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Steroidal saponins from the whole plants of *Agave utahensis* and their cytotoxic activity

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ABSTRACT

Phytochemical investigation of the whole plants of *Agave utahensis* Engelm. (Agavaceae) has resulted in the isolation of 15 steroidal saponins (**1**–**15**), including five spirostanol saponins (**1**–**5**) and three furostanol saponins (**11**–**13**). Structures of compounds **1**–**5** and **11**–**13** were determined by spectroscopic analysis and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

1. Introduction

Agave utahensis Engelm. (Agavaceae) is native to South and Central America (Tsukamoto, 1989). The leaves of this plant are roasted and eaten, and the fiber is twisted for use us ropes and other applications (Irish and Irish, 2000). Previously, we reported the structural characterization of four new steroidal glycosides on the basis of (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin) as the aglycone from the whole plants of *A. utahensis* (Yokosuka and Mimaki, 2007). Our further phytochemical analysis of the polar fraction of the MeOH extract of this plant, in which steroidal saponins are enriched, has resulted in the isolation of five new spirostanol saponins (**1**–**5**) and three new furostanol saponins (**11**–**13**), together with seven known steroidal saponins (**6**–**10**, **14**, and **15**). This paper reports the structural determination of the new saponins on the basis of extensive spectroscopic analysis, including two-dimensional (2D) NMR spectroscopic data and the results of hydrolytic cleavage. The cytotoxic activity of the isolated compounds against HL-60 human promyelocytic leukemia cells is also described.

2. Results and discussion

2.1. Structure elucidation

The fresh whole plants of *A. utahensis* (2.5 kg) were extracted with MeOH. After removal of solvent, the crude extract was fractionated by the combined use of repeated column chromatography on a porous–polymer polystyrene resin (Diaion HP-20), silica gel, and octadecylsilanized (ODS) silica gel to yield **1**–**15**. Compounds **6**–**10**, **14**, and **15** were identified as (25R)-5α-spirostan-3β-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**6**) (Wu et al., 1992; Kuroda et al., 1995), (25R)-2α-hydroxy-5α-spirostan-3β-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**7**) (Achenbach et al., 1994), (25R)-3β-[(O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirostan-12-one (**8**) (Xu et al., 2000), (25R)-3β-[(O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-2α-hydroxy-5α-spirostan-12-one (**9**) (Mimaki et al., 1998), (25R)-2α-hydroxy-5α-spirostan-3β-yl O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**10**) (Yahara et al., 1994), (25R)-26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-5α-furostan-3β-yl O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**14**) (Yahara et al., 1994), and (25R)-26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-5α-furostan-3β-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**15**) (Yahara et al., 1994), based on their physical and spectroscopic data.

Compound **1** was isolated as an amorphous solid, and its molecular formula was deduced to be $C_{44}H_{72}O_{18}$ on the basis of the HRESI–TOFMS (*m/z*: 889.4731 $[M+H]^+$), ^{13}C NMR, and DEPT spectroscopic data. The ^1H NMR spectrum of **1** showed two three-proton singlet signals at δ 0.81 (3H, *s*) and 0.72 (1H, *s*), indicating the presence of two angular methyl groups, as well as two

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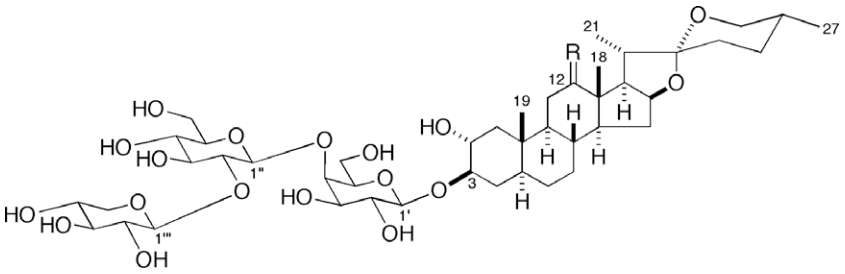
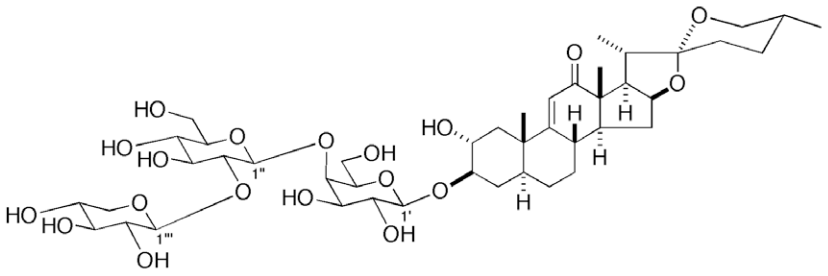


	R
1	H,H
2	O



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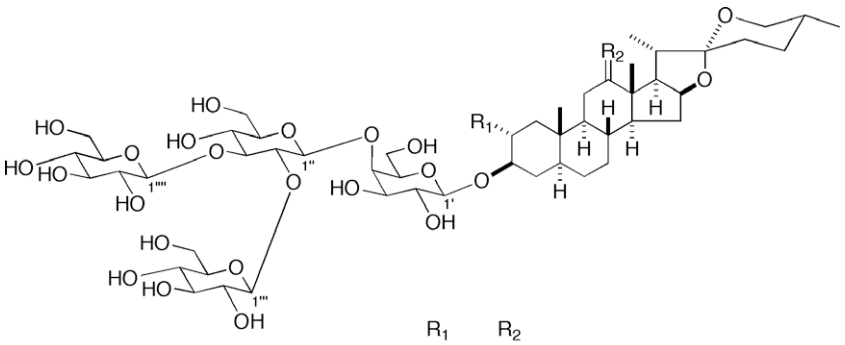
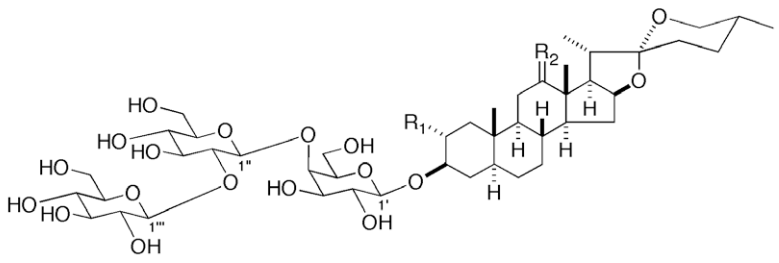
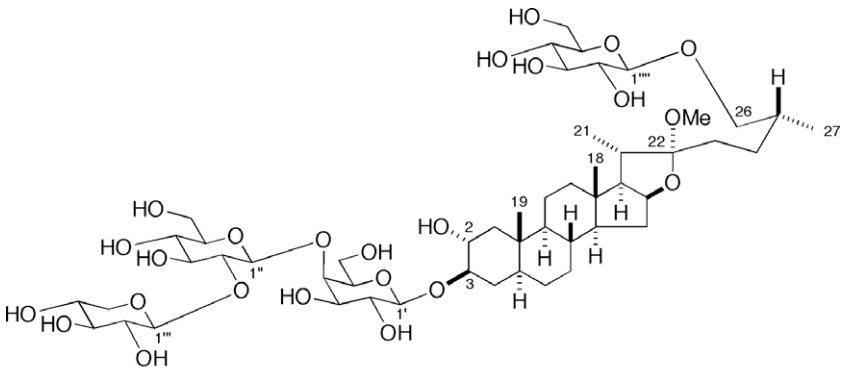


	R_1	R_2
4	OH	O
5	H	O
6	H	H,H
7	OH	H,H



	R_1	R_2
8	H	O
9	OH	O
10	OH	H,H



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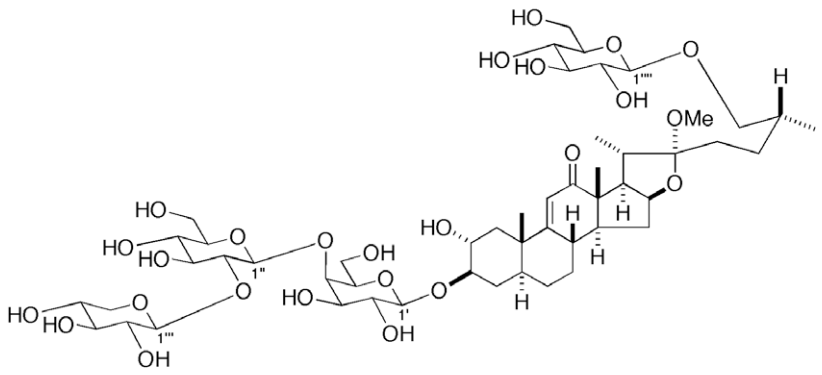

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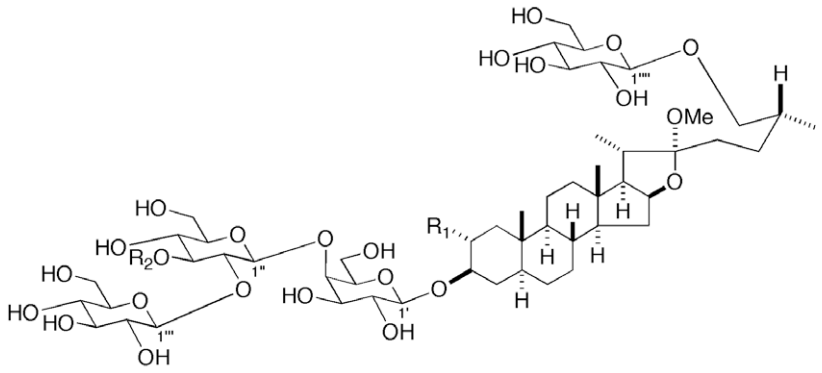

	R_1	R_2
13	H	H
14	OH	H
15	OH	β-D-Glc

three-proton doublet resonances at δ 1.13 (3H, *d*, *J* = 6.9 Hz), 0.69 (3H, *d*, *J* = 5.8 Hz), which were characteristic of the steroidal skeleton. In addition, three anomeric protons were observed at δ 5.18 (1H, *d*, *J* = 7.9 Hz), 5.08 (1H, *d*, *J* = 6.8 Hz), and 4.93 (1H, *d*, *J* = 7.7 Hz), which implied the presence of three monosaccharides. Comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those of **10** showed considerable structural similarity and indicated that the triglycoside moiety included a C-2 substituted β-D-glucopyranosyl unit and a C-4 substituted β-D-galactopyranosyl unit, as in **10**. The difference between **1** and **10** consisted only of resonances due to the terminal monosaccharide constituent. Instead of the resonances for the terminal β-D-glucopyranosyl group, five carbon signals assignable to a terminal β-D-xylopyranosyl group were newly observed at δ 108.0, 76.5, 77.9, 70.4, and 67.4 in **1**, and the anomeric carbon resonance was associated with the δ 5.08 (*d*, *J* = 6.8 Hz) signal by the HMQC spectrum. Acid hydrolysis of **1** with 1 M HCl (dioxane–H_2O, 1:1) gave (25*R*)-5α-spirostane-2α,3β-diol (**1a**, gitogenin) (Agrawal et al., 1985), D-glucose, D-galactose, and D-xylose. Identification of the monosaccharides, including their absolute configurations, was carried out by direct HPLC analysis of the hydrolysate. In the HMBC spectrum of **1**, the anomeric proton (H-1) of the xylosyl group at δ 5.08 showed a long-range correlation with C-2 of the β-D-glucopyranosyl moiety at δ 86.4, of which H-1 at δ 5.18 exhibited a long-range correlation with C-4

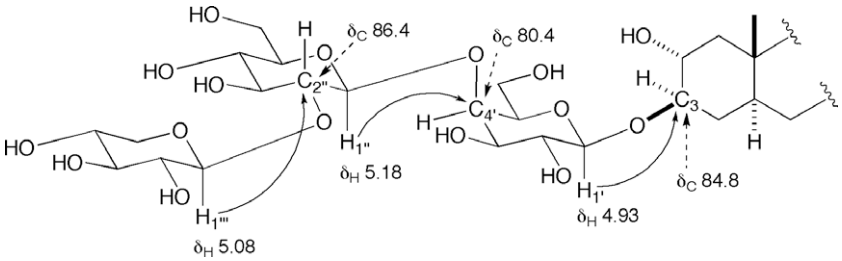

Fig. 1. HMBC correlations of the sugar moiety of **1**.

of the β-D-galactopyranosyl moiety at δ 80.4 (Fig. 1). An HMBC correlation was also observed between H-1 of the β-D-galactopyranosyl moiety at δ 4.93 and C-3 of the aglycone moiety at δ 84.8. The β-orientations of the anomeric centers of the sugar moieties were supported by the relatively large *J* values of their anomeric protons (*J* = 6.8–7.9 Hz). The structure of **1** was formulated as (25*R*)-2α-hydroxy-5α-spirostan-3β-yl *O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. To the best of our knowledge, the structure of the sugar moiety for **1** has not been reported previously.

Compound **2** was shown to have the molecular formula $C_{44}H_{70}O_{19}$ on the basis of the positive-ion HRFABMS and ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum of **2** showed signals for four steroidal methyl groups at δ 1.35 (*d*, *J* = 6.9 Hz), 1.07 (*s*), 0.75 (*s*), and 0.69 (*d*, *J* = 5.8 Hz) and three anomeric protons at δ 5.18 (*d*, *J* = 7.9 Hz), 5.08 (*d*, *J* = 6.3 Hz), and 4.92 (*d*, *J* = 7.7 Hz), as in **1**. The presence of a carbonyl group in **2** was established from the IR (1707 cm^{-1}) and ^{13}C NMR spectra (δ 212.4). Acid hydrolysis of **2** gave (25*R*)-2α,3β-dihydroxy-5α-spirostan-12-one (**2a**) (Mimaki et al., 1995), D-xylose, D-galactose, and D-glucose. The results of acid hydrolysis, and the ^{1}H and ^{13}C NMR spectroscopic data provided evidence that the structure of the sugar moiety of **2** was the same as that of **1**. Thus, **2** was determined to be (25*R*)-2α-hydroxy-3β-[(*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirostan-12-one.

The spectroscopic features of **3** ($C_{44}H_{68}O_{19}$) were quite similar to those of **2**, and the ^{1}H and ^{13}C NMR spectra allowed for the identification of the triglycoside moiety attached to C-3 of the aglycone as being same as that of **2**. However, the molecular formula of **3** was less than by two hydrogen atoms, and the IR (1673 cm^{-1}), UV [λ_{max} 236.4 nm (log ε 4.07)], and ^{13}C NMR [δ 204.2 (C=O), 170.4 (C), 120.1 (CH)] indicated the presence of a conjugated carbonyl group in **3**. In the HMBC spectrum, long-range correlations were observed between the olefinic proton at δ 5.95 (*s*, H-11) and C-8 (δ 36.1)/C-10 (δ 40.5)/C-13 (δ 51.3), and between the carbonyl carbon at δ 204.2 and H-17 (δ 2.63)/Me-18 (δ 1.01)/H-14 (δ 1.72), which are consistent with the 9-en-12-one structure. Acid hydrolysis of **3** gave (25*R*)-2α,3β-dihydroxy-5α-spirost-9-en-12-one (**3a**) (Mimaki et al., 1995), D-xylose, D-galactose, and D-glucose. Accordingly, **3** was formulated as (25R)-2α-hydroxy-3β-[(O-β-D-xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirost-9-en-12-one.

Compound **4** was analyzed for $C_{51}H_{82}O_{25}$ by combined positive-ion HRFABMS and ^{13}C NMR data. The ^{1}H NMR spectrum of **4** showed signals for four anomeric protons at δ 5.60 (*d*, *J* = 7.9 Hz), 5.31 (*d*, *J* = 7.9 Hz), 5.18 (*d*, *J* = 7.9 Hz), and 4.91 (*d*, *J* = 7.8 Hz), as well as signals for four steroid methyl groups at δ 1.36 (*d*, *J* = 6.9 Hz), 1.07 (*s*), 0.74 (*s*), and 0.70 (*d*, *J* = 5.8 Hz). Analysis of the ^{13}C NMR spectrum of **4** and comparison with that of **2** implied that the aglycone of **4** was identical to that of **2**, but differed from **2** in terms of the glycoside structure. Acid hydrolysis of **4** with 1 M HCl (dioxane–H_2O, 1:1) resulted in the production of **2a**, D-galactose, and D-glucose. Thus, **4** was suggested to be a **2a** tetraglycoside. The ^{1}H–^{1}H COSY experiment with **4** allowed the sequential assignments from H-1 to H_2-6 of the monosaccharides, including identification of their multiplet patterns and coupling constants. The HMQC correlated the proton resonances with those of one-bond coupled carbons, leading to unambiguous assignments of the carbon shifts. The ^{1}H and ^{13}C NMR signals thus assigned were indicative of the presence of two terminal β-D-glucopyranosyl units (Glc′) [δ_H 5.60 (*d*, *J* = 7.9 Hz); δ_C 104.8, 76.0, 78.2, 71.3, 78.6, 62.3], (Glc″) [δ_H 5.31 (*d*, *J* = 7.9 Hz); δ_C 104.5, 75.3, 78.7, 71.6, 78.4, 62.6], a C-2 and C-3 disubstituted β-D-glucopyranosyl unit (Glc) [δ_H 5.18 (*d*, *J* = 7.9 Hz); δ_C 104.7, 81.3, 88.7, 70.7, 77.5, 63.0], and a C-4 substituted β-D-galactopyranosyl unit (Gal) [δ_H 4.91 (*d*, *J* = 7.8 Hz); δ_C 103.1, 72.5, 75.5, 79.7, 75.7, 60.5] in **4**. In the HMBC spectrum, long-range correlations were observed between H-1 of Glc′ at δ 5.60 and C-2 of Glc at δ 81.3, H-1 of Glc″ at δ 5.31 and C-3 of Glc at δ 88.7, H-1 of Glc at δ 5.18 and C-4 of Gal at δ 79.7, and between H-1 of Gal at δ 4.91 and C-3 of the aglycone at δ 83.7. Accordingly, **4** was formulated as (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-2α-hydroxy-5α-spirostan-12-one.

Compound **5** was deduced as $C_{51}H_{82}O_{24}$ by the positive-ion HRFABMS and ^{13}C NMR data. Comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of **4** showed that **5** was closely related that of **4**. However, the molecular formula of **5** was lower by one oxygen atom than that of **4**. When the ^{13}C NMR spectrum of **5** was compared with that of **4**, the signals due to the C-2 oxymethine carbon, which was observed at δ 70.2 in **4**, was displaced by a methylene carbon resonance at δ 29.6 in **5**. Acid hydrolysis of **5** gave (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) (Mimaki et al., 1995), D-galactose, and D-glucose. Analysis of the HMBC spectrum of **5** indicated that the tetraglycoside attached to C-3 of the aglycone is the same as that of **4**. Thus, **5** was formulated as (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)-oxy]-5α-spirostan-12-one.

Compound **11** was obtained as an amorphous solid with a molecular formula of $C_{51}H_{86}O_{24}$. The ^{1}H NMR spectrum of **11** showed signals for four anomeric protons at δ 5.17 (1H, *d*, *J* = 7.9 Hz), 5.07 (1H, *d*, *J* = 7.2 Hz), 4.93 (1H, *d*, *J* = 7.7 Hz), and 4.85 (1H, *d*, *J* = 7.8 Hz), as well as signals for four steroid methyl groups at δ 1.18 (*d*, *J* = 6.8 Hz), 1.00 (*d*, *J* = 6.7 Hz), 0.78 (*s*), and 0.72 (*s*), and a methoxy group at δ 3.26 (3H, *s*). From the ^{1}H NMR spectroscopic data, an acetic carbon signal at δ 112.6 in the ^{13}C NMR spectrum, and a positive color reaction in Ehrlich's test (Kiyosawa and Hutoh, 1968; Nohara et al., 1975) indicated **11** to be a 22-methoxyfurostanol saponin with four monosaccharides. Enzymatic hydrolysis of **11** with β-D-glucosidase gave **1** and D-glucose. In addition, a long-range correlation as observed between H-1 of Glc‴ at δ 4.85 and C-26 of the aglycone at δ 75.2. Thus, **11** was demonstrated to be the corresponding 22-methoxyfurostanol saponin of **1** with a glucosyl unit at C-26. A NOE correlation between the methoxy proton at δ 3.26 and the H-16 proton signal at δ 4.45 was consistent with the C-22 δ configuration. Thus, **11**

Table 1
^{1}H and ^{13}C NMR chemical shift assignments for the sugar moieties of compounds **1–6** in C_5D_5N[a].

1				**2**				**3**				**4**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Gal	**1**	4.93 *d* (7.7)	103.8	Gal	**1**	4.92 *d* (7.7)	103.8	Gal	**1**	4.93 *d* (7.7)	103.7	Gal	**1**	4.91 *d* (7.8)	103.1
	2	4.48 *dd* (9.9, 7.7)	72.6		**2**	4.48 *dd* (9.6, 7.7)	72.6		**2**	4.47 *dd* (9.1, 7.7)	72.7		**2**	4.54 *dd* (9.0, 7.8)	72.5
	3	4.15 *dd* (9.9, 2.7)	75.5		**3**	4.15 *dd* (9.6, 3.0)	75.5		**3**	4.14 *dd* (9.1, 3.1)	75.5		**3**	4.12 *dd* (9.0, 3.3)	75.5
	4	4.57 *br d* (2.7)	80.4		**4**	4.57 *br d* (3.0)	80.4		**4**	4.57 *br d* (3.1)	80.4		**4**	4.28 *br d* (3.3)	79.7
	5	4.08 *m*	75.7		**5**	4.07 *m*	75.7		**5**	4.07 *m*	75.7		**5**	4.04 *m*	75.7
	6a	4.75 *dd* (9.7, 9.7)	60.3		**6a**	4.74 *dd* (9.8, 9.8)	60.3		**6a**	4.74 *dd* (9.7, 9.7)	60.3		**6a**	4.63 *dd* (10.5, 9.0)	60.5
	6b	4.23 *dd* (9.7, 5.0)			**6b**	4.22 *dd* (9.8, 5.1)			**6b**	4.23 *dd* (9.7, 5.0)			**6b**	4.33 *m*	
Glc	**1**	5.18 *d* (7.9)	105.2	Glc	**1**	5.18 *d* (7.9)	105.2	Glc	**1**	5.18 *d* (7.8)	105.2	Glc	**1**	5.17 *d* (7.9)	104.7
	2	4.13 *dd* (8.5, 7.9)	86.4		**2**	4.14 *dd* (8.6, 7.9)	86.4		**2**	4.13 *dd* (8.4, 7.8)	86.4		**2**	4.35 *dd* (8.9, 7.9)	81.3
	3	4.27 *dd* (8.5, 8.5)	78.0		**3**	4.28 *dd* (8.6, 8.6)	78.0		**3**	4.27 *dd* (8.4, 8.4)	78.0		**3**	4.19 *dd* (8.9, 8.9)	88.7
	4	3.95 *dd* (8.5, 8.5)	72.2		**4**	3.95 *dd* (8.6, 8.6)	72.2		**4**	3.96 *dd* (8.4, 8.4)	72.2		**4**	3.82 *dd* (8.9, 8.9)	70.7
	5	4.00 *m*	78.0		**5**	3.99 *m*	78.0		**5**	3.99 *m*	78.0		**5**	3.83 *m*	77.5
	6a	4.63 *br d* (9.9)	63.3		**6a**	4.63 *br d* (10.6)	63.3		**6a**	4.63 *br d* (10.6)	63.2		**6a**	4.48 *m*	63.0
	6b	4.10 *m*			**6b**	4.10 *m*			**6b**	4.11 *m*			**6b**	4.02 *m*	
Xyl	**1**	5.08 *d* (6.8)	108.0	Xyl	**1**	5.08 *d* (6.3)	108.0	Xyl	**1**	5.07 *d* (6.1)	108.0	Glc	**1**	5.06 *d* (7.9)	104.8
	2	4.02 *m*	76.5		**2**	4.02 *m*	76.5		**2**	4.02 *m*	76.5		**2**	4.05 *dd* (8.9, 7.9)	76.0
	3	4.01 *m*	77.9		**3**	4.01 *m*	77.9		**3**	4.01 *m*	77.9		**3**	4.17 *dd* (8.9, 8.9)	78.2
	4	3.95 *m*	70.4		**4**	3.95 *m*	70.2		**4**	3.94 *m*	70.4		**4**	4.12 *dd* (8.9, 8.9)	71.3
	5a	4.48 *m*	67.4		**5a**	4.48 *m*	67.4		**5a**	4.47 *m*	67.4		**5**	3.89 *m*	78.6
	5b	3.67 *dd* (11.0, 11.0)			**5b**	3.67 *dd* (10.8, 10.8)			**5b**	3.66 *dd* (11.0, 11.0)	71.6		**6a**	4.54 *dd*	62.3
													6b	4.22 *dd* (12.1, 5.1)	
												Glc	**1**	5.31 *d* (7.9)	104.5
													2	4.04 *dd* (8.8, 7.9)	75.3
													3	4.20 *dd* (8.8, 8.8)	78.7
													4	4.15 *dd* (8.8, 8.8)	71.6
													5	4.04 *m*	78.4
													6a	4.54 *m*	62.6
													6b	4.27 *dd* (11.8, 6.1)	

5				**11**				**12**				**13**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Gal	**1**	4.85 *d* (7.7)	102.4	Gal	**1**	4.93 *d* (7.7)	103.8	Gal	**1**	4.94 *d* (7.7)	103.6	Gal	**1**	4.91 *d* (7.7)	102.4
	2	4.42 *dd* (9.5, 7.7)	73.2		**2**	4.48 *dd* (9.1, 7.7)	72.6		**2**	4.48 *dd* (9.4, 7.7)	72.6		**2**	4.50 *dd* (9.4, 7.7)	73.3
	3	4.10 *dd* (9.5, 3.1)	75.6		**3**	4.15 *dd* (9.1, 3.4)	75.4		**3**	4.15 *dd* (9.4, 3.5)	75.4		**3**	4.12 *dd* (9.4, 4.2)	75.6
	4	4.58 *br d* (3.1)	80.2		**4**	4.57 *br d* (3.4)	80.4		**4**	4.57 *br d* (3.5)	80.3		**4**	4.59 *br d* (4.2)	81.0
	5	4.00 *m*	75.3		**5**	4.09 *m*	75.7		**5**	4.08 *m*	75.7		**5**	4.11 *m*	75.2
	6a	4.68 *dd* (10.1, 9.4)	60.6		**6a**	4.75 *dd* (9.7, 9.7)	60.3		**6a**	4.75 *dd* (9.6, 9.6)	60.3		**6a**	4.78 *dd* (10.1, 9.8)	60.5
	6b	4.20 *m*			**6b**	4.24 *m*			**6b**	4.24 *m*			**6b**	4.26 *dd* (9.9, 4.5)	
Glc	**1**	5.14 *d* (7.9)	105.0	Glc	**1**	5.17 *d* (7.9)	105.2	Glc	**1**	5.18 *d* (7.8)	105.1	Glc	**1**	5.15 *d* (7.8)	105.2
	2	4.38 *dd* (8.9, 7.9)	81.4		**2**	4.13 *dd* (8.6, 7.9)	86.4		**2**	4.14 *dd* (8.8, 7.8)	86.4		**2**	4.15 *dd* (8.8, 7.8)	86.1
	3	4.20 *dd* (8.9, 8.9)	88.4		**3**	4.27 *dd* (8.6, 8.6)	77.9		**3**	4.28 *dd* (8.8, 8.8)	77.9		**3**	4.27 *m*	78.4
	4	3.83 *dd* (8.9, 8.9)	70.8		**4**	3.95 *dd* (8.6, 8.6)	72.2		**4**	3.94 *dd* (8.8, 8.8)	72.2		**4**	3.98 *m*	71.8
	5	3.84 *m*	77.5		**5**	3.97 *m*	78.0		**5**	3.97 *m*	78.0		**5**	3.97 *m*	78.2
	6a	4.48 *m*	63.0		**6a**	4.63 *br d* (9.6)	63.2		**6a**	4.64 *br d* (9.7)	63.2		**6a**	4.63 *br d* (10.3)	63.2
	6b	4.01 *m*			**6b**	4.10 *m*			**6b**	4.10 *dd* (10.6, 7.6)			**6b**	4.12 *m*	
Glc	**1**	5.58 *d* (7.8)	104.9	Xyl	**1**	5.07 *d* (7.2)	108.0	Xyl	**1**	5.07 *d* (7.2)	108.0	Glc	**1**	5.22 *d* (7.5)	106.9
	2	4.06 *dd* (9.2, 7.8)	76.1		**2**	4.02 *m*	76.5		**2**	4.02 *m*	76.5		**2**	4.06 *m*	76.7
	3	4.14 *dd* (9.2, 9.2)	77.9		**3**	4.01 *m*	77.9		**3**	4.01 *m*	77.9		**3**	4.12 *m*	77.6
	4	4.24 *dd* (8.9, 8.9)	70.9		**4**	3.96 *m*	70.4		**4**	3.94 *m*	70.4		**4**	4.22 *m*	70.3
	5	3.85 *m*	78.6		**5a**	4.48 *dd* (11.1, 6.3)	67.4		**5a**	4.46 *m*	67.4		**5**	3.80 *m*	78.9
	6a	4.52 *br d* (12.9)	62.3		**5b**	3.67 *dd* (11.1, 11.1)			**5b**	3.65 *dd* (11.0, 11.0)			**6a**	4.59 m	61.6
	6b	4.37 *m*											**6b**	4.37 *dd* (12.4, 3.5)	
Glc	**1**	5.30 *d* (7.9)	104.5	Glc	**1**	4.85 *d* (7.8)	105.0	Glc	**1**	4.85 *d* (7.8)	105.0	Glc	**1**	4.85 *d* (7.8)	105.0
	2	4.05 *dd* (8.7, 7.9)	75.3		**2**	4.04 *dd* (8.2, 7.8)	75.2		**2**	4.04 *dd* (8.6, 7.8)	75.2		**2**	4.04 *dd* (8.7, 7.8)	75.2
	3	4.20 *dd* (8.7, 8.7)	78.6		**3**	4.26 *dd* (8.2, 8.2)	78.6		**3**	4.24 *dd* (8.6, 8.6)	78.6		**3**	4.24 *m*	78.6
	4	4.14 *dd* (8.7, 8.7)	71.6		**4**	4.23 *dd* (8.2, 8.2)	71.8		**4**	4.23 *dd* (8.6, 8.6)	71.7		**4**	4.24 *m*	71.7
	5	4.03 *m*	78.6		**5**	3.98 *m*	78.5		**5**	3.98 *m*	78.5		**5**	3.96 *m*	78.5
	6a	4.55 *dd* (12.0, 2.2)	62.3		**6a**	4.57 *dd* (11.7, 2.3)	62.9		**6a**	4.58 *dd* (11.7, 2.4)	62.8		**6a**	4.57 *dd* (11.7, 2.4)	62.9
	6b	4.26 *dd* (12.0, 6.3)			**6b**	4.40 *dd* (11.7, 5.4)			**6b**	4.40 *dd* (11.7, 5.4)			**6b**	4.40 *dd* (11.7, 5.4)	

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

was assigned as (25*R*)-26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-5α-furostan-3β-yl *O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **12** ($C_{51}H_{82}O_{25}$) was shown to be a 22-methoxyfurostanol saponin by Ehrlich's test, and ^{1}H NMR [δ 3.27 (3H, *s*)] and ^{13}C NMR [δ 112.9 (C-22), 47.4 (Me)] spectra. The presence of a conjugated carbonyl group in the aglycone moiety of **12** was shown by the IR (1671 cm^{-1}), UV [λ_{max} 236.6 nm (logε 3.93)], and ^{13}C NMR [δ: 204.2 (C=O), 170.4 (C), 120.0 (CH)] spectra. Enzymatic hydrolysis of **12** with β-D-glucosidase gave **3** and D-glucose. Thus, **12** was established as (25*R*)-26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-3-[(*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-furost-9-en-12-one.

Compound **13** ($C_{52}H_{88}O_{24}$) was also a 22-methoxyfurostanol saponin. Treatment of **13** with β-D-glucosidase gave a known spirostanol saponin identified as (25*R*)-5α-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**13a**) (Murakami et al., 1981) and D-glucose. Accordingly, **13** was formulated as (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-methoxy-5α-furostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

2.2. Cytotoxic activity

The isolated compounds were evaluated for their cytotoxic activity against HL-60 cells (Table 3). Compounds **1**, **6**, **7**, and **10** showed moderate cytotoxicity, relative to the control, etoposide, with respective IC_{50} values of 12.3, 9.4, 5.5, and 11.3 μg/ml. In contrast, compounds **2**, **4**, **5**, **8**, and **9** did not exhibit apparent cytotoxicity even at a sample concentration of 20 μg/ml. The above results indicated that the C-12 carbonyl group significantly reduces the cytotoxic activity in these spirostanol saponins. The furostanol saponins (**11**–**15**) were not cytotoxic to HL-60 cells at the sample concentration of 20 μg/ml.

2.3. Conclusions

A number of steroidal saponins with the triglycoside of β-D-glucosyl-(1→2)-[β-D-glucosyl-(1→3)]-β-D-glucosyl-(1→4)-β-D-galactoside and β-D-glucosyl-(1→2)-β-D-glucosyl-(1→4)-β-D-galactoside have been isolated from the Liliaceae, Agavaceae, and Solanaceae plants such as *Allium chinense* (Kuroda et al., 1995), *Allium amperoprasum* (Mimaki et al., 1999), *Chlorophytum malayence* (Li et al., 1990), *Chlorophytum comosum* (Mimaki et al., 1996), *Hosta longips* (Mimaki et al., 1995), and *Hosta sieboldii* (Mimaki et al., 1998) (Liliaceae), *Agave sisalana* (Ding et al., 1993), *Agave uthahensis* (Yokosuka and Mimaki, 2007), and *Polianthes tuberosa* (Jin et al., 2004) (Agavaceae), and *Solanum nigrum* (Ikeda et al., 2000), *Solanum dulcamara* (Lee et al., 1994), and *Capsicum annuum* (Yahara et al., 1994) (Solanaceae). Compounds **1**–**3**, **11**, and **12** are rare type of steroidal saponins with the sugar moiety of β-D-xylosyl-(1→2)-β-D-glucosyl-(1→4)-β-D-galactoside at C-3 of the aglycone. Steroidal saponin with the same sugar moiety as **1**–**3**, **11**, and **12** has been isolated only from *Solanum lyratum* (Solanaceae) (Lee et al., 1997).

3. Experimental

3.1. General

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. UV spectra were recorded on a JASCO V-520 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. FABMS was recorded on a Finnigan MAT TSQ-700 (San Jose, CA, USA). 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 (CC). 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 by using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), a RI-8010 (Tosoh) and 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) (Sigma, St. Louis, MO, USA); fetal bovine serum (FBS) (Bio-Whittaker, Walkersville, MO, USA); penicillin G sodium salt and streptomycin sulfate (Gibco, Grand Island, NY, USA). All other chemicals used were of biochemical reagent grade.

3.2. Plant material

A. utahensis was purchased from a garden center in Japan Cactus Planning Co. (Fukushima, Japan) in 2000 and identified by Dr. Yutaka Sashida, emeritus professor of Medicinal Pharmacognosy at Tokyo University of Pharmacy and Life Sciences, on the basis of its morphological properties. A voucher specimen has been deposited in our laboratory (voucher No. AU-2000-001, Laboratory of Medicinal Pharmacognosy).

3.3. Extraction and isolation

The plant material (2.5 kg) was extracted for 3 h with hot MeOH twice (each 6 l). The combined MeOH extract (12 l) was concentrated under reduced pressure, and the viscous 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 50% MeOH eluate portion was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (40:10:1) to give four fractions (Ia–IVa). Fraction IIIa was applied to an ODS silica gel eluted with MeOH–H_2O (1:1; 7:5) and then silica gel CC with $CHCl_3$–MeOH–H_2O (50:20:1) to yield **12** (27 mg), **13** (58 mg), and **14** (38 mg). The MeOH eluate portion was subjected silica gel CC_1 eluted with a stepwise gradient mixture of $CHCl_3$–MeOH (9:1; 4:1; 3:1; 2:1; 1:1), and finally with MeOH alone, to give five fractions (Ib–Vb).

Table 2
^{13}C NMR chemical shift assignments for the aglycone moiety of compounds **1**–**5** and **11**–**13** in C_5D_5N.

Position	1	2	3	4	5	11	12	13
1	45.7	45.1	43.5	45.0	36.6	45.6	43.5	37.2
2	70.4	70.4	70.3	70.2	29.6	70.4	70.2	30.8
3	84.8	84.5	84.0	83.7	77.1	84.8	83.9	77.4
4	34.1	34.0	33.7	33.9	34.6	34.1	33.7	34.8
5	44.7	44.5	42.5	44.3	44.4	44.7	42.5	44.7
6	28.1	27.8	27.1	27.8	28.5	28.1	27.1	28.9
7	32.2	31.4	32.5	31.4	31.4	32.2	32.4	32.4
8	34.6	33.7	36.1	33.7	34.3	34.5	36.1	35.2
9	54.3	55.7	170.4	55.7	55.5	54.3	170.4	54.4
10	36.9	37.3	40.5	37.2	36.2	36.8	40.5	35.8
11	21.4	38.0	120.1	38.0	37.9	21.3	120.0	21.2
12	40.0	212.4	204.2	212.5	212.8	39.8	204.2	40.0
13	40.7	55.3	51.3	55.3	55.3	41.1	51.7	41.1
14	56.3	55.3	52.6	55.7	55.9	56.2	52.5	56.3
15	32.1	31.8	31.8	31.8	31.7	32.1	31.6	32.1
16	81.1	79.7	80.2	79.7	79.7	81.3	80.3	81.3
17	63.0	54.3	54.5	54.3	54.3	64.3	55.6	64.3
18	16.6	16.1	15.2	16.1	16.0	16.5	15.2	16.5
19	13.4	12.8	19.3	12.8	11.7	13.4	19.3	12.3
20	42.0	42.6	42.9	42.6	42.6	40.5	41.2	40.5
21	15.0	13.9	13.8	13.9	13.9	16.3	14.7	16.3
22	109.2	109.3	109.4	109.3	109.3	112.6	112.9	112.6
23	31.8	31.5	31.8	31.5	31.8	30.8	30.5	30.0
24	29.2	29.2	29.2	29.2	29.2	28.2	28.1	28.2
25	30.6	30.5	30.5	30.5	30.5	34.2	34.1	34.2
26	66.9	66.9	67.0	66.9	66.9	75.2	75.2	75.2
27	17.3	17.3	17.3	17.3	17.3	17.1	17.1	17.1
OMe						47.2	47.4	47.2

Table 3
Cytotoxic activity of the isolated compounds **1–15** on HL-60 leukemia cells.

Compound	IC_{50} (μg/ml)[a]	Compound	IC_{50} (μg/ml)
1	12.3	**9**	–
2	–[b]	**10**	11.3
3	–	**11**	–
4	–	**12**	–
5	–	**13**	–
6	9.4	**14**	–
7	5.5	**15**	–
8	–		
Etoposide	0.20		

[a] Data represent mean of three independent experiments.
[b] Means IC_{50} > 20 μg/ml.

Fraction IIb was applied to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (60:10:1; 50:10:1) and then to ODS silica gel with MeOH–H_2O (8:3; 4:1) and CH_3CN–H_2O (5:7) to give **1** (28 mg), **2** (15 mg), **3** (15 mg), **8** (26 mg), **9** (16 mg), and **10** (9.0 mg). Fraction IVb was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (20:10:1; 7:4:1) and then to ODS silica gel CC with MeOH–H_2O (2:1; 4:1) and CH_3CN–H_2O (1:2; 1:3) to yield **4** (15 mg), **5** (7.0 mg), **6** (10 mg), **7** (32 mg), **11** (49 mg), and **15** (15 mg).

3.4. Compound *1*

(25*R*)-2β-Hydroxy-5α-spirostan-3β-yl *O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**1**); amorphous solid; $[\alpha]_D^{23}$ −46.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3357 (OH), 2928 (CH), 1050; ^1H NMR (500 MHz, C_5D_5N): δ 4.55 (1H, *m*, H-16), 3.98 (1H, *m*, H-2), 3.88 (1H, *ddd*, *J* = 10.8, 9.1, 4.9 Hz, H-3), 1.13 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.81 (3H, *s*, Me-18), 0.72 (3H, *s*, Me-19), 0.69 (3H, *d*, *J* = 5.8 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI–TOFMS *m*/*z*: 889.4731 $[M+H]^+$ (calculated for $C_{44}H_{73}O_{18}$, 889.4797).

3.5. Acid Hydrolysis of *1*

A solution of **1** (9.8 mg) in 1 M HCl (dioxane–H_2O, 1:1; 2 ml) was heated at 95 °C for 2 h under Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and then subjected to silica gel CC_1 eluted with $CHCl_3$–MeOH (9:1) to yield **1a** (3.7 mg) and a sugar fraction (4.5 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) and a Toyopak IC-SP M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. x 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-xylose, 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): 9.70 (D-xylose, positive optical rotation), 13.70 (D-galactose, positive optical rotation), 15.11 (D-glucose, positive optical rotation).

3.6. Compound *2*

(25*R*)-3β-[(*O*-β-D-Glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)-oxy]-2α-hydroxy-5α-spirostan-12-one (**2**); amorphous solid; $[\alpha]_D^{24}$ −8.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2927 (CH), 1707 (C=O), 1072, 1041; ^1H NMR (500 MHz, C_5D_5N): δ 4.49 (1H, *m*, H-16), 3.94 (1H, *m*, H-2), 3.85 (1H, *ddd*, *J* = 11.1, 9.9, 5.4 Hz, H-3), 1.35 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.07 (3H, *s*, Me-18), 0.75 (3H, *s*, Me-19), 0.69 (3H, *d*, *J* = 5.8 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRFABMS (positive mode) *m*/*z*: 925.4409 $[M+Na]^+$ (calculated for $C_{44}H_{70}O_{19}Na$, 925.4409).

3.7. Acid hydrolysis of *2*

A solution of **2** (9.8 mg) was subjected to acid hydrolysis as described for **1** to give **2a** (4.5 mg) and a sugar fraction (2.8 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-xylose, D-galactose, and D-glucose. t_R (min): 10.46 (D-xylose, positive optical rotation), 14.66 (D-galactose, positive optical rotation), 15.86 (D-glucose, positive optical rotation).

3.8. Compound *3*

(25*R*)-2α-Hydroxy-3β-[(*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirost-9-en-12-one (**3**); amorphous solid; $[\alpha]_D^{23}$ −30.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2928 (CH), 1673 (C=O), 1074, 1048; UV λ_{max}: 236.4 nm (MeOH, log ε 4.07); ^1H NMR (500 MHz, C_5D_5N): δ 5.95 (1H, *s*, H-11), 4.53 (1H, *m*, H-16), 4.05 (1H, *m*, H-2), 3.86 (1H, *ddd*, *J* = 10.5, 9.3, 5.3 Hz, H-3), 2.63 (1H, *dd*, *J* = 8.1, 7.7 Hz, H-17), 1.72 (1H, *m*, H-14), 1.40 (3H, *d*, *J* = 6.8 Hz, Me-21), 1.01 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-19), 0.70 (3H, *d*, *J* = 5.2 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI–TOFMS *m*/*z*: 901.4417 $[M+H]^+$ (calculated for $C_{44}H_{68}O_{19}$, 901.4433).

3.9. Acid hydrolysis of *3*

A solution of **3** (10 mg) was subjected to acid hydrolysis as described for **1** to give **3a** (4.7 mg) and a sugar fraction (2.3 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-xylose, D-galactose, and D-glucose. t_R (min): 10.43 (D-xylose, positive optical rotation), 14.63 (D-galactose, positive optical rotation), 15.86 (D-glucose, positive optical rotation).

3.10. Compound *4*

(25*R*)-3β-[(*O*-β-D-Glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)-oxy]-2α-hydroxy-5α-spirostan-12-one (**4**); amorphous solid; $[\alpha]_D^{25}$ −20.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3364 (OH), 2926 (CH), 1706 (C=O), 1074; ^1H NMR (500 MHz, C_5D_5N): δ 4.48 (1H, *m*, H-16), 3.92 (1H, *ddd*, *J* = 11.4, 9.0, 4.6 Hz, H-2), 3.86 (1H, *m*, H-3), 1.36 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.07 (3H, *s*, Me-18), 0.74 (3H, *s*, Me-19), 0.70 (3H, *d*, *J* = 5.8 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRFABMS (positive mode) *m*/*z*: 1117.5019 $[M+Na]^+$ (calculated for $C_{51}H_{82}O_{25}Na$, 1117.5043).

3.11. Acid hydrolysis of *4*

A solution of **4** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give **2a** (2.5 mg) and a sugar fraction (1.5 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose and D-galactose. t_R (min): 17.43 (D-galactose, positive optical rotation), 19.27 (D-glucose, positive optical rotation).

3.12. Compound **5**

(25*R*)-3β-[(*O*-β-D-Glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirostan-12-one (**5**); amorphous solid; $[\alpha]_D^{25}$ −22.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3376 (OH), 2927 (CH), 1707 (C=O), 1071; ^1H NMR (500 MHz, C_5D_5N): δ 4.48 (1H, *m*, H-16), 3.87 (1H, *m*, H-3), 1.35 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.08 (3H, *s*, Me-18), 0.69 (3H, *d*, *J* = 5.8 Hz, Me-27), 0.65 (3H, *s*, Me-19); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRFABMS (positive mode) *m/z*: 1101.5096 $[M + Na]^+$ (calculated for $C_{51}H_{82}O_{24}Na$, 1101.5094).

3.13. Acid hydrolysis of **5**

A solution of **5** (5.0 mg) was subjected to acid hydrolysis as described for **1** to give hecogenin (1.2 mg) and a sugar fraction (2.5 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): 17.39 (D-galactose, positive optical rotation), 19.21 (D-glucose, positive optical rotation).

3.14. Compound **11**

(25*R*)-26-[(β-D-Glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-5α-furostan-3β-yl *O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**11**); amorphous solid; $[\alpha]_D^{28}$ −12.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2928 (CH), 1070; ^1H NMR (500 MHz, C_5D_5N): δ 4.45 (1H, *m*, H-16), 3.97 (1H, *m*, H-2), 3.88 (1H, *m*, H-3), 3.26 (3H, *s*, OMe), 1.18 (3H, *d*, *J* = 6.8 Hz, Me-21), 1.00 (3H, *d*, *J* = 6.7 Hz, Me-27), 0.78 (3H, *s*, Me-18), 0.72 (3H, *s*, Me-19); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI–TOFMS *m/z*: 1051.5308 $[M - OMe]^+$ (calculated for $C_{50}H_{83}O_{23}$, 1051.5325); FABMS (positive mode) *m/z*: 1105 $[M + Na]^+$ (Found: C, 53.67; H, 8.05. $C_{51}H_{86}O_{24} \cdot 3H_2O$ requires, C, 53.86; H, 8.15%).

3.15. Enzymatic hydrolysis of **11**

Compound **11** (7.7 mg) was treated with β-D-glucosidase (Sigma, EC 3.2.1.21, 10 mg) in HOAc/NaOAc buffer (pH 5.0, 10 ml) at room temperature for 10 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 **4** (2.6 mg) and D-glucose (0.8 mg).

3.16. Compound **12**

(25*R*)-26-[(β-D-Glucopyranosyl)oxy]-2α-hydroxy-22α-methoxy-3-[(*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-furost-9-en-12-one (**12**); amorphous solid; $[\alpha]_D^{28}$ −26.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3376 (OH), 2930 (CH), 1671 (C=O), 1073, 1049; UV λ_{max}: 236.6 nm (MeOH, $\log\varepsilon$ 3.93); ^1H NMR (500 MHz, C_5D_5N): δ 5.93 (1H, *s*, H-11), 4.43 (1H, *m*, H-16), 4.04 (1H, *m*, H-2), 3.86 (1H, *ddd*, *J* = 11.1, 9.2, 5.3 Hz, H-3), 3.27 (3H, *s*, OMe), 1.46 (3H, *d*, *J* = 6.8 Hz, Me-21), 0.99 (3H, *d*, *J* = 6.7 Hz, Me-27), 0.98 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-19); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI–TOFMS *m/z*: 1117.5029 $[M + Na]^+$ (calculated for $C_{51}H_{82}O_{25}$, 1117.5043).

3.17. Enzymatic hydrolysis of **12**

Compound **12** (11 mg) was subjected to enzymatic hydrolysis as described for **11** to yield **5** (8.0 mg) and D-glucose (1.0 mg).

3.18. Compound **13**

(25*R*)-26-[(β-D-Glucopyranosyl)oxy]-22α-methoxy-5α-furostan-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**13**); amorphous solid; $[\alpha]_D^{24}$ −36.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3376 (OH), 2929 (CH), 1070; ^1H NMR (500 MHz, C_5D_5N): δ 4.46 (1H, *m*, H-16), 3.95 (1H, *m*, H-3), 3.27 (3H, *s*, OMe), 1.19 (3H, *d*, *J* = 6.8 Hz, Me-21), 1.00 (3H, *d*, *J* = 6.7 Hz, Me-27), 0.80 (3H, *s*, Me-18), 0.65 (3H, *s*, Me-19); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI–TOFMS *m/z*: 1065.5441 $[M - OMe]^+$ (calculated for $C_{51}H_{85}O_{23}$, 1065.5482); FABMS (positive mode) *m/z*: 1119 $[M + Na]^+$ (Found: C, 54.78; H, 8.26. $C_{52}H_{88}O_{24} \cdot 5/2H_2O$ requires, C, 54.68; H, 8.21%).

3.19. Enzymatic hydrolysis of **13**

Compound **13** (8.6 mg) was subjected to enzymatic hydrolysis as described for **11** to yield **13a** (3.0 mg) and D-glucose (1.0 mg).

3.20. HL-60 cell culture assay

The cell growth was measured with an MTT reduction assay as described in a previous paper (Yokosuka et al., 2002). Briefly, HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) FBS supplemented with L-glutamine, 100 unit/ml penicillin G sodium salt, and 100 μg/ml streptomycin sulfate. The cells (4×10^4 cells/ml) were continuously treated with each compounds for 72 h, and cell growth was measured with an MTT reduction assay procedure. A dose-response curve was plotted for **1**, **6**, **7**, and **10**, and the concentration giving 50% inhibition (IC_{50}) was calculated.

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