic bodies, which were membrane enclosed vesicles that have budded off the cytoplasmic extension, were also observed. These apoptotic cells, excluding trypan blue dye, suggested that they were not necrosing. Agarose-gel electrophoresis of DNA extracted from **3**-treated HL-60 cells showed apparently increased DNA fragmentation. DNA fragmentation in apoptotic cells has been reported to be due to Ca^{2+} dependent endonucleases, and Zn^{2+} inhibits the enzyme activity, which results in blocks of apoptosis.[10)] The addition of 50 μM Zn^{2+} significantly (approximately 3 times) attenuated the inhibitory effect of **3** on HL-60 cell growth, while Zn^{2+} alone showed no effect on HL-60 cell growth or morphology. Compound **3** was thus suggested to induce Ca^{2+} dependent endonuclease activity, which at least partially resulted in apoptosis of HL-60 cells. Compound **1** showed no activity toward HL-60 cells. The activity of **2**, which has the same aglycone structure with **3**, was significantly less potent than that of **3**, suggesting that a structural requirement for the potent activity is the acyl group attached to the diglycoside moiety.

Table 3. Effects of **1** - **3** and Anticancer Agents on Leukemia Cell Proliferation

agents	IC_{50} (nM = x 10^{-9} M)	
	HL-60	MOLT-4
1	10,000.0<	4,300.0
2	1,800.0	1,300.0
3	9.2	3.2
anticancer agents		
etoposide	25.0	38.0
methotrexate	12.0	48.0
adriamycin	7.2	35.0

Experimental

General. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrophotometer and MS on a VG AutoSpec E or a Hitachi M-80 instrument. Elemental analysis was carried out using an Elementar Vario EL elemental analyzer. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^1H NMR) and 2D NMR on a Bruker AM-500 (500 MHz for ^1H NMR) or JEOL-LA 600 (600 MHz for ^1H NMR). Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei), ODS silica-gel (Nacalai Tesque), and Sephadex LH-20 (Pharmacia) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) 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 Tosoh HPLC system comprising of a CCPM pump, a CCP controller PX-8010, a UV-8000 or an RI-8010 detector, and 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 (Shiseido, 10 mm i.d. x 250 mm, ODS, 5 µm) was used for preparative HPLC, and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. x 250 mm, ODS, 5 µm) employed for analytical HPLC. Computer calculations were performed using the molecular-modeling software Macro-model 4.0 on Silicon Graphics work stations. The following materials and reagents were used for cell culture and assay for cytostatic activity; microplate reader: Corona MT P-32, 96-well flat-bottom plate: Corning., HL-60 and MOLT-4 cells: ICN Biomedicals, RPMI 1640 medium: GIBCO, MTT: Sigma, NP-40: Sigma, RNase A: Sigma, and proteinase K: Sigma. All other chemicals used were of biochemical reagent grade.

Extraction and isolation. Commercially available fresh bulbs of *O. saundersiae* (16.2 kg) were cut into pieces and extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble phase was fractionated on a Diaion HP-20 column eluting with increased amounts of MeOH in H_2O. Fractions eluted with 80% MeOH and MeOH were combined, and further fractionated by silica-gel column chromatography, eluting with stepwise gradients of $CHCl_3$-MeOH (9 : 1; 4 : 1; 2 : 1) and finaly with MeOH, to give six fractions (I - VI). Fraction III was subjected to ODS silica-gel column chromatography eluting with MeOH-H_2O (4 : 1) and preparative HPLC with MeOH-H_2O (3 : 2) to yield compound **3** (13.0 mg, 0.000080%). Fraction V was chromatographed on silica-gel eluting with $CHCl_3$-MeOH-H_2O (40 : 10 : 1) and ODS silica-gel with MeOH-H_2O (3 : 1; 7 : 3), followed by purification by preparative HPLC with MeCN-H_2O (3 : 1; 7 : 3) to yield compounds **1** (2.54 g, 0.016%) and **2** (90.0 mg, 0.00056%).

Compound 1. An amorphous solid, $[\alpha]_D$ -64.0° (MeOH; *c* = 0.25). Anal. Calcd for $C_{45}H_{74}O_{17}\cdot H_2O$: C, 59.72; H, 8.46. Found: C, 59.78; H, 8.33. Negative-ion FABMS *m/z* 885 $[M - H]^-$, 739 $[M - \text{rhamnosyl}]^-$; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2925 (CH), 1440, 1370, 1160, 1120, 1065, 1030, 995, 905, 890, 835, 805; ^1H NMR (pyridine-*d5*): δ 6.30 (1H, d, *J* = 1.2 Hz, 1'''-H), 5.86 (1H, d, *J* = 7.2 Hz, 1''-H), 5.82 (1H, br d, *J* = 8.7 Hz, 24-H), 5.00 (1H, d, *J* = 7.7 Hz, 1'-H), 4.60 (1H, dd, *J* = 8.7, 7.8 Hz, 23-H), 4.32 (1H, br s, 3-H), 4.30 (1H, m, 16-H), 3.60 (1H, dd, *J* = 10.9, 7.8 Hz, 22-H), 1.82 (3H, slightly br s, 27-Me), 1.81 (3H, d, *J* = 6.2 Hz, 6'''-Me), 1.72 (3H, slightly br s, 26-Me), 1.38 (3H, d, *J* = 6.4 Hz, 21-Me), 1.03 (3H, s, 19-Me), 1.02 (3H, s, 18-Me); ^{13}C NMR (pyridine-*d5*): δ 30.9, 27.3, 74.9, 30.6, 36.1, 27.0, 27.0, 35.5, 40.4, 35.3, 21.2, 41.0, 42.4, 53.6, 34.4, 72.7, 60.4, 15.4, 24.0, 34.1, 17.6, 76.6, 77.8, 126.1, 135.6, 26.2 and 18.7 (C-1 - C-27), 101.2, 78.2, 79.5, 71.9, 78.7 and 63.7 (C-1' - C-6'), 101.9, 79.8, 79.3, 73.2, 77.8 and 62.7 (C-1'' - C-6''), and 102.6, 72.4, 72.5, 74.4, 69.6 and 19.0 (C-1''' - C-6''').

Acid hydrolysis of 1 and identification of monosaccharides. A solution of **1** (40 mg) in 1 M HCl (dioxane-H_2O, 1 : 1, 4 mL) was heated at 100°C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column, and then transferred to a silica-gel column, eluting with $CHCl_3$-MeOH (19 : 1) followed by MeOH, to give a sugar fraction (13.5 mg). The sugar fraction (2 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 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 (Waters) 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) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[11) which was then analyzed by HPLC under the following conditions: solvent, MeCN-H_2O (2 : 3); flow rate, 0.8 mL min^{-1}; detection, UV 230 nm. The derivatives of D-glucose and L-rhamnose were detected. R_t (min): 23.00 (derivative of D-glucose); 26.10 (derivative of L-rhamnose).

Acetylation of 1. Compound **1** (25 mg) was acetylated with Ac_2O (0.5 mL) in pyridine (0.5 mL), and the crude acetate was chromatographed on silica-gel, eluting with hexane-Me_2CO (2 : 1), to yield the corresponding decaaceate (**1a**) (28.1 mg). Compound **1a**: an amorphous solid. IR ν_{max} (KBr) cm^{-1}: 2925 (CH), 1740 (C=O), 1435, 1365, 1225, 1170, 1130, 1035, 985; ^{1}H NMR (pyridine-d_5): δ 5.83 (1H, dd, *J* = 9.4, 9.4 Hz, 3''-H), 5.78 (1H, dd, *J* = 10.0, 3.4 Hz, 3'''-H), 5.71 (1H, dd, *J* = 9.4, 9.4 Hz, 3'-H), 5.70 (1H, br dd, *J* = 8.4, 0.9 Hz, 24-H), 5.66 (1H, dd, *J* = 3.4, 1.8 Hz, 2'''-H), 5.65 (1H, dd, *J* = 10.0, 10.0 Hz, 4'''-H), 5.43 (1H, dd, *J* = 9.4, 9.4 Hz, 4''-H), 5.41 (1H, d, *J* = 1.8 Hz, 1'''-H), 5.40 (1H, d, *J* = 9.4, 9.4 Hz, 4'-H), 5.26 (1H, d, *J* = 7.6 Hz, 1''-H), 5.07 (1H, dd, *J* = 11.6, 8.4 Hz, 22-H), 4.97 (1H, d, *J* = 7.5 Hz, 1'-H), 4.73 (1H, dd, *J* = 12.3, 5.0 Hz, 6''a-H), 4.66 (1H, dq, *J* = 10.0, 6.2 Hz, 5'''-H), 4.58 (1H, dd, *J* = 12.3, 4.6 Hz, 6'a-H), 4.50 (1H, dd, *J* = 8.4, 8.4 Hz, 23-H), 4.42 (1H, dd, *J* = 12.3, 2.7 Hz, 6''b-H), 4.39 (1H, dd, *J* = 9.4, 7.5 Hz, 2'-H), 4.38 (1H, dd, *J* = 12.3, 2.1 Hz, 6'b-H), 4.32 (1H, br s, $W_{1/2}$ = 7.6 Hz, 3-H), 4.27 (1H, m, 16-H), 4.26 (1H, ddd, *J* = 9.4, 5.0, 2.7 Hz, 5''-H), 4.07 (1H, ddd, *J* = 9.4, 4.6, 2.1 Hz, 5'-H), 4.05 (1H, dd, *J* = 9.4, 7.6 Hz, 2''-H), 2.21, 2.18, 2.17, 2.13, 2.10, 2.05 x 2, 2.04, 2.03 and 2.00 (each 3H, s, Ac), 1.71 (3H, d, *J* = 0.9 Hz, 27-Me), 1.68 (3H, br s, 26-Me), 1.61 (3H, d, *J* = 6.2 Hz, 6'''-Me), 1.15 (3H, s, 19-Me), 1.06 (3H, d, *J* = 6.5 Hz, 21-Me), 0.99 (3H, s, 18-Me).

Catalytic hydrogenation of 1. A mixture of **1** (160 mg) and PtO_2 (12 mg) in MeOH (5 mL) was stirred with an H_2 atmosphere at ambient temperature for 12 h. The reaction mixture, after removal of the catalyst by filtration, was subjected to preparative HPLC, eluting with MeOH-H_2O (4 : 1), to yield the dihydro derivative (**1b**) (118 mg) of **1**. Compound **1b**: an amorphous solid, $[\alpha]_D$ -40.0° (MeOH; *c* = 0.10). Negative-ion FABMS *m/z* 888 $[M]^-$; IR ν_{max} (KBr) cm^{-1}: 3375 (OH), 2925 (CH), 1445, 1360, 1260, 1150, 1070, 1035, 910, 895, 805; ^{1}H NMR (pyridine-d_5): δ 6.30 (1H, br s, 1'''-H), 5.85 (1H, d, *J* = 7.2 Hz, 1''-H), 5.00 (1H, d, *J* = 7.4 Hz, 1'-H), 4.32 (1H, br s, 3-H), 1.80 (3H, d, *J* = 6.1 Hz, 6'''-Me), 1.36 (3H, d, *J* = 6.3 Hz, 21-Me), 1.03 (3H, d, *J* = 6.2 Hz, 26-Me or 27-Me), 1.02 (3H, s, 19-Me), 1.00 (3H, s, 18-Me), 0.99 (3H, d, *J* = 6.2 Hz, 26-Me or 27-Me); ^{13}C NMR (pyridine-d_5): δ 30.8, 27.0, 74.8, 30.5, 36.1, 27.2, 27.0, 35.5, 40.4, 35.3, 21.2, 41.0, 42.4, 53.6, 34.1, 71.3, 60.1, 15.3, 24.0, 34.1, 17.5, 76.8, 79.4, 40.7, 25.5, 24.1 and 22.3 (C-1 - C-27), 101.1, 78.1, 79.5, 71.9, 78.7 and 63.6 (C-1' - C-6'), 101.9, 79.8, 79.3, 73.1, 77.7 and 62.7 (C-1'' - C-6''), and 102.5, 72.4, 72.7, 74.4, 69.6 and 19.0 (C-1''' - C-6''').

Acid hydrolysis of 1b. A solution of **1b** (55 mg) in 1 M HCl (dioxane-H_2O, 1 : 1, 5 mL) was heated at 100°C for 1 h under an Ar atmosphere. After neutralization of the reaction mixture by passage through an Amberlite IRA-93ZU column, it was chromatographed on silica-gel, eluting with $CHCl_3$-MeOH (19 : 1), to give an aglycone (**1c**) (19.5 mg). Compound **1c**: an amorphous solid, $[\alpha]_D$ -48.0° ($CHCl_3$; *c* = 0.10). CIMS *m/z* (%): 401 $[M - OH]^+$ (77), 383 (45), 360 (42), 285 (71), 273 (100), 154 (95), 113 (37); IR ν_{max} (KBr) cm^{-1}: 3380 (OH), 2920 (CH), 1445, 1375, 1260, 1160, 1075, 1055, 1030, 1000, 950, 930, 880, 805; ^{1}H NMR (pyridine-d_5): δ 6.29 (1H, br d, *J* = 4.2 Hz, 22-OH), 5.54 (1H, br s, 3-OH), 4.38 (1H, br s, 3-H), 4.15 (1H, q-like, *J* = 6.8 Hz, 16-H), 3.94 (1H, ddd, *J* = 8.9, 8.9, 2.5 Hz, 23-H), 3.38 (1H, ddd, *J* = 12.9, 6.9, 4.2 Hz, 22-H), 1.37 (3H, d, *J* = 6.4 Hz, 21-Me), 1.04 (3H, d, *J* = 6.5 Hz, 26-Me or 27-Me), 1.03 (3H x 2, s, 18-Me and 19-Me), 0.98 (3H, d, *J* = 6.6 Hz, 26-Me or 27-Me); ^{13}C NMR (pyridine-d_5): δ 30.7, 28.7, 66.1, 34.5, 37.1, 27.2, 27.0, 35.5, 40.3, 35.6, 21.3, 41.1, 42.5, 53.7, 34.1, 71.3, 60.1, 15.7, 24.3, 34.1, 17.6, 76.8, 79.5, 40.7, 25.5, 24.1 and 22.3 (C-1 - C-27).

Preparation of (S)- and (R)-MTPA esters of 1c. Compound **1c** (4.6 mg) was dissolved in dry CH_2Cl_2 (1.5 mL), to which was added large excess of MTPA (63.7 mg), 4-(dimethylamino)pyridine (19.6 mg), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (52.1 mg), and the solution was allowed to stand at room temperature for 10 h under an Ar atmosphere. The reaction mixture, after dilution with H_2O, was extracted with Et_2O, and subjected to silica-gel column chromatography, eluting with hexane-EtOAc (15 : 1), to yield (*S*)-MTPA ester (**1d**) (6.4 mg) as an amorphous solid. ^{1}H NMR of **1d** (chloroform-*d*): δ 7.57 - 7.38 (5H x 2, aromatic protons), 5.34 (1H, br s, 3-H), 4.73 (1H, dd, *J* = 11.6, 7.4 Hz, 22-H), 3.99 (1H, m, 16-H), 3.54 (7H, overlapping, 23-H and OMe x 2), 0.91 (3H, d, *J* = 6.5 Hz, 21-Me), 0.89 (3H, s, 18-Me), 0.85 (3H, d, *J* = 6.9 Hz, 26-Me or 27-Me), 0.84 (3H, s, 19-Me), 0.72 (3H, d, *J* = 6.5 Hz, 26-Me or 27-Me). The (*R*)-MTPA ester (**1e**) (1.5 mg) was prepared from **1c** (4.6 mg) by the same method as described for the preparation of the (*S*)-MTPA ester. ^{1}H NMR of **1e** (chloroform-*d*): δ 7.58 - 7.37 (5H x 2, aromatic protons), 5.34 (1H, br s, 3-H), 4.76 (1H, dd, *J* = 11.6, 7.4 Hz, 22-H), 4.00 (1H, m, 16-H), 3.66 (1H, m, 23-H), 3.55 (3H x 2, OMe x 2), 0.90 (3H, d, *J* = 6.6 Hz, 26-Me or 27-Me), 0.88 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 0.82 (3H, d, *J* = 6.3 Hz, 21-Me), 0.78 (3H, d, *J* = 6.4 Hz, 26-Me or 27-Me).

Compound 2. An amorphous solid, $[\alpha]_D$ -78.0° (MeOH; *c* = 0.10). Anal. Calcd for $C_{46}H_{72}O_{19} \cdot 3H_2O$: C, 56.20; H, 8.00. Found: C, 56.50; H, 8.01. Negative-ion FABMS *m/z* 927 $[M - H]^-$, 783 $[M - rhamnosyl]^-$; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 (CH), 1440, 1365, 1255, 1215, 1160, 1065, 1025, 835, 805; ^{1}H NMR (pyridine-d_5): δ 6.37 (1H, br s, 1'''-H), 5.84 (1H, d, *J* = 7.4 Hz, 1''-H), 5.69 (1H, br d, *J* = 10.3 Hz, 24-H), 5.50 (1H, dd, *J* = 8.3, 6.3 Hz, 23-H), 5.41 (1H, br d, *J* = 4.5 Hz, 6-H), 5.14 (1H, d, *J* = 7.5 Hz, 1'-H), 4.75 (1H, s, 18-H), 4.55 (1H, ddd, *J* = 9.2, 9.2, 6.6 Hz, 16-H), 3.97 (1H, m, 3-H), 3.37 (3H, s, OMe), 2.67 (1H, dd, *J* = 10.3, 8.3 Hz, 22-H), 2.00 (1H, d, *J* = 9.2 Hz, 17-H), 1.82 (3H, d, *J* = 6.2 Hz, 6'''-Me), 1.81 (3H, slightly br s, 26-Me), 1.78 (3H, slightly br s, 27-Me), 1.43 (3H, s, 21-Me), 0.89 (3H, s, 19-Me); ^{13}C NMR (pyridine-d_5): δ 37.4, 30.1, 78.9, 39.4, 141.5, 121.3, 32.2, 32.3, 50.0, 37.2, 22.9, 34.5, 57.5, 51.1, 29.6, 77.1, 53.2, 108.5, 19.4, 85.7, 27.7, 50.7, 90.9, 122.8, 134.5, 26.4 and 19.0, 55.4 (C-1 - C-27, OMe), 101.3, 80.8, 79.1, 71.8, 77.7 and 62.8 (C-1' - C-6'), 102.3, 78.8, 79.3, 72.3, 78.1 and 63.0 (C-1'' - C-6''), and 102.1, 72.3, 72.6, 74.4, 69.8 and 19.0 (C-1''' - C-6''').

Acid hydrolysis of 2. Compound **2** (10 mg) was subjected to acid hydrolysis using the same procedures as described for **1** to give a sugar fraction (3.3 mg). The monosaccharide constituents 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 and L-rhamnose were detected.

Compound 3. An amorphous solid, $[\alpha]_D$ -62.0° (MeOH; c = 0.10). Anal. Calcd for $C_{48}H_{68}O_{16}\cdot 4H_2O$: C, 59.25; H, 7.87. Found: C, 59.38; H, 8.11. Negative-ion FABMS *m/z* 899 $[M - H]^-$; UV λ_{max} nm (MeOH): 257 (log ε = 4.02); IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2920 (CH), 1705 (C=O), 1600 and 1505 (aromatic ring), 1445, 1375, 1310, 1255, 1160, 1095, 1065, 1020, 920, 840, 800, 760; ^1H NMR (pyridine-d_5): δ 8.28 (2H, d, J = 8.9 Hz, 2'''-H and 6'''-H), 7.00 (2H, d, J = 8.9 Hz, 3'''-H and 5'''-H), 6.47 (1H, br s, 1''-H), 6.15 (1H, dd, J = 9.6, 9.6 Hz, 4''-H), 5.73 (1H, br d, J = 10.3 Hz, 24-H), 5.55 (1H, dd, J = 8.3, 6.9 Hz, 23-H), 5.51 (1H, br d, J = 5.0 Hz, 6-H), 5.11 (1H, d, J = 7.5 Hz, 1'-H), 4.63 (1H, s, 18-H), 4.60 (1H, ddd, J = 9.2, 9.2, 6.7 Hz Hz, 16-H), 4.02 (1H, m, 3-H), 3.72 (3H, s, aromatic OMe), 3.42 (3H, s, OMe), 2.71 (1H, dd, J = 10.3, 8.3 Hz, 22-H), 2.05 (1H, d, J = 9.2 Hz, 17-H), 1.83 (3H, slightly br s, 26-Me), 1.80 (3H, slightly br s, 27-Me), 1.60 (3H, d, J = 6.3 Hz, 6'''-Me), 1.46 (3H, s, 21-Me), 0.98 (3H, s, 19-Me); ^{13}C NMR (pyridine-d_5): δ 37.3, 30.3, 77.8, 39.2, 141.2, 121.7, 32.4, 32.5, 49.9, 37.2, 22.8, 34.5, 57.5, 51.0, 29.6, 77.1, 53.1, 108.6, 19.6, 85.8, 27.7, 50.8, 90.9, 122.8, 134.5, 26.5 and 19.0, 55.4 (C-1 - C-27, OMe), 100.4, 77.5, 79.7, 71.8, 78.4 and 62.7 (C-1' - C-6'), 101.7, 72.6, 70.5, 76.7, 67.0 and 18.1 (C-1'' - C-6''), and 123.6, 132.3, 114.1, 163.8, 114.1, 132.3 and 166.4, 55.4 (C-1''' - C-6''', OMe).

Acid hydrolysis of 3. Compound **3** (12 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (0.6 mg). The monosaccharide constituents 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 and L-rhamnose were detected.

Alkaline hydrolysis of 3. Compound **3** (2 mg) was treated with 3% NaOMe in MeOH (2 mL) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo) column and then chromatographed over Sephadex LH-20, eluting with MeOH, to yield methyl *p*-methoxybenzoate (0.2 mg).

Conformational analysis. 1000-Step systematic Monte Carlo conformation searches were carried out with the MM2 force field as implemented in Macro-model 4.0 to predict the fully optimized lowest energy structure.12) Energies were minimized with the PR conjugate gradient minimizer, and convergence was obtained when the gradient root mean square was less than 0.001 $kJ\text{Å}^{-1}M$. Throughout this article, all MM calculations assumed a dielectric constant of 1.0. The MD simulations were carried out with Macro-model beginning with the lowest energy structures obtained by the Monte Carlo conformation search. The following options were used in the MD calculations; time step: 0.001 ps, equilibration time period: 10 ps, and production run time period: 100 ps. Initial kinetic energy was added to all atoms as random velocities. Translational and rotational momentum was reset to zero every 0.1 ps. To maintain a constant temperature, the system was coupled to an external temperature bath set at 296 K. Coupling between bath and molecule was updated every 0.2 ps. In the production run time at 296 K the conformers were sampled every 1 ps, followed by energy minimizations using the MM2 force field. The final MM calculations provided the fully optimized lowest energy structures as shown in this article.

Leukemia cell culture. HL-60 and MOLT-4 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum supplemented with L-glutamine, 100 μg mL^{-1} streptomycin, and 100 units mL^{-1} penicillin. The cells were washed and resuspended in the above medium to 2 - 5 x 10^4 cells mL^{-1}, and 200 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated for 24 h at 37 °C in 5% CO_2/air. After incubation, 4 μL of EtOH solution containing the glycoside was added to give final concentrations of 1 - 10,000 ng mL^{-1}. 4 μL of EtOH was added into control wells. The cells were incubated for a further 24 - 96 h in the presence of each agent, and then cell growth or viability was evaluated with an MTT assay procedure. Cell viability was estimated with a general dye exclusion test using trypan blue as a dye.

MTT assay. The assay was carried out according to a modified method of Sargent and Taylor as follows.[13] After termination of cell culture, 10 μL of 5 mg mL^{-1} MTT in phosphate buffered saline was added to every well, and the plate was reincubated at 37 °C in 5% CO_2/air for a further 4 h. The plate was then centrifuged at 800 x g for 5 min to precipitate cells and formazan. 150 μL of the supernatant was removed from every well, and 175 μL of 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. A dose response curve was plotted for each drug, and a concentration which gave 50% inhibition of cell growth (IC_{50}) was calculated.

Agarose gel electrophoresis. DNA electrophoresis was carried out according to a modified method of Gorczyca *et al.*[14] In brief, HL-60 cells treated with the glycoside (**3**) were collected by centrifugation, resuspended in 0.5 mL of 45 mM Tris-borate buffer/1 mM EDTA, pH 8.0, and lysed with the same buffer containing 0.25% NP-40 and 0.1% RNase A for 30 min at 37 °C. The lysate was further treated with 1 mg mL^{-1} of proteinase K for 30 min at 37 °C, and then 0.1 mL of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol was added. An aliquot (10 - 20 μL) of this solution was transferred to an 1.0% agarose gel containing 0.5 μg mL^{-1} of ethidium bromide, and electrophoresis was carried out at 2 V cm^{-1} for 3 h. The DNA in gels was visualized under UV light.

References and Notes

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3. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Tetrahedron Lett.* **1993**, 34, 6073-6076. In the preliminary communication reported by us, the unusual steroidal skeleton of **2** was named 22-homo-23-norcholestane. But from a biosynthetic point of view, the designation "24(23→22)*abeo*-cholestane" is considered to be more preferable since this modified skeleton was possibly generated through the pinacol type rearrangement. It is unlikely that the skeleton came from introduction of a C-1

unit to 23-norcholestane. In this report, we correctly use the 24(23→22)*abeo*-cholestane designation for the unusual aglycone.

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6. Acid hydrolysis of **2** gave several unidentified artifactual sapogenols, and the absolute stereostructure of the aglycone of **2** could not be ascertained by the same procedures as described for **1**. However, the modified steroidal skeleton of **2** was biosynthetically produced through the pinacol type rearrangement via a C-22 and C-23 oxygenated precursor like the aglycone of **1**, and presumed to have a usual steroidal absolute stereostructure.
7. Unfortunately, no $^3J_{C,H}$ correlation between the carbonyl carbon of the *p*-methoxybenzoyl group and the 4''-H proton of the rhamnose was observed in the HMBC spectra optimized for $^nJ_{C,H} = 5$ Hz and 8 Hz. The 4''-H proton of the rhamnose, however, was shifted downfield by 1.83 ppm in comparison of that of a steroidal saponin bearing the same saccharide sequence as **3** isolated by us from a *Lilium* plant: Mimaki, Y.; Ishibashi, N.; Ori, K.; Sashida, Y. *Phytochemistry* **1992**, 31, 1753-1758. This is believed to be a confirmative evidence for the ester linkage position.
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