Steroidal Glycosides from *Agave utahensis*

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Three new spirostanol glycosides (1—3) and a new furostanol glycoside (4), together with two known spirostanol glycosides (5 and 6) were isolated from the whole plants of *Agave utahensis* (Agavaceae). The structures of the new compounds were determined by spectroscopic analysis and the results of hydrolytic cleavage.

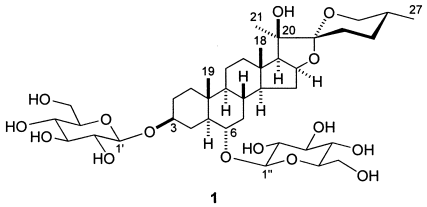
Key words *Agave utahensis*; Agavaceae; spirostanol glycoside; furostanol glycoside

The family Agavaceae, with about 19 genera, is distributed in tropical and subtropical regions of the world. The occurrence of steroidal glycosides in several Agavaceae species, especially those belonging to such representative genera as *Agave*, *Dracaena*, and *Yucca*, is well documented.[1—4] Our previous chemical studies on *Agave americana*,[5] *Dracaena surculosa*,[6,7] and *Polianthes tuberosa*[8,9] led to the isolation of a variety of steroidal glycosides, some of which showed cytotoxic activity against cultured tumor cells. As part of our continuing chemical investigation of Agavaceae plants aimed at the steroidal glycoside constituents, we have performed phytochemical screening of the fresh whole plants of *Agave utahensis* Engelmann. *A. utahensis* is native to South and Central America, and is the most northern *Agave* species in distribution. The leaves of *A. utahensis* are roasted and eaten, and the fiber is twisted into ropes and other uses.[10] A literature survey concerning the secondary metabolites of *A. utahensis* showed that no systematic chemical work has been carried out on the plant and only a few steroidal sapogenins such as tigogenin, hecogenin, chlorogenin, and mannogenin have been reported.[11] This paper mainly deals with the structural determination of four new steroidal glycosides (**1**—**4**) isolated from *A. utahensis* on the basis of extensive spectroscopic analysis and the results of hydrolysis cleavage.

Results and Discussion

The fresh whole plants of *A. utahensis* (2.5 kg) were extracted twice with hot MeOH. After removal of solvent, the MeOH extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, eluted with MeOH–H_2O gradients, EtOH, and EtOAc. The MeOH eluate portion was subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography to afford compounds **1**—**6**. Compounds **5** and **6** were identified as (25*R*)-3β-hydroxy-5α-spirostan-6α-yl β-D-glucopyranoside (**5**)[12] and (23*S*,25*R*)-3β,23-dihydroxy-5α-spirostan-6α-yl β-D-glucopyranoside (**6**),[13] respectively.

Compound **1** was isolated as an amorphous solid. The high-resolution (HR)-electrospray ionization (ESI)-time of flight (TOF)-MS of **1** showed an $[M+Na]^+$ peak at *m*/*z* 795.4142, corresponding to the empirical molecular formula of $C_{39}H_{64}O_{15}$, which was also deduced by analysis of its ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data. The ^{1}H-NMR spectrum of **1** showed signals for four methyl groups at δ 1.49 (s), 0.92 (s), 0.73 (d, J=5.6 Hz), and 0.72 (s), as well as signals for two anomeric protons at δ 5.14 (d, J=7.6 Hz) and 4.89 (d, J=7.7 Hz). Enzymatic hydrolysis of **1** with naringinase gave an aglycone (**1a**) and D-glucose. Identification of D-glucose, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate. The ^{1}H- and ^{13}C-NMR spectral data of **1a** ($C_{27}H_{44}O_5$) were similar to those of chlorogenin [(25*R*)-5α-spirostane-3β,6α-diol].[14] However, the ^{1}H-NMR spectrum of **1a** contained signals for three exchangeable protons at δ 6.08 (br d, J=3.5 Hz), 5.85 (br d, J=5.2 Hz), and 5.06 (br s), which were removed by the addition of HCl vapor. When the ^{1}H-NMR spectrum of **1a** was compared with that of chlorogenin, the signal assignable to Me-21 of chlorogenin disappeared and a three-proton singlet signal could be observed at δ 1.53 in the ^{1}H-NMR spectrum of **1a**. Furthermore, an oxygenated quaternary carbon was



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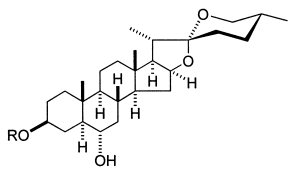
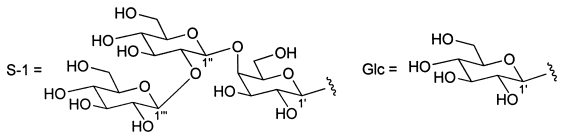
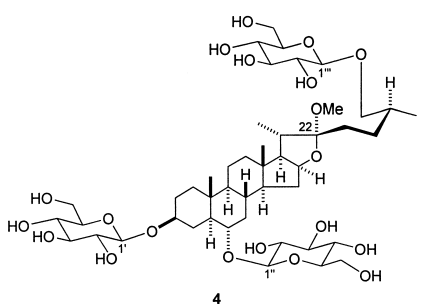


R
2 S-1
3 Glc





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observed at δ 81.7 instead of the C-20 methine carbon of chlorogenin in the ^{13}C-NMR spectrum of **1a**. The ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum of **1a** exhibited long-range correlations between the quaternary carbon and H-16 (δ 4.71)/H-17 (δ 2.24)/Me-21. These data imply that **1a** is a chlorogenin derivative with one more hydroxy group at C-20 in addition to the C-3β (δ_H 3.93; δ_C 71.0) and C-6α (δ_H 3.68; δ_C 68.6) hydroxy moieties. In the phase-sensitive NOE correlation spectroscopy (NOESY) spectrum of **1a**, NOE correlations between H-3 and H-5 (δ 1.33), H-6 and Me-19, H-8 (δ 1.64) and Me-18/Me-19, H-9 (δ 0.64) and H-5/H-14 (δ 1.01), H-14 and H-16/H-17, H-16 and H-17/H-26axial(ax) (δ 3.60), Me-18 and Me-21/H-23equatrial(eq) (δ 1.80), Me-21 and H-12eq (δ 1.97)/H-17, and between H-23ax (δ 1.79) and Me-25 (δ 1.59) gave evidence for the ring junctions of A/B *trans*, B/C *trans*, C/D *trans*, and D/E *cis*, and the 3β, 6α, 20*R*, 22α, and 25*R* configurations (Fig. 1). Thus, the structure of the new steroidal sapogenin **1a** was assigned to be (20*R*,25*R*)-5α-spirostane-3β,6α,20-triol. The ^{13}C-NMR spectrum of **1** showed the presence of two β-D-glucopyranosyl units in **1**. In the HMBC spectrum of **1**, one anomeric proton at δ 5.14 showed a $^{3}J_{C-H}$ correlation with C-3 (δ 77.0) of the aglycone, whereas another anomeric proton at δ 4.89 was correlated with C-6 (δ 79.7) of the aglycone, indicating that the C-3 and C-6 hydroxy groups of the aglycone are glucosylated. All of these data are consistent with the structure (20*R*,25*R*)-20-hydroxy-5α-spirostane-3β,6α-diyl bis-β-D-glucopyranoside, which was assigned to **1**.

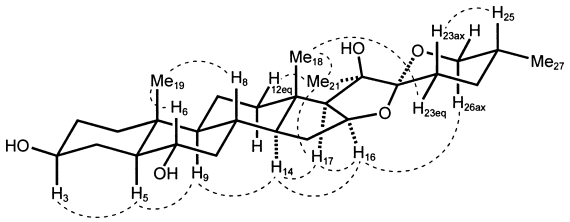

Fig. 1. Important NOE Correlations of **1a**

Compound **2** was shown to have the molecular formula of $C_{45}H_{74}O_{19}$ from its HR-ESI-TOF-MS and ^{13}C-NMR spectral data. The ^{1}H-NMR spectrum of **2** showed signals for four steroid methyl groups at δ 1.15 (d, J=6.9 Hz), 0.84 (s), 0.72 (s), and 0.70 (d, J=5.4 Hz), as well as signals for three anomeric protons at δ 5.24 (d, J=7.4 Hz), 5.15 (d, J=7.8 Hz), and 4.92 (d, J=7.7 Hz). Acid hydrolysis of **2** with 1 M HCl in dioxane–H_2O (1 : 1) gave chlorogenin, D-glucose, and D-galactose. Thus **2** was suggested to be a chlorogenin triglycoside. The ^{1}H–^{1}H shift correlation spectroscopy (COSY) experiment with **2** allowed the sequential assignments from H-1 to H_2-6 of monosaccharide, including identification of their multiplet patterns and coupling constants. The ^{1}H-detected heteronuclear multiple quantum coherance (HMQC) correlated the proton resonances with those of one-bond coupled carbons, leading to the unambiguous assignments of the carbon shifts. The ^{1}H- and ^{13}C-NMR signals thus assigned were indicative of the presence of a terminal β-D-glucopyranosyl unit (Glc‴) [δ_H 5.24 (d, J=7.4 Hz); δ_C 107.0, 76.8, 77.7, 70.3, 79.0, 61.6], a C-2 substituted β-D-glucopyranosyl unit (Glc″) [δ_H 5.15 (d, J=7.8 Hz); δ_C 105.2, 86.1, 78.5, 71.9, 78.2, 63.0], and a C-4 substituted β-D-galactopyranosyl unit (Gal′) [δ_H 4.92 (d, J=7.7 Hz); δ_C 102.3, 73.3, 75.6, 81.1, 75.0, 60.4] in **2**.[15] The β-orientations of the anomeric centers of the sugar moieties were supported by the relatively large J values of their anomeric protons (J=7.4—7.8 Hz). In the HMBC spectrum, long-range correlations were observed from δ 5.24 (H-1 of Glc‴) to δ 86.1 (C-2 of Glc″), δ 5.15 (H-1 of Glc″) to δ 81.1 (C-4 of Gal′), and δ 4.92 (H-1 of Gal′) to δ 77.7 (C-3 of the aglycone). Accordingly, the structure of **2** was formulated as (25*R*)-6α-hydroxy-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **3** ($C_{33}H_{54}O_9$) was suggested to be a chlorogenin monoglucoside based on its ^{1}H- and ^{13}C-NMR spectra, which showed signals for four steroid methyl groups at δ_H 1.15 (d, J=7.0 Hz), 0.85 (s), 0.76 (s), and 0.70 (d, J=5.6 Hz),

Table 1. ^{1}H-NMR Chemical Shift Assignments for the Sugar Moieties of Compounds **1**—**4** in C_5D_5N

Position		**1**	**2**	**3**	**4**
1′		5.14 d (7.6)[a)]	4.92 d (7.7)	5.09 d (7.7)	5.14 d (7.7)
2′		4.06 dd (8.3, 7.6)	4.51 dd (9.5, 7.7)	4.06 dd (8.3, 7.7)	4.06 dd (8.7, 7.7)
3′		4.29 dd (8.9, 8.3)	4.04 dd (9.5, 3.0)	4.24 dd (8.9, 8.3)	4.29 m
4′		4.28 m	4.56 br d (3.0)	4.28 dd (8.9, 8.9)	4.26 m
5′		3.86 m	3.90 dd-like (9.9, 4.5)	3.90 m	3.86 m
6′	a	4.44 dd (11.7, 2.2)	4.77 dd (9.9, 9.9)	4.57 br d (12.6)	4.45 dd (11.7, 2.4)
	b	4.33 dd (11.7, 5.1)	4.21 dd (9.9, 4.5)	4.41 dd (12.6, 5.4)	4.33 dd (11.7, 5.1)
1″		4.89 d (7.7)	5.15 d (7.8)		4.91 d (7.7)
2″		4.04 dd (8.3, 7.7)	4.17 dd (8.5, 7.8)		4.05 dd (8.7, 7.7)
3″		4.23 dd (8.8, 8.3)	4.29 dd (8.5, 8.5)		4.23 m
4″		4.26 dd (8.8, 8.8)	3.97 dd (9.6, 8.5)		4.25 m
5″		3.93 m	3.98 m		3.93 m
6″	a	4.51 dd (11.7, 1.8)	4.64 br d (10.2)		4.51 dd (11.6, 2.4)
	b	4.40 dd (11.7, 4.8)	4.11 m		4.39 dd (11.6, 6.3)
1‴			5.24 d (7.4)		4.85 d (7.8)
2‴			4.07 dd (8.6, 7.4)		4.04 dd (9.1, 7.8)
3‴			4.13 dd (9.3, 8.6)		4.26 m
4‴			4.23 dd (9.5, 9.3)		4.24 m
5‴			3.80 m		3.97 m
6‴	a		4.59 dd (12.5, 1.6)		4.57 dd (11.7, 2.2)
	b		4.37 dd (12.5, 3.4)		4.40 dd (11.7, 5.7)

a) The values in parentheses are coupling constants in Hz.

Table 2. ^{13}C-NMR Chemical Shift Assignments for Compounds **1**—**4** in C_5D_5N

Position	1	2	3	4
1	37.5	37.7	37.7	37.5
2	29.8	29.6	29.5	29.8
3	77.0	77.7	77.6	77.0
4	28.5	30.0	30.0	28.6
5	50.9	52.3	52.2	50.9
6	79.7	68.4	68.4	79.7
7	41.1	42.7	42.7	41.3
8	33.6	34.3	34.3	34.0
9	53.6	54.1	54.1	53.8
10	36.6	36.6	36.6	36.7
11	20.7	21.3	21.3	21.1
12	40.2	40.1	40.1	39.8
13	41.3	40.8	40.8	41.0
14	57.0	56.3	56.3	56.2
15	31.5	32.2	32.2	31.9
16	79.8	81.1	81.1	81.2
17	71.4	63.0	63.0	64.2
18	16.3	16.6	16.6	16.5
19	13.4	13.5	13.6	13.4
20	81.6	42.0	42.0	40.4
21	22.2	15.0	15.0	16.2
22	106.5	109.2	109.2	112.5
23	30.6	31.8	31.8	30.7
24	28.9	29.3	29.3	28.2
25	30.5	30.6	30.6	34.2
26	68.4	66.9	66.9	75.2
27	17.0	17.3	17.3	17.1
OMe				47.2
1′	101.6	102.3	102.2	101.6
2′	75.5	73.3	75.4	75.5
3′	78.6	75.6	78.6	78.6
4′	71.7	81.1	71.8	71.7
5′	78.1	75.0	78.4	78.1
6′	62.7	60.4	62.9	62.7
1″	106.2	105.2		106.1
2″	75.7	86.1		75.7
3″	78.5	78.5		78.5
4″	71.7	71.9		71.7
5″	78.0	78.2		78.0
6″	63.0	63.0		62.9
1‴		107.0		104.9
2‴		76.8		75.2
3‴		77.7		78.6
4‴		70.3		71.7
5‴		79.0		78.5
6‴		61.6		62.9

an anomeric proton and carbon at δ_H 5.09 (d, J=7.7 Hz)/δ_C 102.2, and an acetalic carbon at δ 109.2. Acid hydrolysis of **3** with 1 M HCl in dioxane–H_2O (1 : 1) gave chlorogenin and D-glucose. The anomelic proton signal of the glucosyl residue at δ 5.09 exhibited an HMBC correlation with the δ 77.6 resonance assignable to C-3 of the aglycone. The structure of **3** was assigned to be (25*R*)-6α-hydroxy-5α-spirostan-3β-yl β-D-glucopyranoside.

Compound **4** was obtained as an amorphous solid with the molecular formula of $C_{46}H_{78}O_{20}$. The ^{1}H-NMR spectrum showed signals for four steroid methyl groups at δ 1.17 (d, J=6.9 Hz), 1.01 (d, J=6.6 Hz), 0.74 (s), and 0.72 (s), and a methoxy group at δ 3.27 (s), as well as signals for three anomeric protons at δ 5.14 (d, J=7.7 Hz), 4.91 (d, J=7.7 Hz), and 4.85 (d, J=7.8 Hz). The above ^{1}H-NMR data, an acetalic carbon signal at δ 112.5 in the ^{13}C-NMR spectrum, and a positive color reaction in Ehrlich's test, indicated **4** to be a furostanol saponin with three monosaccharides. Enzymatic hydrolysis of **4** with β-D-glucosidase gave a known spirostanol saponin identified as (25*R*)-5α-spirostane-3β,6α-diyl bis-β-D-glucopyranoside[16] and D-glucose. An ROE correlation between the methoxy protons at δ 3.27 and the H-16 proton at δ 4.31 was consistent with the C-22α configuration.[17] As is usual with naturally occurring furostanol glycosides, one glucosyl group was shown to be linked to the C-26 hydroxy group of the aglycone by an HMBC correlation of the anomeric proton at δ 4.85 with C-26 of the aglycone at δ 75.2. Thus, **4** was determined to be (25*R*)-22α-methoxy-5α-furostane-3β,6α,26-triyl tris-β-D-glucopyranoside.

The structures of the isolated glycosides are based on (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin) as the aglycone. The chlorogenin glycosides have been obtained in good yields from the plants of the genus *Camassia* (Liliaceae)[14,18,19] and *Agave* (Agavaceae),[5] most of which are chlorogenin 6-*O*-glycosides. Chlorogenin 3-*O*-glycosides such as **2** and **3** are rarely found in plants. Compounds **1**—**6** did not show any apparent cytotoxic activity against HL-60 leukemia cells at a sample concentration of 10 μg/ml.

Experimental

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrophotometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. FAB-MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA, U.S.A.) and HR-ESI-TOF-MS were recorded on a Micromass LCT mass spectrometer (Manchester, U.K.). Elemental analysis was carried out using an Elementar Vario EL elemental analyzer (Hanau, Germany). 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 column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254} S plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4, 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.

Plant Material *A. utahensis* was obtained from a garden center (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 twice with hot MeOH (each 6 l). After the MeOH extract was concentrated under reduced pressure, the viscous concentrate (190 g) was passed through a Diaion HP-20 column and successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (each 4 l). Column chromatography of the MeOH-eluate portion on silica 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 four fractions (I—IV). 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** (11 mg), **2** (12 mg), **3** (5.0 mg), **5** (6.0 mg), and **6** (12 mg). Fraction IV was subjected to column chromatography 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 **4** (210 mg).

Compound **1**: Amorphous solid, $[\alpha]_D^{24}$ −34.0° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 795.4142 $[M+Na]^+$ (Calcd for $C_{39}H_{64}O_{15}Na$: 795.4143). IR ν_{max} (film) cm^{-1}: 3398 (OH), 2926 (CH), 1069, 1024. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 4.58 (1H, m, H-16), 3.98 (1H, m, H-3), 3.64 (1H, overlapping, H-6), 1.49 (3H, s, Me-21), 0.92 (3H, s, Me-18), 0.73 (3H, d, J=5.6 Hz, Me-27), 0.72 (3H, s, Me-19). Signals for the sugar moieties: see Table 1. ^{13}C-NMR: see Table 2.

Table 3. ^{1}H- and ^{13}C-NMR Chemical Shift Assignments for Compound **1a**

1a (C_5D_5N)			**1a** (C_5D_5N+HCl vapor)		
Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1 eq	1.67	38.0	1 eq	1.66	38.0
ax	1.01		ax	1.01	
2 eq	2.08	32.3	2 eq	2.08	32.3
ax	1.76		ax	1.76	
3	3.93 m	71.0	3	3.93 m	71.0
4 eq	3.02 br d (12.4)*a)*	33.7	4 eq	3.02 br d (12.4)	33.7
ax	1.68 q-like (12.4)		ax	1.69 q-like (12.4)	
5	1.33	52.7	5	1.33	52.7
6	3.68 m	68.6	6	3.68 ddd (10.7, 10.7, 4.1)	68.6
7 eq	2.23	42.5	7 eq	2.23	42.5
ax	1.19		ax	1.19	
8	1.64	33.9	8	1.64	33.9
9	0.64 ddd (10.9, 10.9, 3.8)	54.1	9	0.64 ddd (10.8, 10.8, 3.7)	54.1
10	—	36.5	10	—	36.5
11 eq	1.46	20.9	11 eq	1.46	20.9
ax	1.22		ax	1.23	
12 eq	1.97	40.4	12 eq	1.97	40.4
ax	1.23		ax	1.23	
13	—	41.4	13	—	41.4
14	1.01	57.1	14	1.01	57.1
15 α	2.06	31.7	15 α	2.05	31.7
β	1.45		β	1.45	
16	4.71	79.9	16	4.71	79.9
17	2.24 d (6.5)	71.5	17	2.24 d (6.4)	71.5
18	1.01 s	16.3	18	1.01 s	16.3
19	0.88 s	13.7	19	0.88 s	13.7
20	—	81.7	20	—	81.7
21	1.53 s	22.3	21	1.53 s	22.3
22	—	106.6	22	—	106.6
23 eq	1.80	30.6	23 eq	1.80	30.6
ax	1.79		ax	1.79	
24 (2H)	1.58	28.9	24 (2H)	1.58	28.9
25	1.59	30.5	25	1.59	30.5
26 eq	3.67 dd (10.8, 5.8)	68.4	26 eq	3.67 dd (11.0, 4.1)	68.4
ax	3.60 dd (10.8, 10.3)		ax	3.60 dd (11.0, 10.4)	
27	0.69 d (5.6)	17.0	27	0.69 d (5.2)	17.0
3-OH	6.08 br d (3.5)				
6-OH	5.85 br d (5.2)				
20-OH	5.06 br s				

a) The values in parentheses are coupling constants in Hz.

Enzymatic Hydrolysis of 1 Compound **1** (6.0 mg) was treated with naringinase (Sigma, St. Louis, MO, U.S.A., 41 mg) in HOAc/KOAc buffer (pH 4.3, 10 ml) at room temperature for 12 h. The reaction mixture was chromatographed on Diaion HP-20 and eluted with H_2O–MeOH (3 : 2) followed by Me_2CO–EtOH (1 : 1) to yield a sugar fraction (2.0 mg) and aglycone fraction. The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$–MeOH (19 : 1) to yield **1a** (3.4 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) and a Toyopak IC-SP M cartridge (Tosoh), 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-glucose present in the sugar fraction was carried out by comparison of its retention time and optical rotation with those of an authentic sample. t_R (min): 15.86 (D-glucose, positive optical rotation).

Compound **1a**: Amorphous solid, $[\alpha]_D^{24}$ −38.0° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 431.3158 $[M-OH]^+$ (Calcd for $C_{27}H_{43}O_4$: 431.3161). IR ν_{max} (film) cm^{-1}: 3333 (OH), 2928 (CH), 1261, 1037. ^{1}H- and ^{13}C-NMR: see Table 3.

Compound **2**: Amorphous solid, $[\alpha]_D^{24}$ −34.0° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 941.4751 $[M+Na]^+$ (Calcd for $C_{45}H_{74}O_{19}Na$: 941.4722). IR ν_{max} (film) cm^{-1}: 3375 (OH), 2928 (CH), 1074. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 4.55 (1H, m, H-16), 4.02 (1H, m, H-3), 3.58 (1H, ddd, J=10.8, 10.1, 4.7 Hz, H-6), 1.15 (3H, d, J=6.9 Hz, Me-21), 0.84 (3H, s, Me-18), 0.72 (3H, s, Me-19), 0.70 (3H, d, J=5.4 Hz, Me-27). Signals for the sugar moieties: see Table 1. ^{13}C-NMR: see Table 2.

Acid Hydrolysis of 2 A solution of **2** (11 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 (Organo, Tokyo, Japan) column and was chromatographed on silica gel eluted with $CHCl_3$–MeOH (9 : 1) to yield chrologenin (2.3 mg) and a sugar fraction (2.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-galactose and D-glucose. t_R (min): 14.57 (D-galactose, positive optical rotation), 15.86 (D-glucose, positive optical rotation).

Compound **3**: Amorphous solid, $[\alpha]_D^{24}$ −18.0° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 617.3666 $[M+Na]^+$ (Calcd for $C_{33}H_{54}O_9Na$: 617.3666). IR ν_{max} (film) cm^{-1}: 3364 (OH), 2927 (CH), 1038. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 4.55 (1H, m, H-16), 4.07 (1H, m, H-3), 3.60 (1H, overlapping, H-6), 1.15 (3H, d, J=7.0 Hz, Me-21), 0.85 (3H, s, Me-18), 0.76 (3H, s, Me-19), 0.70 (3H, d, J=5.6 Hz, Me-27). Signals for the sugar moiety: see Table 1. ^{13}C-NMR: see Table 2.

Acid Hydrolysis of 3 A solution of **3** (4.5 mg) was subjected to acid hydrolysis as described for **2** to give chrologenin (2.0 mg) and a sugar fraction (1.0 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose. t_R (min): 15.86 (D-glucose, positive optical rotation).

Compound **4**: Amorphous solid, $[\alpha]_D^{28}$ −24.0° (c=0.10, MeOH). FAB-MS (negative mode) m/z 949 $[M-H]^-$. *Anal.* Calcd for $C_{46}H_{78}O_{20}\cdot 3H_2O$: C, 54.97; H, 8.42. Found: C, 55.01; H, 8.47. IR ν_{max} (KBr) cm^{-1}: 3375 (OH),

2933 (CH), 1076, 1029. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 4.31 (1H, m, H-16), 3.97 (1H, m, H-3), 3.27 (3H, s, OMe), 3.65 (1H, ddd, J=11.3, 10.3, 4.6 Hz, H-6), 1.17 (3H, d, J=6.9 Hz, Me-21), 1.01 (3H, d, J=6.6 Hz, Me-27), 0.74 (3H, s, Me-19), 0.72 (3H, s, Me-18). Signals for the sugar moieties: see Table 1. ^{13}C-NMR: see Table 2.

Enzymatic Hydrolysis of 4 Compound **4** (10 mg) was treated with β-D-glucosidase (Sigma, St. Louis, U.S.A., 5.8 mg) in HOAc/NaOAc buffer (pH 5.0, 10 ml) at room temperature for 12 h. The reaction mixture was chromatographed on Diaion HP-20, eluted with H_2O–MeOH (3 : 2) followed by Me_2CO–EtOH (1 : 1), and on silica gel eluted with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to yield (25*R*)-5α-spirostan-3β,6α-diyl bis-β-D-glucopyranoside (5.6 mg) and D-glucose (2.0 mg).

HL-60 Cell Culture Assay HL-60 cell growth was measured with an MTT reduction assay as described in a previous paper.[20]

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17) Compound **4** was obtained as a mixture of the C-22 hydroxy and C-22 methoxy forms. The C-22 hydroxy form present in the mixture was completely converted to the C-22 methoxy form by treatment with hot MeOH, and the structural elucidation of **4** was carried out with the C-22 methoxy form.
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