Steroidal Glycosides from *Agave utahensis* and Their Cytotoxic Activity

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Eight new spirostanol saponins (**1−8**) and three new furostanol saponins (**9−11**) were isolated from the whole plants of *Agave utahensis*. The structures of **1−11** were determined by analysis of extensive spectroscopic data. The saponins were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells. Compound **1** showed cytotoxicity against HL-60 cells with an IC_{50} value of 4.9 μg/mL, induced apoptosis in HL-60 cells, and markedly activated caspase-3.

Agave utahensis Engelm. (Agavaceae) is native to South and Central America. The leaves of this plant are roasted and eaten, and the fiber has various uses.[1] Previously, we reported four new steroidal glycosides, based on (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin) as the aglycone, from the whole plants of *A. utahensis.*[2] Further phytochemical analysis of the saponin-enriched fraction prepared from the MeOH extract of this plant has resulted in the isolation and structure determination of eight new spirostanol saponins (**1−8**) and three new furostanol saponins (**9−11**). The cytotoxic activity of **1−11** against HL-60 human promyelocytic leukemia cells and the apoptosis induction properties of saponin (1) for HL-60 cells are also reported.

Results and Discussion

Fresh whole plants of *A. utahensis* (2.5 kg) were extracted with MeOH. After removal of solvent, the crude extract was fractionated by repeated column chromatography (CC) on porous-polymer polystyrene resin (Diaion HP-20), silica gel, and octadecylsilanized (ODS) silica gel to yield **1−11**.

Compound **1** was obtained as an amorphous solid with the molecular formula $C_{39}H_{64}O_{13}$ from its HRESITOFMS (*m/z* 741.4417 $[M + H]^+$) and ^{13}C NMR data. The ^1H NMR spectrum showed signals for two anomeric protons at δ 5.27 (d, *J* = 7.9 Hz) and 4.78 (d, *J* = 7.7 Hz), along with signals for four methyl groups at δ 1.15 (d, *J* = 6.9 Hz), 0.82 (s), 0.82 (s), and 0.70 (d, *J* = 5.6 Hz). Acid hydrolysis of **1** with 1 M HCl in dioxane−H_2O (1:1) yielded a sapogenin identified as (25*R*)-5β-spirostan-3β-ol (smilagenin: **1a**),[3] D-galactose, and D-glucose. Identification of the monosaccharides was carried out by direct HPLC analysis of the hydrolysate, using a combination of refractive index (RI) and optical rotation (OR) detectors. The above data, along with anomeric carbon signals at δ 107.1 and 103.7, suggested that **1** was a smilagenin diglycoside. Analysis of the NMR data implied that the sugar moiety of **1** was composed of a β-D-galactopyranosyl ($^4C_1$) unit (Gal) and a β-D-glucopyranosyl ($^4C_1$) unit (Glc) (Table 1). The glucosyl residue was the terminal unit, as indicated by the absence of any glycosylation shift for its carbon resonances (Table 2).[3,4] In the HMBC spectrum of **1**, the anomeric proton (H-1) of Glc at δ 5.27 showed a long-range correlation with C-4 of Gal at δ 80.1, of which H-1 at δ 4.78 in turn showed an HMBC correlation with C-3 of the aglycone at δ 74.7. Thus, **1** was determined to be (25*R*)-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **2** was obtained as an amorphous solid, $C_{39}H_{64}O_{13}$, by HRESITOFMS (*m/z* 741.4417 $[M + H]^+$). Acid hydrolysis of **2** gave **1a**, D-galactose, and D-glucose. The ^1H and ^{13}C NMR spectra of **1** and **2** were similar; however the terminal β-D-glucopyranosyl unit was attached to C-3 of the inner β-D-galactopyranosyl unit [δ_H 4.88 (d, *J* = 7.7 Hz); δ_C 103.2, 71.8, 85.1, 70.0, 76.7, and 62.4] instead of C-4 of Gal. In the HMBC spectrum of **2**, long-range correlations were observed between H-1 of Glc and C-3 of Gal and between H-1 of Gal and C-3 of the aglycone. Thus, **2** was formulated as (25*R*)-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside.

Compound **3** analyzed for $C_{45}H_{74}O_{18}$ by HRESITOFMS (*m/z* 903.4917 $[M + H]^+$), higher than that of **1** by $C_6H_{10}O_5$, and the ^1H NMR spectrum showed signals for three anomeric protons (δ 5.20, 5.14, and 4.80). Acid hydrolysis of **3** yielded **1a**, D-glucose, and D-galactose. Comparison of the ^{13}C NMR spectrum of **3** with that of **1** showed a set of six additional signals corresponding to a terminal β-D-glucopyranosyl moiety (Glc′, see Table 2), and the signal due to C-2 of the glucose moiety linked to C-4 of the inner galactosyl moiety and its neighboring carbons varied, while all other signals remained almost unaffected. In the HMBC spectrum of **3**, long-range correlations were observed between H-1 of Glc′ and C-2 of Glc, between H-1 of Glc and C-4 of Gal, and between H-1 of Gal and C-3 of the aglycone. Compound **3** was thus (25*R*)-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **4** had the molecular formula $C_{39}H_{62}O_{14}$ (HRESITOFMS). Analysis of the ^{13}C NMR spectrum of **4** and comparison with that of **1** revealed that **4** possessed a diglycoside moiety identical to that of **1**, but differed slightly from **1** in the aglycone structure. The IR (1707 cm^{-1}) and the ^{13}C NMR (δ 213.0) spectra demonstrated that the aglycone contained a carbonyl group and that it was located at C-12 by HMBC correlations between C-12 (δ 213.0) and H_2-11 (δ 2.37) and δ 2.21 (dd, *J* = 14.0, 4.9 Hz)/H-17 at δ 2.82 (dd, *J* = 8.6, 6.8 Hz)/Me-18 at δ 1.09 (s). Acid hydrolysis of **4** gave (25*R*)-3β-hydroxy-5β-spirostan-12-one (**4a**),[5] D-galactose, and D-glucose. Thus, **4** was determined to be (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5β-spirostan-12-one.

Compound **5** ($C_{39}H_{62}O_{14}$) furnished **4a**, D-galactose, and D-glucose, when **5** was subjected to acid hydrolysis. The ^1H and ^{13}C NMR spectra of **5** indicated that the diglycoside attached to C-3 of the aglycone was the same as that of **2**. Thus, compound **5** was (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)oxy]-5β-spirostan-12-one.

Compound **6** had the molecular formula $C_{39}H_{64}O_{14}$ (HRESITOFMS), which was higher than that of **1** by one oxygen atom. The spectroscopic properties of **6** were similar to those of **1**, except

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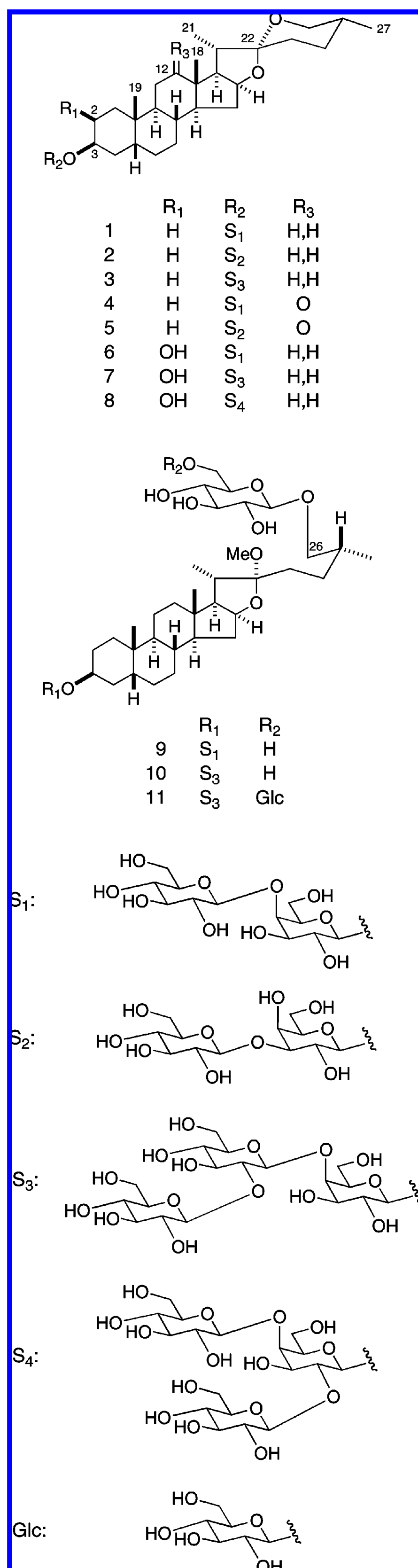

for differences between the carbon signals attributed to ring A (C-1−C-4). Acid hydrolysis of **6** gave an aglycone, identified as (25*R*)-5*β*-spirost-2*β*,3*β*-diol (**6a**),[6] together with D-galactose and D-glucose. The structure of **6** was thus characterized as (25*R*)-2*β*-hydroxy-5*β*-spirostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compound **7** ($C_{45}H_{74}O_{19}$) gave **6a**, D-galactose, and D-glucose on acid hydrolysis. Analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra of **7** indicated that the sugar moiety of **7** comprised a terminal *β*-D-glucopyranosyl unit, a 2-substituted *β*-D-glucopyranosyl unit, and a 4-substituted *β*-D-galactopyranosyl unit and that their sequence is the same as that of **3**. The structure of **7** was determined to be (25*R*)-2*β*-hydroxy-5*β*-spirostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compound **8** ($C_{45}H_{74}O_{19}$) gave **6a**, D-galactose, and D-glucose on acid hydrolysis. Comparison of the ^{1}H and ^{13}C NMR spectra of **8** with those of **7** showed their structural similarity, except for the triglycoside moiety linked to C-3 of the aglycone. The ^{1}H−^{1}H COSY and HMQC spectra indicated that the triglycoside moiety of **8** included a C-2 and C-4 disubstituted *β*-D-galactopyranosyl unit [δ_H 4.91 (d, J = 7.6 Hz); δ_C 102.7, 81.8, 75.0, 79.0, 75.6, and 60.7] and two terminal *β*-D-glucopyranosyl units (Glc and Glc′). In the HMBC spectrum of **8**, long-range correlations were observed between H-1 of Glc′ at *δ* 5.23 and C-4 of Gal at *δ* 79.0, between H-1 of Glc at *δ* 5.24 and C-2 of Gal at *δ* 81.8, and between H-1 of Gal at *δ* 4.91 and C-3 of the aglycone at *δ* 81.4. The structure of **8** was assigned as (25*R*)-2*β*-hydroxy-5*β*-spirostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-galactopyranoside.

Compound **9** ($C_{46}H_{78}O_{19}$) was shown to be a 22-methoxyfurostanol saponin by Ehrlich's test,[7,8] and by ^{1}H NMR [*δ* 3.28 (3H, s)] and ^{13}C NMR [*δ* 112.6 (C-22) and 47.3 (Me)] spectra.[9] The ^{1}H NMR spectrum of **9** showed signals for three anomeric protons at *δ* 5.28 (1H, d, J = 7.9 Hz), 4.85 (1H, d, J = 7.8 Hz), and 4.79 (1H, d, J = 7.7 Hz), as well as signals for four steroid methyl groups at *δ* 1.21 (d, J = 6.9 Hz), 1.01 (d, J = 6.6 Hz), 0.83 (s), and 0.81 (s). Enzymatic hydrolysis of **9** with *β*-D-glucosidase gave **1** and D-glucose. An NOE correlation from the methoxy signal at *δ* 3.28 to the H-16 signal at *δ* 4.52 (m) was consistent with the C-22α configuration. Thus, the structure of **9** was determined to be (25*R*)-26-[(*β*-D-glucopyranosyl)oxy]-22α-methoxy-5*β*-furostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compound **10** ($C_{52}H_{88}O_{24}$) was suggested to be the corresponding 22-methoxyfurostanol saponin of **3** with a glucosyl unit at C-26. Enzymatic hydrolysis of **10** with *β*-D-glucosidase afforded **3** and D-glucose. The structure of **10** was thus (25*R*)-26-[(*β*-D-glucopyranosyl)oxy]-22α-methoxy-5*β*-furostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compound **11** ($C_{58}H_{98}O_{29}$) showed spectral features closely related to those of **10**. However, the molecular formula of **11** was higher than that of **10** by $C_6H_{10}O_5$, and the ^{1}H NMR spectrum showed signals for five anomeric protons. On comparison of the ^{13}C NMR spectrum of **11** with that of **10**, six signals corresponding to a terminal *β*-D-glucopyranosyl moiety (Glc‴) were observed at *δ* 105.4 (CH), 75.2 (CH), 78.4 (CH), 71.6 (CH), 78.4 (CH), and 62.7 (CH_2), and the signal due to C-6 of the glucosyl moiety (Glc″) attached at C-26 of the aglycone was shifted downfield by 7.2 ppm in **11**. In the HMBC spectrum of **11**, a long-range correlation was observed between H-1 of Glc‴ at *δ* 5.11 and C-6 of Glc″ at *δ* 70.1 and between H-1 of Glc″ at *δ* 4.77 and C-26 of the aglycone at *δ* 75.3. Accordingly, the structure of **11** was formulated as (25*R*)-26-[(*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-22α-methoxy-5*β*-furostan-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compounds **1**−**11** were evaluated for their cytotoxic activity against HL-60 leukemia cells using a modified MTT assay method. Compounds **1**−**3** were cytotoxic against HL-60 cells with IC_{50} values of 4.9, 7.0, and 4.9 μg/mL, respectively, whereas **4**−**11** exhibited no apparent cytotoxicity at sample concentrations of 10 μg/mL. The above results indicate that the C-2 hydroxy group and the C-12 carbonyl group significantly reduce cytotoxic

Table 1. ^{1}H NMR Chemical Shift Assignments for the Sugar Moieties of Compounds **1**−**11** in C_5D_5N[a]

	position		**1**	**2**	**3**	**4**	**5**	**6**
Gal	1		4.78 d (7.7)	4.88 d (7.7)	4.80 d (7.8)	4.76 d (7.7)	4.85 d (7.7)	4.86 d (7.8)
	2		4.39 dd (9.4, 7.7)	4.65 dd (9.7, 7.7)	4.57 dd (9.8, 7.8)	4.39 dd (9.5, 7.7)	4.64 dd (9.8, 7.7)	4.47 dd (8.8, 7.8)
	3		4.21 dd (9.4, 3.2)	4.30 dd (9.7, 2.9)	4.07 dd (9.8, 3.3)	4.20 dd (9.5, 3.2)	4.29 dd (9.8, 3.2)	4.23 dd (8.8, 3.1)
	4		4.70 br d (3.2)	4.76 br d (2.9)	4.56 br d (3.3)	4.69 br d (3.2)	4.76 br d (3.2)	4.68 br d (3.1)
	5		4.02 m	4.04 m	3.96 m	4.00 m	4.02 m	4.09 m
	6	a	4.66 dd (10.6, 8.8)	4.41 m	4.73 dd (9.9, 9.9)	4.65 dd (10.6, 8.6)	4.37 m	4.65 dd (10.5, 8.7)
		b	4.22 m	4.36 m	4.19 dd (9.9, 5.1)	4.20 m	4.35 dd (10.9, 5.6)	4.26 m
Glc	1		5.27 d (7.9)	5.45 d (7.8)	5.14 d (7.8)	5.27 d (7.9)	5.44 d (7.8)	5.28 d (7.9)
	2		4.12 dd (8.5, 7.9)	4.04 m	4.11 dd (8.7, 7.8)	4.13 dd (8.9, 7.9)	4.04 m	4.14 dd (9.0, 7.9)
	3		4.22 dd (8.5, 8.5)	4.26 m	4.26 dd (8.7, 8.7)	4.23 dd (8.9, 8.9)	4.26 m	4.24 dd (9.0, 9.0)
	4		4.06 dd (8.5, 8.5)	4.26 m	3.97 m	4.07 dd (8.9, 8.9)	4.27 m	4.09 dd (9.0, 9.0)
	5		4.01 m	3.99 m	3.97 m	4.01 m	3.98 m	4.02 m
	6	a	4.60 dd (11.1, 2.4)	4.52 dd (11.6, 2.2)	4.62 m	4.60 dd (11.1, 2.4)	4.52 br d (11.6)	4.61 m
		b	4.20 m	4.42 m	4.11 m	4.21 m	4.40 dd (11.6, 5.4)	4.22 m
Glc′	1				5.20 d (7.6)			
	2				4.04 dd (9.1, 7.6)			
	3				4.11 dd (9.1, 9.1)			
	4				4.20 dd (9.1, 9.1)			
	5				3.77 m			
	6	a			4.59 m			
		b			4.37 dd (12.3, 3.5)			
Glc″	1							
	2							
	3							
	4							
	5							
	6	a						
		b						
Glc‴	1							
	2							
	3							
	4							
	5							
	6	a						
		b						

	position		**7**	**8**	**9**	**10**	**11**
Gal	1		4.88 d (7.9)	4.91 d (7.6)	4.79 d (7.7)	4.80 d (7.7)	4.80 d (7.7)
	2		4.65 dd (10.0, 7.9)	4.63 dd (9.2, 7.6)	4.40 m	4.57 m	4.57 dd (8.9, 7.7)
	3		4.08 dd (10.0, 3.0)	4.31 dd (9.2, 3.1)	4.22 m	4.07 m	4.07 dd (8.9, 3.3)
	4		4.56 br d (3.0)	4.66 br d (3.1)	4.70 br d (3.2)	4.57 br d (3.3)	4.57 br d (3.3)
	5		4.24 m	4.04 m	4.04 m	3.96 m	3.98 m
	6	a	4.72 dd (9.9, 9.9)	4.56 m	4.67 dd (10.1, 9.0)	4.73 dd (10.5, 9.8)	4.73 dd (10.1, 9.8)
		b	4.23 dd (9.9, 5.4)	4.23 m	4.24 m	4.19 dd (10.5, 5.5)	4.19 m
Glc	1		5.15 d (7.8)	5.24 d (7.7)	5.28 d (7.9)	5.14 d (7.8)	5.14 d (7.8)
	2		4.10 dd (8.6, 7.8)	4.06 dd (8.8, 7.7)	4.13 dd (8.3, 7.9)	4.11 dd (8.9, 7.8)	4.11 dd (8.9, 7.8)
	3		4.26 dd (8.6, 8.6)	4.16 dd (8.8, 8.8)	4.23 m	4.26 dd (8.9, 8.9)	4.26 dd (8.9, 8.9)
	4		3.97 dd (8.6, 8.6)	4.26 dd (8.8, 8.8)	4.07 dd (9.5, 9.5)	3.96 dd (8.9, 8.9)	3.96 m
	5		3.96 m	3.79 m	4.02 m	3.97 m	3.96 m
	6	a	4.62 m	4.49 dd (11.4, 2.2)	4.61 dd (11.4, 2.1)	4.56 dd (9.2, 2.7)	4.63 br d (11.0)
		b	4.10 m	4.41 dd (11.4, 4.5)	4.22 m	4.10 m	4.11 m
Glc′	1		5.25 d (7.6)	5.23 d (7.7)	4.85 d (7.8)	5.20 d (7.5)	5.20 d (7.6)
	2		4.62 dd (8.6, 7.6)	4.11 dd (8.6, 7.7)	4.04 m	4.04 dd (8.9, 7.5)	4.05 dd (8.9, 7.6)
	3		4.14 dd (9.3, 8.6)	4.22 dd (9.2, 8.6)	4.24 m	4.11 dd (8.9, 8.9)	4.11 dd (8.9, 8.9)
	4		4.19 dd (9.3, 9.3)	4.09 dd (9.2, 9.2)	4.23 m	4.20 dd (8.9, 8.9)	4.21 dd (9.3, 8.9)
	5		3.89 m	3.97 m	3.97 m	3.78 m	3.78 m
	6	a	4.64 m	4.57 m	4.57 dd (12.0, 2.3)	4.60 dd (12.0, 2.3)	4.60 m
		b	4.42 dd (12.3, 3.8)	4.22 m	4.41 m	4.37 dd (12.0, 3.4)	4.37 m
Glc″	1					4.85 d (7.8)	4.77 d (7.8)
	2					4.04 dd (9.0, 7.8)	3.98 dd (8.4, 7.8)
	3					4.25 dd (9.0, 9.0)	4.19 dd (8.4, 8.4)
	4					4.22 dd (9.0, 9.0)	4.16 dd (9.0, 8.4)
	5					3.96 m	4.08 m
	6	a				4.63 m	4.84 m
		b				4.39 dd (11.9, 5.4)	4.34 m
Glc‴	1						5.11 d (7.7)
	2						4.47 dd (9.1, 7.7)
	3						4.22 dd (9.1, 9.1)
	4						4.24 dd (9.1,9.1)
	5						3.93 m
	6	a					4.51 br d (9.1)
		b					4.37 m

[a] Values in parentheses are coupling constants in Hz.

activity in these 5β-spirostanol glycosides. The morphorogical observation (data not shown) suggested that HL-60 cell death caused by **1** and **3** is mediated partially through induction of apoptosis. In agarose gel electrophoresis of DNA, a typical ladder pattern of the internucleosomal fragmentation of DNA was observed when HL-60 cells were treated with 20 μg/mL of **1**

Table 2. ^{13}C NMR Chemical Shift Assignments for Compounds **1**−**11** in C_5D_5N

position		1	2	3	4	5	6	7	8	9	10	11
1		30.9	30.9	30.8	30.4	30.3	40.5	40.5	40.5	30.8	30.9	30.8
2		27.0	27.0	27.0	26.7	26.7	66.8	66.9	67.1	27.0	27.0	27.0
3		74.7	74.4	74.7	74.3	74.0	80.2	79.7	81.4	74.7	74.7	74.8
4		30.6	30.5	30.7	30.6	30.6	31.9	32.0	31.5	30.6	30.6	30.7
5		36.9	36.9	36.9	36.5	36.5	36.4	36.4	36.4	36.9	36.9	36.9
6		27.0	27.0	27.0	26.7	26.8	26.1	26.2	26.2	27.0	27.0	27.0
7		26.7	26.8	26.7	26.3	26.4	26.7	26.7	26.8	26.7	26.7	26.7
8		35.5	35.6	35.5	34.7	34.7	35.5	35.5	35.6	35.5	35.5	35.5
9		40.2	40.2	40.2	41.9	41.9	41.3	41.4	41.4	40.2	40.2	40.2
10		35.2	35.2	35.2	35.6	35.7	36.9	36.9	37.0	35.2	35.2	35.2
11		21.1	21.2	21.1	37.7	37.8	21.3	21.3	21.3	21.1	21.1	21.1
12		40.3	40.3	40.3	213.0	213.0	40.2	40.2	40.2	40.2	40.1	40.1
13		40.9	40.9	40.9	55.6	55.6	40.8	40.8	40.8	41.2	41.2	41.2
14		56.4	56.5	56.5	56.0	56.0	56.3	56.3	56.3	56.4	56.4	56.4
15		32.2	32.2	32.1	31.4	31.5	32.1	32.1	32.1	32.1	32.1	32.1
16		81.2	81.3	81.2	79.8	79.8	81.2	81.2	81.2	81.4	81.4	81.4
17		63.1	63.1	63.1	54.3	54.3	63.1	63.1	63.1	64.4	64.4	64.5
18		16.6	16.6	16.6	16.0	16.1	16.5	16.5	16.5	16.5	16.5	16.5
19		23.8	23.9	23.9	23.0	23.0	23.7	23.8	23.9	23.8	23.8	23.9
20		42.0	42.0	42.0	42.6	42.7	42.0	42.0	42.0	40.5	40.5	40.6
21		15.0	15.1	15.0	13.9	13.9	15.0	15.0	15.0	16.3	16.3	16.4
22		109.2	109.2	109.2	109.3	109.3	109.2	109.2	109.2	112.6	112.6	112.6
23		31.8	31.9	31.8	31.8	31.8	31.8	31.8	31.9	30.8	30.8	30.8
24		29.2	29.3	29.2	29.2	29.2	29.3	29.2	29.3	28.2	28.2	28.2
25		30.6	30.6	30.6	30.5	30.5	30.6	30.6	30.6	34.2	34.2	34.1
26		66.9	66.9	66.9	66.9	67.0	66.9	66.9	66.9	75.2	75.2	75.3
27		17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.1	17.1	17.1
OMe										47.3	47.3	47.3
Gal	1	103.7	103.2	103.4	103.6	103.1	104.6	104.2	102.7	103.7	103.4	103.4
	2	73.5	71.8	73.2	73.5	71.7	72.8	72.4	81.8	73.5	73.2	73.2
	3	75.2	85.1	75.6	75.2	85.1	75.1	75.4	75.0	75.2	75.6	75.6
	4	80.1	70.0	80.9	80.1	69.9	80.0	80.9	79.0	80.1	81.0	80.9
	5	75.3	76.7	75.0	75.4	76.7	75.8	75.5	75.6	75.4	75.0	75.0
	6	60.9	62.4	60.5	60.9	62.4	60.8	60.4	60.7	60.9	60.5	60.5
Glc	1	107.1	106.7	105.0	107.1	106.7	107.2	105.0	105.6	107.1	105.0	105.0
	2	75.9	75.9	86.3	75.9	75.9	75.9	86.2	76.8	75.9	86.3	86.3
	3	78.7	78.4	78.5	78.7	78.4	78.7	78.5	78.0	78.7	78.5	78.4
	4	72.3	71.5	71.8	72.3	71.5	72.2	71.7	71.9	72.3	71.7	71.8
	5	78.5	78.7	78.1	78.5	78.7	78.5	78.2	78.3	78.5	78.1	78.1
	6	63.1	62.6	63.2	63.1	62.6	63.1	63.2	62.9	63.1	63.2	63.2
Glc′	1			107.0				106.9	106.9	105.0	107.0	107.0
	2			76.6				76.5	75.9	75.2	76.6	76.6
	3			77.7				77.9	78.6	78.6	77.7	77.7
	4			70.2				70.3	72.2	71.8	70.2	70.2
	5			79.0				79.0	78.5	78.5	79.0	79.0
	6			61.5				61.7	63.1	62.9	61.5	61.5
Glc″	1										104.9	104.9
	2										75.2	75.0
	3										78.6	78.5
	4										71.7	71.6
	5										78.5	77.2
	6										62.9	70.1
Glc‴	1											105.4
	2											75.2
	3											78.4
	4											71.6
	5											78.4
	6											62.7

for 18 h (Figure 1). Caspases are cysteine proteases and play essential roles in the apoptotic signaling pathway. Among caspases, caspase-3 has been shown to be the executing enzyme of apoptosis. Caspase-3 was markedly activated when HL-60 cells were treated with **1** and **3** at sample concentrations of 20 μg/mL for 6 h (Figure 2). Although the structure of **2** is very similar to that of **1**, **2** did not induce apoptosis in HL-60 cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrophotometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESITOFMS were recorded on a Waters-Micromass LCT mass spectrometer. Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for CC. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and RP_{18} F_{254} S (0.25 mm, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed by using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 (Tosoh) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. HL-60 cells were obtained from the Human Science Research Resources Bank (JCRB 0085, Osaka, Japan). The following reagents were obtained from the indicated companies: RPMI 1640 medium and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)

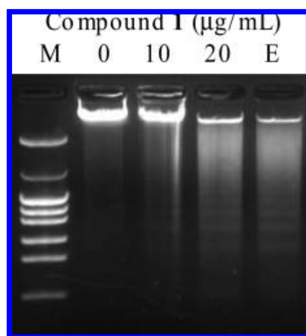

Figure 1. Induction of DNA fragmentation by **1** in HL-60 cells. HL-60 cells were incubated at 37 °C for 18 h with 0, 10, or 20 μg/mL of **1** or 10 μg/mL of etoposide (E). DNA was then extracted and applied to agarose gel electrophoresis. M: DNA marker.

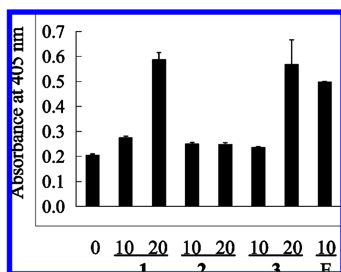

Figure 2. Caspase-3 activity in **1**, **2**, **3**, or etoposide-treated HL-60 cell lysates. HL-60 cells were incubated at 37 °C for 6 h with 0, 10, or 20 μg/mL of **1**, **2**, or **3**, or 10 μg/mL of etoposide (E). Each value represents mean ± SE from triplicate determinations.

(Sigma-Aldrich, St. Louis, MO); FBS (Bio-Whittaker, Walkersville, MO); penicillin G sodium salt and streptomycin sulfate (Gibco, Grand Island, NY). All other chemicals used were of biochemical reagent grade.

Plant Material. *A. utahensis* plants were purchased from a garden center at Japan Cactus Planning Co. (Fukushima, Japan) in 2000 and identified by Dr. Yutaka Sashida, emeritus professor of the Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher no. AU-2000-001, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation. The plant material (2.5 kg) was extracted with hot MeOH twice (each 6 L). The MeOH extract was concentrated under reduced pressure, and the concentrate (190 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (each 4 L). The MeOH eluate was chromatographed on silica gel and eluted with a stepwise gradient of $CHCl_3$−MeOH (9:1; 4:1; 3:1; 2:1; 1:1) and finally with MeOH alone, yielding fractions (I−V). Fraction II was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (60:10:1; 50:10:1) and ODS silica gel with MeOH−H_2O (8:3; 4:1) and CH_3CN−H_2O (5:7) to give **1** (150 mg), **2** (14 mg), **3** (820 mg), **4** (13 mg), **5** (4.5 mg), **6** (78 mg), **7** (12 mg), **8** (8.5 mg), and **9** (50 mg). Fraction IV was subjected to CC on silica gel eluted with $CHCl_3$−MeOH−H_2O (20:10:1; 7:4:1) and ODS silica gel with MeOH−H_2O (2:1; 4:1) and CH_3CN−H_2O (1:2; 1:3) to give **10** (42 mg) and **11** (7.0 mg). The furostanol saponins **9** and **11** were obtained as mixtures of C-22 methoxy and C-22 hydroxy forms. The C-22 hydroxy form was converted to the C-22 methoxy form by treatment with hot MeOH, and the structure elucidation was carried out with the C-22 methoxy form.

Compound 1: amorphous solid; $[\alpha]_D^{28}$ −48.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3347 (OH), 2926 (CH), 1058 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.63 (1H, m, H-16), 4.31 (1H, br s, H-3), 1.15 (3H, d, J = 6.9 Hz, Me-21), 0.82 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.70 (3H, d, J = 5.6 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 741.4417 [M + H]$^+$ (calcd for $C_{39}H_{65}O_{13}$, 741.4425).

Acid Hydrolysis of 1. A solution of **1** (9.7 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 by passage through an Amberlite IRA-93ZU column and was chromatographed on silica gel eluted with $CHCl_3$−MeOH (9:1) to yield **1a** (3.0 mg) and a sugar fraction (4.2 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge and a Toyopak IC-SP M cartridge, which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 0.9 mL/min; detection, RI and OR. Identification of D-galactose and D-glucose present in the sugar fraction was carried out by comparison of their retention times and specific optical rotations with those of authentic samples.

Compound 2: amorphous solid; $[\alpha]^{28}_D$ −10.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3363 (OH), 2927 (CH), 1075 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.62 (1H, m, H-16), 4.37 (1H, br s, H-3), 1.16 (3H, d, J = 6.9 Hz, Me-21), 0.85 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.70 (3H, d, J = 5.3 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 741.4417 [M + H]$^+$ (calcd for $C_{39}H_{65}O_{13}$, 741.4425).

Compound 3: amorphous solid; $[\alpha]^{28}_D$ −44.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3376 (OH), 2928 (CH), 1072 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.61 (1H, m, H-16), 4.32 (1H, br s, H-3), 1.15 (3H, d, J = 6.9 Hz, Me-21), 0.87 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.69 (3H, d, J = 5.5 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 903.4917 [M + H]$^+$ (calcd for $C_{45}H_{75}O_{18}$, 903.4953).

Compound 4: amorphous solid; $[\alpha]^{28}_D$ −10.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3376 (OH), 2926 (CH), 1707 (C=O), 1074, 1030 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.55 (1H, m, H-16), 4.27 (1H, br s, H-3), 2.82 (1H, dd, J = 8.6, 6.8 Hz, H-17), 2.37 (1H, dd, J = 14.0, 14.0 Hz, H-11a), 2.21 (1H, dd, J = 14.0, 4.9 Hz, H-11b), 1.37 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, s, Me-18), 0.83 (3H, s, Me-19), 0.70 (3H, d, J = 5.9 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 777.3985 [M + Na]$^+$ (calcd for $C_{39}H_{62}O_{14}Na$, 777.4037).

Compound 5: amorphous solid; $[\alpha]_D^{24}$ −4.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3356 (OH), 2927 (CH), 1707 (C=O), 1074 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.55 (1H, m, H-16), 4.31 (1H, br s, H-3), 2.83 (1H, br t, J = 7.8 Hz, H-17), 2.37 (1H, dd, J = 13.8, 13.8 Hz, H-11a), 2.20 (1H, dd, J = 13.8, 4.7 Hz, H-11b), 1.37 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, s, Me-18), 0.85 (3H, s, Me-19), 0.70 (3H, d, J = 5.5 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 777.3981 [M + Na]$^+$ (calcd for $C_{39}H_{62}O_{14}Na$, 777.4037).

Compound 6: amorphous solid; $[\alpha]^{24}_D$ −52.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3346 (OH), 2929 (CH), 1055 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.60 (1H, m, H-16), 4.42 (1H, br s, H-3), 3.87 (1H, br d, J = 12.7 Hz, H-2), 1.14 (3H, d, J = 6.9 Hz, Me-21), 0.84 (3H, s, Me-19), 0.81 (3H, s, Me-18), 0.70 (3H, d, J = 5.5 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 757.4426 [M + H]$^+$ (calcd for $C_{39}H_{65}O_{14}$, 757.4374).

Compound 7: amorphous solid; $[\alpha]_D^{25}$ −34.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3348 (OH), 2926 (CH), 1073 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.59 (1H, m, H-16), 4.46 (1H, br s, H-3), 3.88 (1H, m, H-2), 1.13 (3H, d, J = 6.9 Hz, Me-21), 0.90 (3H, s, Me-19), 0.80 (3H, s, Me-18), 0.69 (3H, d, J = 5.5 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 941.4734 [M + Na]$^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

Compound 8: amorphous solid; $[\alpha]^{25}_D$ −2.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3375 (OH), 2928 (CH), 1074 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.61 (1H, m, H-16), 4.34 (1H, br s, H-3), 3.82 (1H, br d, J = 12.8 Hz, H-2), 1.14 (3H, d, J = 6.8 Hz, Me-21), 0.99 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.70 (3H, d, J = 4.9 Hz, Me-27), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 941.4689 [M + Na]$^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

Acid Hydrolysis of 2−8. Compounds **2** (7.0 mg), **3** (10 mg), **4** (13 mg), **5** (4.6 mg), **6** (10 mg),**7** (10 mg), and **8** (6.0 mg) were independently subjected to acid hydrolysis as described for **1** to give aglycones (**1a**: 3.5 and 2.9 mg from **2** and **3**, **4a**: 7.0 and 1.7 mg from **4** and **5**, **6a**: 4.0, 4.8, and 2.4 mg from **6**, **7**, and **8**, respectively) and a sugar fractions (**2**: 2.6 mg, **3**: 4.3 mg, **4**: 5.0 mg, **5**: 1.6 mg, **6**: 3.8 mg, **7**: 4.1 mg, **8**: 3.3 mg). HPLC analysis of the sugar fractions under the same conditions as in the case of **1** showed the presence of D-galactose and D-glucose in those of **2**−**8**.

Compound 9: amorphous solid; $[\alpha]^{23}_D$ −36.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3364 (OH), 2929 (CH), 1069 cm^{-1}; ^{1}H NMR (500 MHz,

C_5D_5N) δ 4.52 (1H, m, H-16), 4.31 (1H, br s, H-3), 3.28 (3H, s, OMe), 1.21 (3H, d, $J = 6.9$ Hz, Me-21), 1.01 (3H, d, $J = 6.6$ Hz, Me-27), 0.83 (3H, s, Me-19), 0.81 (3H, s, Me-18), signals for the sugar moieties, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 957.5071 [M + Na]$^+$ (calcd for $C_{46}H_{78}O_{19}Na$, 957.5035).

Enzymatic Hydrolysis of 9. Compound **9** (9.3 mg) was treated with β-D-glucosidase (6.0 mg) in HOAc/NaOAc buffer (pH 5.0, 10 mL) at room temperature for 12 h. The reaction mixture was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (20:10:1) to yield **1** (3.0 mg) and D-glucose (1.0 mg).

Compound 10: amorphous solid; $[\alpha]^{28}_D$ −50.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3375 (OH), 2927 (CH), 1070 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.52 (1H, m, H-16), 4.32 (1H, br s, H-3), 3.27 (3H, s, OMe), 1.20 (3H, d, $J = 6.9$ Hz, Me-21), 1.00 (3H, d, $J = 6.6$ Hz, Me-27), 0.87 (3H, s, Me-19), 0.79 (3H, s, Me-18), signals for the sugar moieties, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 1119.5522 [M + Na]$^+$ (calcd for $C_{52}H_{88}O_{24}Na$, 1119.5563).

Enzymatic Hydrolysis of 10. Compound **10** (11 mg) was treated with β-D-glucosidase (12 mg) as described for **9** to yield **3** (9.0 mg) and D-glucose (1.0 mg).

Compound 11: amorphous solid; $[\alpha]^{28}_D$ −22.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3376 (OH), 2927 (CH), 1084, 1029 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.51 (1H, m, H-16), 4.31 (1H, br s, H-3), 3.27 (3H, s, OMe), 1.20 (3H, d, $J = 6.8$ Hz, Me-21), 1.00 (3H, d, $J = 6.4$ Hz, Me-27), 0.87 (3H, s, Me-19), 0.80 (3H, s, Me-18), signals for the sugar moieties, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 1281.6122 [M + Na]$^+$ (calcd for $C_{58}H_{98}O_{29}Na$, 1281.6091).

HL-60 Cell Culture Assay. HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) fetal bovine serum (FBS) supplemented with 100 units/mL penicillin G sodium salt and 100 μg/mL streptomycin sulfate. For cytotoxicity assay, the cells were washed and resuspended in the above medium to 4×10^4 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 a humidified air/CO_2 (19:1) atmosphere 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; 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. Then cell growth was evaluated by the modified MTT assay procedure established by Sargent and Taylor.[9] A dose−response curve was plotted for **1**−**3**, which showed less than 50% of cell growth at the sample concentration of 10 μg/mL, and the concentration giving 50% inhibition (IC_{50}) was calculated. Each assay was done in triplicate.

Assay for Detection of DNA Fragmentation. The cells were incubated at 37 °C for 18 h with varying the concentrations of test samples. DNA was extracted with a commercially available kit (Wizard Genomic DNA Purification Kit, Promega, WI). In brief, cells (2×10^6 cells) were centrifuged for 5 min at 10000*g*. The cell pellet was resuspended in 600 μL of nuclei lysis solution. Then, 3 μL of 4 mg/mL RNaseA solution was added to the cell lysate, and the solution was incubated at 37 °C for 15 min. Protein precipitation solution (200 μL) was added into the RNaseA-treated cell lysate, and the mixture was incubated for 5 min on ice and centrifuged at 10000*g* for 5 min. The supernatant was transferred to a clean 1.5 mL microcentrifuged tube containing 600 μL of 2-propanol and mixed by inversion. After centrifugation at 10000*g* for 5 min, DNA was visible as a small white pellet, and it was washed with 70% EtOH. Finally, the pellet was resuspended in 25 μL of DNA rehydration solution and incubated at 65 °C for 1 h, which was stored at −20 °C until use. The sample (10−15 μL) was applied to 2% agarose gel electrophoresis in 40 mM Tris-acetate buffer (pH 7.4) at 50 V for 1 h. DNA molecular weight marker (pH marker, Takara, Shiga, Japan) and DNA from apoptotic HL-60 cells induced by 10 μg/mL etoposide were used for calibration. The DNA fragmentation pattern was examined in photographs taken under UV illumination.

Assay for Caspase-3 Activation. The activity of caspase-3 was measured using a commercially available kit (APPOCYTO Caspase-3 Colorimetric Assay Kit, MBL, Aichi, Japan). HL-60 cells (2×10^6) were treated with test samples for 6 h, and the cells were centrifuged and collected. Cell pellets were suspended in 60 μL of ice cold cell lysis buffer and incubated on ice for 10 min. This cell pellet suspension was centrifuged at 10000*g* for 5 min, and the supernatant was collected. The cell lysate (50 μL, equivalent to 200 μg of protein) was mixed with 50 μL of 2 × reaction buffer containing the substrates for caspase-3 (DEVD-*p*NA (*p*-nitroanilide)). After incubation for 2 h at 37 °C, the absorbance at 405 nm of the liberated chromophore *p*NA was measured using a microplate reader. The activity of caspase-3 was evaluated in triplicate.

Supporting Information Available: ^{1}H NMR chemical shift assignments for the aglycone moieties of **1**−**11**. This information is available free of charge via the Internet at http://pubs.acs.org.

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