Steroidal Glycosides from *Furcraea foetida* and Their Cytotoxic Activity

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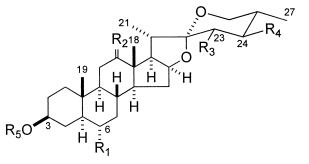
Two new spirostanol glycosides (1, 2) and a new furostanol glycoside (3), together with nine known steroidal glycosides (4—12) were isolated from the leaves of *Furcraea foetida* (Agavaceae). The structures of the new compounds were determined by spectroscopic analysis and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activities against HL-60 human leukemia cells, A549 human lung adenocarcinoma cells, HSC-2 human oral squamous carcinoma cells, and HSC-4 human oral squamous carcinoma cells.

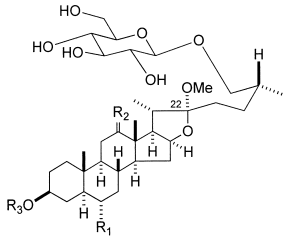
Key words *Furcraea foetida*; Agavaceae; spirostanol glycoside; furostanol glycoside

The genera *Agave*, *Dracaena*, *Polianthes*, and *Yucca* belong to the Agavaceae family and are known to be rich sources of steroidal sapogenins and saponins. Our previous chemical studies on *Agave americana*,[1] *Agave utahensis*,[2,3] *Dracaena surculosa*,[4,5] and *Polianthes tuberosa*[6,7] led the isolation of a variety of steroidal glycosides, some of which show cytotoxic activities against cultured tumor cells. The genus *Furcraea* is also classified in the Agavaceae family, has about 20 species, and is mainly cultivated as ornamental plants.[8] *Furcraea foetida* (L.) HAW. is native to northern South America and is distributed widely in frost-free climates. This plant is called "Mauritius hemp" and its leaves are used as a commercial fiber crop on Mauritius Island in the Indian Ocean.[9] Although a few steroidal components such as furcreastatin were isolated from *F. foetida*,[10] there has been no systematic exploration of its secondary metabolites. As part of our continuing chemical investigation of Agavaceae plants aimed at the steroidal glycoside constituents, phytochemical screening was performed on the leaves of *F. foetida*, resulting in the isolation of 12 steroidal glycosides including two new spirostanol glycosides (**1**, **2**) and a new furostanol glycoside (**3**). In this paper, we describe the structural elucidation of the three new compounds on the basis of spectroscopic analysis and the results of hydrolytic cleavage. The cytotoxic activities of the isolated compounds against HL-60 human leukemia cells, A549 human lung adenocarcinoma cells, HSC-2 human oral squamous carcinoma cells, and HSC-4 human oral squamous carcinoma cells are also reported.

The leaves of *F. foetida* (13 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 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. The MeOH-eluate fraction was repeatedly subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography to afford compounds **1**—**12**. Compounds **4**—**12** were identified as (25*R*)-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-

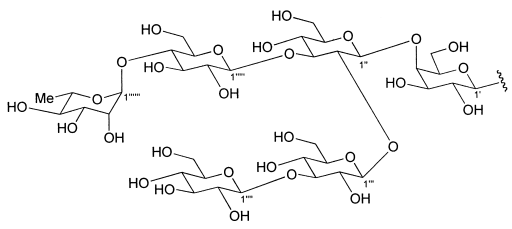


	R_1	R_2	R_3	R_4	R_5
1	H	H,H	H	-O-Glc	S
2	-O-Glc	H,H	OH	H	Fuc
4	H	H,H	H	H	S
5	H	O	H	H	S
6	OH	H,H	H	H	S
7	-O-Glc	H,H	OH	H	H
8	-O-Glc	H,H	H	H	Glc
9	-O-Glc	H,H	OH	H	Glc

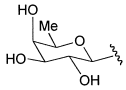


	R_1	R_2	R_3
3	OH	H,H	S
10	H	H,H	S
11	H	O	S
12	-O-Glc	H,H	Glc

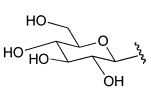
S:



Fuc:



Glc:



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glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**4**),[11] (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirostan-12-one (furcreastatin, **5**),[10] (25*R*)-6α-hydroxy-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**6**),[12] (23*S*,25*R*)-3β,23-dihydroxy-5α-spirostan-6α-yl β-D-glucopyranoside (**7**),[13] (25*R*)-5α-spirostane-3β,6α-diyl bis-β-D-glucopyranoside (**8**),[14] (23*S*,25*R*)-23-hydroxy-5α-spirostane-3β,6α-diyl bis-β-D-glucopyranoside (**9**),[15] (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-methoxy-5α-furostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**10**),[16] (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-26α-[(β-D-glucopyranosyl)oxy]-22-methoxy-5α-furostan-12-one (**11**),[17] and (25*R*)-22α-methoxy-5α-furostane-3β,6α,26-triyl tris-β-D-glucopyranoside (**12**)[3] based on their physical and spectral data.

Compound **1** was isolated as an amorphous solid. Its molecular formula, $C_{69}H_{114}O_{38}$, was determined from high-resolution (HR)-electrospray ionization (ESI)-time of flight (TOF)-MS (*m/z* 1551.7115 $[M+H]^+$). The ^{1}H-NMR spectrum of **1** showed signals for four steroidal methyl groups at δ 1.14 (d, *J*= 6.6 Hz), 1.05 (d, *J*= 6.6 Hz), 0.72 (s), and 0.62 (s), as well as signals for seven anomeric protons at δ 5.77 (br s), 5.53 (d, *J*=7.5 Hz), 5.18 (d, *J*=7.6 Hz), 5.12 (d, *J*=7.7 Hz), 5.09 (d, *J*=7.6 Hz), 4.90 (d, *J*=7.6 Hz), and 4.85 (d, *J*=7.2 Hz), and the methyl group of a 6-deoxyhexopyranosyl moiety at δ 1.68 (d, *J*=6.1 Hz). Acid hydrolysis of **1** with 1 M HCl in dioxane–H_2O (1 : 1) gave D-galactose, D-glucose, and L-rhamnose, while the aglycone was decomposed under acidic conditions. Identification of monosaccharides, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate. Comparison of the ^{1}H- and ^{13}C-NMR assignments of the aglycone moiety of **1**, which were established by analysis of the ^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC), and ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectra, with those of the known compound **4** revealed that the structures of the ring A—E portions (C-1—C-21) and hexaglycosyl moiety attached to C-3 of the aglycone are identical to those of **4** including the orientation of the C-3 oxygen atom (3β-equatorial) and ring junctions (B/C *trans*, C/D *trans*, and D/E *cis*). However, significant differences were recognized in the signals for the ring F portion (C-22—C-27). The ^{1}H–^{1}H COSY spectrum was carefully inspected to assign the structure of the ring F residue, with a three-proton doublet signal at δ 1.14 (d, *J*=6.6 Hz), attributable to Me-27, used as the starting point for analysis. The Me-27 protons showed a spin-coupling correlation with a broad multiplet signal centered at δ 1.89, which was assigned to H-25, and exhibited correlations with a pair of oxymethylene protons at δ 3.63 and 3.57,

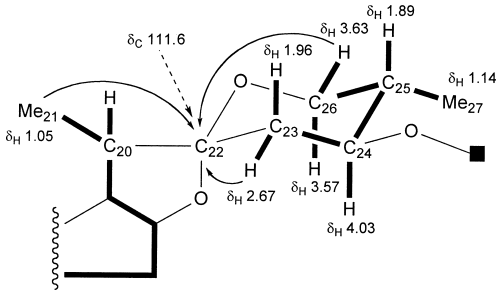

Fig. 1. Partial Structure of **1**

Bold lines indicate the ^{1}H–^{1}H spin couplings and arrows indicate ^{1}H/^{13}C long-range correlations.

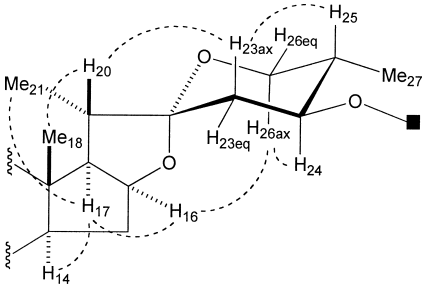

Fig. 2. Important NOE Correlations of **1**

and with an oxymethine proton at δ 4.03. The oxymethine proton displayed correlations with the terminal methylene protons at δ 2.67 and 1.96. These subsequent correlations led us to propose the ring F fragment of **1** to be $-C_{(23)}H_2-C_{(24)}H(O)-C_{(25)}H(Me_{(27)})-C_{(26)}H_2-O-$. Evidence for the connectivity of the terminal free bonds of the fragment to C-22 was obtained by observation of ^{1}H/^{13}C long-range correlations from the methylene protons at δ 2.67 and 1.96 and the oxymethylene protons at δ 3.63 and 3.57 to C-22 at δ 111.6 in the HMBC spectrum (Fig. 1). Thus the presence of an oxygen atom at C-24 is evident. The proton multiplicity of H-24, with *J* values of 12.0 Hz (H-24/H-23ax), 10.2 Hz (H-24/H-25), and 4.2 Hz (H-24/H-23eq), and NOE correlations between H-23ax and H-20/H-25, and between H-26ax and H-24/H-16 in the phase-sensitive NOESY spectrum were consistent with the 22α, 24*S*, and 25*S* configurations (Fig. 2). The exact structures of the sugar moieties and their linkage positions to the aglycone were resolved by detailed analysis of the one-dimensional (1D) totally correlated spectroscopy (TOCSY) and two-dimensional (2D) NMR spectra. The ^{1}H-NMR subspectra of individual monosaccharide units were obtained using selective irradiation of easily identifiable anomeric proton signals, as well as nonoverlapping proton signals in a series of 1D TOCSY experiments.[18—20] Subsequent analysis of the ^{1}H–^{1}H COSY spectrum resulted in the sequential assignments of all the proton resonances due to the seven glycosyl units, including identification of their multiplet patterns and coupling constants (Table 1). The HMQC and ^{1}H-detected heteronuclear single-quantum coherence (HSQC)-TOCSY spectra correlated the proton resonances with those of the corresponding one-bond coupled carbons, leading to unambiguous assignments of the carbon shifts. Comparison of the carbon chemical shifts thus assigned with those of reference methyl glycosides,[21,22] taking into account the known effects of *O*-glycosylation, indicated

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shift Assignments of the Sugar Moieties for Compounds **1**—**3**[a)]

1				**2**				**3**			
Position	^{1}H	*J* (Hz)	^{13}C	Position	^{1}H	*J* (Hz)	^{13}C	Position	^{1}H	*J* (Hz)	^{13}C
Gal 1′	4.85 d	7.2	102.4	Fuc 1	4.92 d	7.9	101.9	Gal 1′	4.82 d	7.8	102.4
2′	4.39 dd	9.6, 7.2	73.1	2	4.35 dd	9.0, 7.9	72.4	2′	4.34 dd	9.6, 7.8	73.1
3′	4.10 br d	9.6	75.3	3	4.07 dd	9.0, 3.0	75.4	3′	4.02 br d	9.6	75.4
4′	4.58 br s		80.0	4	3.99 br s		72.8	4′	4.53 br s		80.2
5′	3.99 m		75.4	5	3.64 q-like	6.4	71.1	5′	3.82 m		75.2
6′a	4.66 m		60.6	6	1.46 d	6.4	17.4	6′a	4.61 dd	9.0, 9.0	60.5
b	4.21 m							b	4.14 m		
Glc 1″	5.12 d	7.7	104.8	6-Glc 1	4.88 d	7.7	106.4	Glc 1″	5.10 d	7.8	104.8
2″	4.31 dd	8.0, 7.7	80.8	2	4.04 dd	8.5, 7.7	75.6	2″	4.29 dd	8.5, 7.8	80.8
3″	4.12 dd	8.0, 8.0	88.1	3	4.24 dd	8.5, 7.4	78.6	3″	4.15 dd	8.5, 8.5	88.1
4″	3.73 dd	8.0, 8.0	70.6	4	4.25 dd	7.4, 7.4	71.9	4″	3.73 m		70.4
5″	3.78 m		77.4	5	3.97 m		78.1	5″	3.80 m		77.5
6″a	4.42 br d	13.4	62.9	6a	4.54 br d	11.0	63.1	6″a	4.40 br d	11.3	62.8
b	3.96 br d	13.4		b	4.41 br d	11.0		b	3.96 dd	11.3, 3.2	
Glc 1‴	5.53 d	7.5	104.0					Glc 1‴	5.54 d	7.4	103.9
2‴	4.09 dd	9.3, 7.5	74.6					2‴	4.07 dd	8.6, 7.4	74.6
3‴	4.02 dd	9.3, 8.8	88.1					3‴	4.03 dd	8.6, 8.6	88.1
4‴	4.09 m		69.2					4‴	4.11 dd	8.6, 7.8	69.2
5‴	3.77 m		78.0					5‴	3.76 m		78.0
6‴a	4.42 br d	9.7	62.0					6‴a	4.36 m		61.9
b	4.26 br d	9.7						b	4.25 br d	13.0	
Glc 1⁗	5.09 d	7.6	105.6					Glc 1⁗	5.06 d	7.6	105.6
2⁗	4.01 dd	7.6, 7.2	75.6					2⁗	4.00 dd	8.2, 7.6	75.4
3⁗	4.16 dd	7.2, 7.2	78.0					3⁗	4.10 dd	8.2, 7.9	77.9
4⁗	4.15 dd	7.2, 7.2	71.5					4⁗	4.10 dd	7.9, 7.9	71.5
5⁗	3.87 m		78.5					5⁗	3.87 m		78.4
6⁗a	4.49 br d	11.6	62.4					6⁗a	4.45 br d	13.3	62.4
b	4.25 br d	11.6						b	4.22 br d	13.3	
Glc 1⁗′	5.18 d	7.6	104.2					Glc 1⁗′	5.18 d	6.6	104.2
2⁗′	3.95 dd	9.2, 7.6	75.6					2⁗′	3.94 dd	9.2, 6.6	75.5
3⁗′	4.07 dd	9.2, 9.2	76.5					3⁗′	4.04 dd	9.2, 9.2	76.4
4⁗′	4.34 dd	9.2, 8.5	77.8					4⁗′	4.29 dd	9.2, 7.6	78.0
5⁗′	3.78 m		77.2					5⁗′	3.78 m		77.2
6⁗′a	4.21 br d	12.0	61.0					6⁗′a	4.18 br d	13.0	61.0
b	4.02 br d	12.0						b	4.01 br d	13.0	
Rha 1⁗″	5.77 br s		102.6					Rha 1⁗″	5.70 br s		102.6
2⁗″	4.61 br s		72.5					2⁗″	4.54 br s		72.4
3⁗″	4.51 br d	8.6	72.7					3⁗″	4.45 br d	8.3	72.5
4⁗″	4.32 dd	9.6, 8.6	73.9					4⁗″	4.28 dd	9.5, 8.3	73.8
5⁗″	4.93 dq	9.6, 6.1	70.3					5⁗″	4.87 dq	9.5, 6.6	70.4
6⁗″	1.68 d	6.1	18.5					6⁗″	1.67 d	6.6	18.5
24-Glc 1	4.90 d	7.6	106.4					26-Glc 1	4.82 d	7.8	105.0
2	4.04 dd	8.5, 7.6	75.7					2	4.02 dd	8.8, 7.8	75.1
3	4.20 dd	8.9, 8.5	78.6					3	4.19 dd	8.8, 8.8	78.5
4	4.26 dd	8.9, 8.9	71.7					4	4.17 dd	8.8, 7.4	71.7
5	3.85 m		78.0					5	3.93 m		78.5
6a	4.49 br d	13.1	62.8					6a	4.52 br d	12.0	62.8
b	4.36 br d	13.1						b	4.34 br d	12.0	

a) Compounds **1** and **2** were measured in C_5D_5N, and **3** in C_5D_5N–CD_3OD (10 : 1).

that **1** contains two C-4 substituted β-D-galactopyranosyl moieties (Gal′ and Glc⁗′), a C-2 and C-3 disubstituted β-D-glucopyranosyl moiety (Glc″), a C-3 substituted β-D-glucopyranosyl moiety (Glc‴), two terminal β-D-glucopyranosyl moieties (Glc⁗ and Glc), and a terminal α-L-rhamnopyranosyl moiety (Rha⁗″). The anomeric conformations of the Gal and Glc groups were ascertained by the relatively large *J* values of their anomeric protons (7.2—7.7 Hz).[23)] For the Rha moiety, the large $^{1}J_{C-H}$ value (171.9 Hz) provides evidence that the anomeric proton is equatorial, thus possessing an α-pyranoid anomeric form.[24)] In the HMBC spectrum of **1**, long-range correlations were observed between the anomeric proton (H-1) of Glc⁗ at δ_H 5.09 and C-3 of Glc‴ at δ_C 88.1, H-1 of Glc‴ at δ_H 5.53 and C-2 of Glc″ at δ_C 80.8, H-1 of Rha⁗″ at δ_H 5.77 and C-4 of Glc⁗ at δ_C 77.8, H-1 of Glc⁗′ at δ_H 5.18 and C-3 of Glc″ at δ_C 88.1, H-1 of Glc″ at δ_H 5.12 and C-4 of Gal′ at δ_C 80.0, H-1 of Gal′ at δ_H 4.85 and C-3 of the aglycone at δ_C 77.3, and between H-1 of Glc at δ_H 4.90 and C-24 of the aglycone at δ_C 81.6. Accordingly, the structure of **1** was characterized as (24*S*,25*S*)-24-[(β-D-glucopyranosyl)oxy]-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **2** was determined to be $C_{39}H_{64}O_{14}$ from its HR-ESI-TOF-MS data. The ^{1}H-NMR spectrum of **2** con-

tained signals for four steroid methyl groups at δ 1.19 (3H, d, J=7.1 Hz), 0.97 (3H, s), 0.75 (3H, d, J=6.2 Hz), and 0.64 (3H, s), two anomeric protons at δ 4.92 (1H, d, J=7.9 Hz) and 4.88 (1H, d, J=7.7 Hz), and the methyl group of a 6-deoxyhexopyranosyl moiety at δ 1.46 (1H, d, J=6.4 Hz). Acid hydrolysis of **2** with 1 M HCl gave (23*S*,25*R*)-5α-spirostane-3β,6α,23-triol (chrysogenin),[25] D-fucose, and D-glucose. Thus **2** is a chrysogenin diglycoside. The ^{1}H- and ^{13}C-NMR spectra of **2** showed the presence of a terminal β-D-fucopyranosyl unit (Fuc) [$\delta_{H\text{-}1}$ 4.92 (1H, d, J=7.9 Hz); δ_C 101.9, 72.4, 75.4, 72.8, 71.1, and 17.4] and a terminal β-D-glucopyranosyl unit (Glc) [$\delta_{H\text{-}1}$ 4.88 (1H, d, J=7.7 Hz); δ_C 106.4, 75.6, 78.6, 71.9, 78.1, and 63.1] in **2**. In the HMBC spectrum, long-range correlations were observed between H-1 of Fuc at δ 4.92 and C-3 of the aglycone at δ 76.7, and between H-1 of Glc at δ 4.88 and C-6 of the aglycone at δ 80.1. Accordingly, the structure of **2** was formulated as (23*S*,25*R*)-6α-[(β-D-glucopyranosyl)oxy]-23-hydroxy-5α-spirostan-3β-yl β-D-fucopyranoside.

Compound **3** was obtained as an amorphous solid with the molecular formula of $C_{70}H_{118}O_{39}$. The ^{1}H-NMR spectrum of **3** contained signals for four steroid methyl groups at δ 1.19 (3H, d, J=6.6 Hz), 1.01 (3H, d, J=6.6 Hz), 0.81 (3H, s), and 0.72 (3H, s), seven anomeric protons at δ 5.70 (1H, br s), 5.54 (1H, d, J=7.4 Hz), 5.18 (1H, d, J=6.6 Hz), 5.10 (1H, d, J=7.8 Hz), 5.06 (1H, d, J=7.6 Hz), and 4.82 (2H, d, J=7.8 Hz), a methoxy group at δ 3.27 (3H, s), and the methyl group of a 6-deoxyhexopyranosyl moiety at δ 1.67 (d, J=6.6 Hz). The above ^{1}H-NMR data, an acetalic carbon signal at δ 112.7 in the ^{13}C-NMR spectrum, and a positive color reaction in Ehrlich's test[26,27] indicate **3** to be a furostanol saponin with sugar moieties composed of seven monosaccharides. Enzymatic hydrolysis of **3** with β-D-glucosidase gave **6** and D-glucose. The ^{1}H- and ^{13}C-NMR signals due to the sugar moieties were assigned as shown in Table 1 on the basis of the 1D TOCSY, ^{1}H–^{1}H COSY, HMQC, and HSQC-TOCSY results. In the HMBC spectrum, a correlation peak between H-1 of Glc (terminal glucosyl) at δ 4.82 and C-26 of the aglycone at δ 75.3 implied that one glucose unit is attached to C-26 of the aglycone, which is a structural feature often encountered in plant furostanol saponins. The hexaglycoside attached to C-3 of the aglycone was ascertained by HMBC correlations between H-1 of Glc$''''$ at δ 5.06 and C-3 of Glc$'''$ of δ 88.1, H-1 of Glc$'''$ at δ_H 5.54 and C-2 of Glc$''$ at δ 80.8, H-1 of Rha$''''$ at δ 5.70 and C-4 of Glc$''''$ at δ 78.0, H-1 of Glc$''''$ at δ 5.18 and C-3 of Glc$''$ at δ 88.1, H-1 of Glc$''$ at δ 5.10 and C-4 of Gal$'$ at δ 80.2, and between H-1 of Gal$'$ at δ 4.82 and C-3 of the aglycone at δ 77.9. An NOE correlation between the methoxy protons at δ 3.27 and the H-16 proton at δ 4.45 is consistent with the C-22α configuration.[28] Thus, **3** was determined to be (25*R*)-26-[(β-D-glucopyranosyl)oxy]-6α-hydroxy-22α-methoxy-5α-furostan-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compounds **1**—**3** are new steroidal saponins. Compound **2** is believed to be the first representative of a steroidal glycoside with a β-D-fucopyranosyl unit at C-3 of the spirostanol skeleton.

The isolated compounds (**1**—**12**) were evaluated for their cytotoxic activity against HL-60 cells. The spirostanol saponins (**4**, **5**) showed cytotoxic activity with IC_{50} values of 3.5 and 3.5 μM, respectively, whereas the other spirostanol saponins (**1**, **2**, **6**—**9**) did not exhibit apparent cytotoxicity even at the sample concentration of 20 μM. The above results indicate that the C-6 hydroxy group or C-6 *O*-glucosyl group significantly reduces the cytotoxic activity in these spirostanol saponins. Although furostanol saponins usually exhibit no cytotoxicity against HL-60 cells except for the furostanol glycosides with an α-L-rhamnosyl-(1→2)-*O*-β-D-glucosyl unit or an α-L-rhamnosyl-(1→2)-[α-L-rhamnosyl-(1→4)]-*O*-β-D-glucosyl unit at C-3 of the proto-diosgenin-type aglycone ((25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-methoxyfurost-5-ene),[29] compound **10**, the corresponding bisdesmosidic furostanol saponin of **4** was cytotoxic to HL-60 cells with IC_{50} values of 5.0 μM. Compounds **4**, **5**, and **10** were also evaluated for their cytotoxic activities against three solid tumor cell lines. All three compounds showed considerable cytotoxicity, with IC_{50} values ranging from 1.4 to 4.6 μM against A549 cells, HSC-2 cells, and HSC-4 cells, whereas these cells were resistant to the anticancer agents etoposide and cisplatin used as positive controls (Table 2).

Table 2. Cytotoxic Activity of Compounds **1**—**12** against Four Human Cancer Cell Lines[a,b]

Compound	IC_{50} (μM)			
	HL-60	A549	HSC-2	HSC-4
4	3.5±0.16	2.4±0.18	1.4±0.13	3.0±0.19
5	3.5±0.07	3.0±0.02	3.5±0.09	2.8±0.05
10	5.0±0.35	2.9±0.03	4.6±0.22	1.6±0.06
Cisplatin[c]	1.6±0.03	6.4±0.43	16.9±0.60	>20
Etoposide[c]	0.38±0.02	>20	>20	>20
Doxorubicin[c]	0.07±0.01	1.7±0.03	0.25±0.03	0.88±0.04

a) Data represent the mean±SEM of three independent experiments. *b*) Compounds **1**—**3**, **6**—**9**, **11**, and **12** were inactive against HL-60 cells (IC_{50}>20 μM). *c*) Positive control substances.

Experimental

Optical rotations were measured using a JASCO P-1030 (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) and a Bruker DRX-600 (600 MHz for ^{1}H-NMR) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HR-ESI-TOF-MS was recorded on a Waters-Micromass LCT mass spectrometer (Manchester, U.K.). 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 solution, followed by heating. HPLC was performed using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a PX-8010 controller (Tosoh), an RI-8010 (Tosoh) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. A Capcell Pak C_{18} UG120 Å column (10 mm i.d.×250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: RPMI-1640 medium, minimum essential medium (MEM), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, U.S.A.); fetal bovine serum (FBS) (Bio-Whittaker, Walkersville, MD, U.S.A.); and Dulbecco's modified Eagle's medium (DMEM), penicillin G sodium salt, and streptomycin sulfate (Gibco, Grand Island, NY, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material *F. foetida* was purchased from a nursery in Exotic Plants

(Chiba, Japan) in June 2002 and identified by Dr. Yutaka Sashida, emeritus professor of Medicinal Pharmacognosy, Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher no. FF-2002-001, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation The plant material (fresh weight, 13 kg) was extracted twice with hot MeOH (each 30 l). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (1.0 kg) was passed through a Diaion HP-20 column (85 mm i.d.×200 mm) and successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (each 15 l). Column chromatography of the MeOH-eluate portion (190 g) on silica gel (85 mm i.d.×200 mm), elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (9 : 1 : 0; 30 : 10 : 1; 7 : 4 : 1), and finally with MeOH alone, gave nine fractions (I—IX). Fraction IV was chromatographed on ODS silica gel (40 mm i.d.×240 mm) eluted with MeOH–H_2O (4 : 1) to give **7** (34.0 mg). Fraction V was subjected to column chromatography on ODS silica gel (40 mm i.d.×260 mm) eluted with MeOH–H_2O (3 : 1) and on silica gel (25 mm i.d.×150 mm) eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1; 30 : 10 : 1) to give **2** (2.3 mg) and **9** (48.1 mg). Fraction VI was suspended in MeOH, and the insoluble solid was filtered to give **8** (20 g). Fraction VIII was separated on a silica gel column (75 mm i.d.×240 mm) eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) to give eight subfractions (VIIIa—VIIIh). Fraction VIIId was further separated on an ODS silica gel column (40 mm i.d.×260 mm) eluted with MeCN–H_2O (1 : 2) to give seven fractions (VIIId-1—VIIId-7). Fraction VIIId-1 was subjected to a silica gel column (25 mm i.d.×210 mm) eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and an ODS silica gel column (20 mm i.d.×230 mm) with MeOH–H_2O (3 : 2) to afford **12** (48.4 mg). Fraction VIIId-7 was subjected to ODS silica gel column chromatography (40 mm i.d.×240 mm) eluted with MeOH–H_2O (3 : 1) to give **5** (33.8 mg). Fraction VIIIh was subjected to column chromatography on ODS silica gel (55 mm i.d.×220 mm) eluted with MeCN–H_2O (2 : 5) to give eight fractions (VIIIh-1—VIIIh-8). Fraction VIIIh-2 was subjected to an ODS silica gel column (40 mm i.d.×220 mm) eluted with MeCN–H_2O (1 : 4) to give **11** (32.9 mg). Fraction VIIIh-3 was subjected to an ODS silica gel column (40 mm i.d.×230 mm) eluted with MeCN–H_2O (1 : 4; 2 : 7) and an ODS silica gel column (25 mm i.d.×180 mm) with MeOH–H_2O (1 : 1) to give **3** (22.0 mg). Fraction VIIIh-6 was purified by column chromatography on ODS silica gel (40 mm i.d.×220 mm) eluted with MeCN–H_2O (1 : 3; 2 : 5) and ODS silica gel (40 mm i.d.×230 mm) eluted with MeOH–H_2O (3 : 2) to give **1** (8.8 mg) and **10** (400 mg). Fraction VIIIh-8 was subjected to an ODS silica gel column (20 mm i.d.×210 mm) eluted with MeOH–H_2O (7 : 3) to give **6** (11.5 mg). Fraction IX was subjected to column chromatography on silica gel (75 mm i.d.×270 mm) eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 15 : 10 : 1; 7 : 4 : 1), ODS silica gel (40 mm i.d.×250 mm) eluted with MeCN–H_2O (1 : 2), and on ODS silica gel (40 mm i.d.×250 mm) eluted with MeOH–H_2O (3 : 1) to furnish **4** (35.3 mg).

Compound **1**: Amorphous solid, $[\alpha]_D^{25}$ −36.7° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 1551.7115 $[M+H]^+$ (Calcd for $C_{69}H_{115}O_{38}$: 1551.7066). IR ν_{max} (film) cm^{-1}: 3400 (OH), 2954, 2921, and 2850 (CH), and 1157 and 1065 (C–O). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 4.50 (1H, m, H-16), 4.03 (1H, ddd, J=12.0, 10.2, 4.2 Hz, H-24), 3.89 (1H, m, $W_{1/2}$=23.1 Hz, H-3), 3.63 (1H, dd, J=11.4, 4.5 Hz, H-26eq), 3.57 (1H, dd, J=11.4, 10.8 Hz, H-26ax), 2.67 (1H, dd, J=12.0, 4.2 Hz, H-23eq), 1.96 (1H, dd, J=12.0, 12.0 Hz, H-23ax), 1.90 (1H, m, H-20), 1.89 (1H, m, H-25), 1.72 (1H, m, H-17), 1.14 (3H, d, J=6.6 Hz, Me-27), 1.05 (3H, d, J=6.6 Hz, Me-21), 0.72 (3H, s, Me-18), 0.62 (3H, s, Me-19). Signals for the sugar moieties: see Table 1. ^{13}C-NMR (150 MHz, C_5D_5N) δ: 37.1 (C-1), 29.8 (C-2), 77.3 (C-3), 34.7 (C-4), 44.6 (C-5), 28.9 (C-6), 32.3 (C-7), 35.1 (C-8), 54.3 (C-9), 35.8 (C-10), 21.2 (C-11), 40.0 (C-12), 40.7 (C-13), 56.4 (C-14), 32.0 (C-15), 81.5 (C-16), 62.5 (C-17), 16.5 (C-18), 12.3 (C-19), 42.1 (C-20), 14.9 (C-21), 111.6 (C-22), 40.8 (C-23), 81.6 (C-24), 38.2 (C-25), 65.1 (C-26), 13.5 (C-27). Signals for the sugar moieties: see Table 1.

Acid Hydrolysis of 1 A solution of **1** (5.0 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 3 ml) was heated at 95 °C for 1.5 h under an Ar atomosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column and chromatographed on Diaion HP-20, eluted with MeOH–H_2O (2 : 3) followed by EtOH–Me_2CO (1 : 1), to yield a sugar fraction (1.2 mg). The sugar fraction was analyzed using HPLC under the following conditions: column, Capcell Pak NH2 UG80 Å (4.6 mm i.d.×250 mm, 5 mm, Shiseido); solvent, MeCN–H_2O (17 : 3); flow rate, 0.9 ml/min; and detection, RI and OR. Identification of L-rhamnose, D-galactose, and D-glucose present in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min): 8.28 (L-rhamnose, negative optical rotation), 17.45 (D-galactose, positive optical rotation), and 18.05 (D-glucose, positive optical rotation).

Compound **2**: Amorphous solid, $[\alpha]_D^{25}$ −22.0° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 779.4140 $[M+Na]^+$ (Calcd for $C_{39}H_{64}O_{14}$ Na: 779.4194). IR ν_{max} (film) cm^{-1}: 3389 (OH), 2952, 2926, 2871, and 2853 (CH), and 1163, 1095 and 1067 (C–O). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 4.56 (1H, m, H-16), 4.00 (1H, m, $W_{1/2}$=20.2 Hz, H-3), 3.85 (1H, m, H-23), 3.56 (1H, ddd, J=10.7, 10.4, 4.3 Hz, H-6), 3.54 (1H, dd, J=10.7, 10.7 Hz, H-26ax), 3.48 (1H, d, J=10.7, 4.3 Hz, H-26eq), 1.19 (3H, d, J=7.1 Hz, Me-21), 0.97 (3H, s, Me-18), 0.75 (3H, d, J=6.2 Hz, Me-21), 0.64 (3H, s, Me-19). Signals for the sugar moieties: see Table 1. ^{13}C-NMR (125 MHz, C_5D_5N) δ: 37.6 (C-1), 29.9 (C-2), 76.7 (C-3), 28.6 (C-4), 51.0 (C-5), 80.1 (C-6), 41.5 (C-7), 33.9 (C-8), 53.8 (C-9), 36.7 (C-10), 21.2 (C-11), 40.4 (C-12), 41.3 (C-13), 56.3 (C-14), 32.1 (C-15), 81.6 (C-16), 62.5 (C-17), 16.9 (C-18), 13.3 (C-19), 35.8 (C-20), 14.7 (C-21), 111.7 (C-22), 67.5 (C-23), 38.8 (C-24), 31.7 (C-25), 66.0 (C-26), 16.9 (C-27). Signals for the sugar moieties: see Table 1.

Acid Hydrolysis of 2 A solution of **2** (2.0 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction and a sugar fraction (1.2 mg). The aglycone fraction was chromatographed on silica gel eluted with $CHCl_3$–MeOH (19 : 1) to give **2a** (chrysogenin, 0.5 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-fucose and D-glucose. t_R (min): 8.92 (D-fucose, positive optical rotation) and 18.09 (D-glucose, positive optical rotation).

Compound **3**: Amorphous solid, $[\alpha]_D^{25}$ −37.8° (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 1605.7183 $[M+Na]^+$ (Calcd for $C_{70}H_{118}O_{39}$Na: 1605.7148). IR ν_{max} (film) cm^{-1}: 3270 (OH), 2920 and 2850 (CH), and 1153 and 1051 (C–O). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 4.45 (1H, m, H-16), 3.96 (1H, m, H-26a), 3.95 (1H, m, H-3), 3.83 (1H, m, H-26b), 3.58 (1H, m, H-6), 3.27 (3H, s, OMe-22), 1.19 (3H, d, J=6.6 Hz, Me-21), 1.01 (3H, d, J=6.6 Hz, Me-27), 0.81 (3H, s, Me-18), 0.72 (3H, s, Me-19). Signals for the sugar moieties: see Table 1. ^{13}C-NMR (150 MHz, C_5D_5N) δ: 37.8 (C-1), 29.9 (C-2), 77.9 (C-3), 29.4 (C-4), 52.2 (C-5), 68.5 (C-6), 42.6 (C-7), 34.3 (C-8), 54.2 (C-9), 36.6 (C-10), 21.3 (C-11), 40.0 (C-12), 41.2 (C-13), 56.3 (C-14), 32.2 (C-15), 81.4 (C-16), 64.4 (C-17), 16.6 (C-18), 13.6 (C-19), 40.6 (C-20), 16.4 (C-21), 112.7 (C-22), 30.9 (C-23), 28.3 (C-24), 34.3 (C-25), 75.3 (C-26), 17.2 (C-27), 47.3 (OMe-22). Signals for the sugar moieties: see Table 1.

Enzymatic Hydrolysis of 3 Compound **3** (9.3 mg) was treated with β-D-glucosidase (Sigma, 10 mg) in HOAc/NaOAc buffer (pH 5.0, 5 ml) at room temperature for 22 h. The reaction mixture was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (7 : 4 : 1) to yield **3a** (3.8 mg) and D-glucose (1.2 mg).

Cell Culture and Assay for Cytotoxic Activity against HL-60 Cells HL-60 cells, which were obtained from the Human Science Research Resources Bank (JCRB 0085, Osaka, Japan) were maintained in RPMI-1640 medium containing 10% heat-inactivated FBS and antibiotics (penicillin sodium salt 100 units/ml and streptomycin sulfate 100 μg/ml) in a 5% CO_2 humidified incubator at 37 °C. The cells were washed and resuspended in the medium to 4×10^4 cells/ml, and 196 μl of this cell suspension was divided into 96-well flat-bottomed plates (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μl of EtOH–H_2O (1 : 1) solution containing the sample was added to give the final concentration of 0.01—20 μM, and 4 μl of EtOH–H_2O (1 : 1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated using a modified MTT reduction assay.[30] At the end of incubation, 10 μl of MTT 5 mg/ml in phosphate-buffered saline (PBS) was added to each well, and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. Then the plate was centrifuged at 1500 ***g*** for 5 min to precipitate MTT formazan. An aliquot of 150 μl of supernatant was removed from esch well, and 175 μl of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microplate mixer for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as the IC_{50} value, which reduces the viable cell number by 50%.

Cell Culture and Assay for Cytotoxic Activity against A549, HSC-2, and HSC-2 Cells A549 (JCRB 0076) cells were incubated at 37 °C in MEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. HSC-2 and HSC-4 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. Cells were trypsinized and resuspended in the medium to 1×10^5 cells/ml, and 100 μl of this cell suspension was divided into 96-well flat-bottomed plates and incubated for 24 h. After washing once with PBS, they were treated for 24 h in the presence of test compounds. They were washed once with PBS and incubated for 4 h with MTT 0.2 mg/ml in MEM (A549 cells) and DMEM (HSC-2 and HSC-4 cells) sup-

plemented with 10% FBS. After the medium was removed, the MTT formazan crystals were dissolved with 100 μl of DMSO. The plate was mixed on a microplate mixer for 10 min and then read on a microplate reader at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as the IC_{50} value.

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