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Steroidal glycosides from the underground parts of *Yucca glauca* and their cytotoxic activities

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ABSTRACT

Six steroidal glycosides and 14 known compounds were isolated from the underground parts of *Yucca glauca* (Agavaceae). Their structures were determined from extensive spectroscopic analysis, including analysis of two-dimensional NMR data, and from chemical transformations. The compounds were also evaluated for cytotoxic activities against HL-60 human leukemia cells and A549 human lung adenocarcinoma cells. Four spirostanol glycosides and three furostanol glycosides exhibited cytotoxic activities against both HL-60 and A549 cells. Two of the compounds induced apoptosis in HL-60 cells.

Introduction

Plants of the family Agavaceae are widely distributed in tropical and subtropical regions throughout the world. Plants of the genera *Agave*, *Cordyline*, *Dracaena*, and *Yucca* in Agavaceae are rich sources of steroidal sapogenins and saponins (Mahato et al., 1982; Minaki et al., 1998; Miyakoshi et al., 2000; Yokosuka and Mimaki, 2009a). Previous studies of *Agave utahensis*, *Dracaena thalioides*, and *Cordyline terminalis* led to isolation of a variety of steroidal glycosides, some of which showed cytotoxic activities against cultured tumor cells (Yokosuka and Mimaki, 2007, Yokosuka et al., 2012, 2013a). For example, the 5β-spirostanol glycosides isolated from whole plants of *A. utahensis* induced apoptotic cell death in HL-60 human promyelocytic leukemia cells via caspase-3 activation (Yokosuka et al., 2009b).

The genus *Yucca* comprises about 60 species, and *Yucca glauca* Nutt. ex J. Fraser is a perennial plant native to the southwest of the United States (Tsukamoto, 1988). Roots of the plant are known as soapweed and are an herbal medicine used for the treatment of inflammation, wounds, and bleeding cuts. As part of a series of phytochemical studies on plants of the family Agavaceae, the steroidal glycosides of the underground parts of *Y. glauca* were investigated. Six new steroidal glycosides (**1**–**5** and **13**) and 14 known compounds (**6**–**12** and **14**–**20**) were isolated. Reported herein is the structural determination of the new glycosides on the basis of extensive spectroscopic analysis, including two-dimensional NMR spectroscopy, and chemical transformations. Cytotoxic activities of the isolated compounds against HL-60 cells and A549 human lung adenocarcinoma cells and the apoptotic induction properties of **1** and **16** in HL-60 cells are also described.

Results and discussion

Structural elucidation

The underground parts of *Y. glauca* (2.0 kg) were extracted with MeOH (5 l). After the solvent was removed, the crude extract was fractionated by repeated column chromatography on porous polystyrene resin (Diaion HP-20), silica gel, and octadecylsilanized (ODS) silica gel to yield compounds **1**–**20**. Compounds **6**–**12** and **14**–**20** were identified as (25*R*)-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**6**) (Agrawal et al., 1985; Nakano et al., 1989); (25*R*)-12β-hydroxy-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**7**) (Nakano et al., 1991); (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl)oxy]-5β-spirostan-12-one (**8**) (Pant et al., 1986); 2β-hydroxy-5β-spirost-25

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(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**9**) (Miyakoshi et al., 2000); (25*R*)-2β-hydroxy-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**10**) (Nakano et al., 1989); 5β-spirost-25(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranoside (**11**) (Miyakoshi et al., 2000); (25*R*)-5β-spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranoside (**12**) (Miyakoshi et al., 2000; Inoue et al., 1995); (25*R*)-26-[(β-D-glucopyranosyl)oxy]-2β,22α-dihydroxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**14**) (Peng et al., 1994); (25*S*)-26-[(β-D-glucopyranosyl)oxy]-2β,22α-dihydroxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**15**) (Kang et al., 2006); 26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5β-furost-25(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**16**) (Chen et al., 2007); (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**17**) (Chen et al., 2007); (25*S*)-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside (**18**) (Nagumo et al., 1991); (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5β-furostan-12-one (**19**) (Zhang et al., 2007); and (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**20**) (Cheng et al., 2009), respectively. Compounds **6** and **12** also contain the corresponding 25*S* isomers by analysis of the ^{1}H and ^{13}C NMR spectra (Fig. 1).

Compound **1** was obtained as an amorphous solid with a molecular formula of $C_{39}H_{62}O_{13}$, as determined by HRESI–TOFMS (*m*/*z*: 739.4244 $[M+H]^+$), ^{13}C NMR, and DEPT (Table 1). The ^{1}H NMR spectrum of **1** contained signals for two tertiary methyl groups at δ_H 0.95 (3H, *s*) and 0.81 (3H, *s*), a secondary methyl group at δ_H 1.09 (3H, *d*, *J* = 6.8 Hz), an exomethylene group at δ_H 4.81 and 4.77 (each 1H, *br s*), and two anomeric protons at δ_H 5.28 (1H, *d*, *J* = 7.7 Hz) and 4.89 (1H, *d*, *J* = 7.5 Hz). Acid hydrolysis of **1** with 1 M HCl yielded a sapogenin identified as 5β-spirost-25(27)-en-3β-ol (**1a**) (Miyakoshi et al., 2000), D-galactose and D-glucose. Identification of monosaccharides, including their absolute configurations, was carried out by direct HPLC analysis of the hydrolysate. These data suggested that **1** was a 5β-spirostan diglycoside. The NMR spectroscopic data for **1** implied that its sugar moiety was composed of a β-D-galactopyranosyl ($^4C_1$) unit (Gal) [δ_H 4.89 (1H, *d*, *J* = 7.5 Hz);

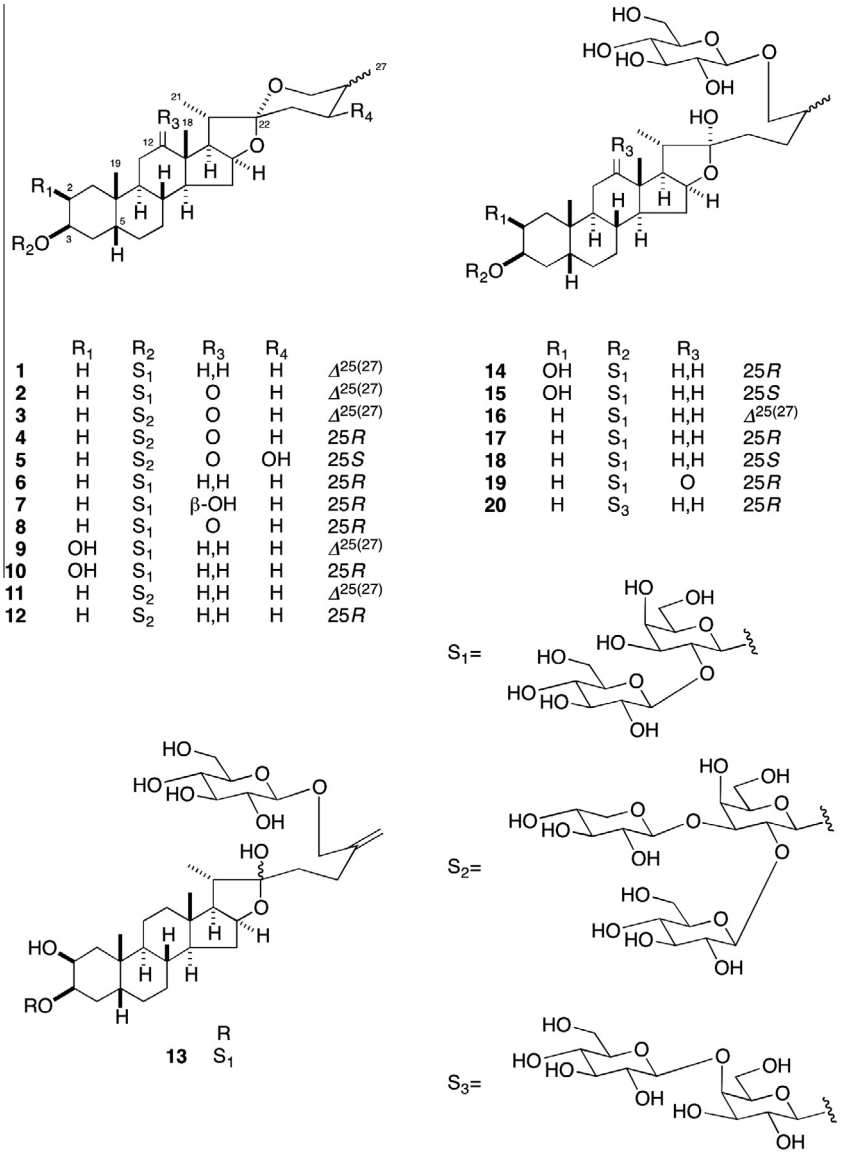

Fig. 1. Structures of compounds **1**–**20**.

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

Position	1	2	3	4	5	13
1	30.7	30.6	30.3	30.3	30.3	40.4
2	26.6	26.5	26.5	26.5	26.5	67.2
3	75.2	75.2	74.8	74.8	74.8	81.7
4	30.7	30.7	30.5	30.5	30.5	31.7
5	36.7	36.5	36.0	36.1	36.0	36.4
6	26.6	26.8	26.7	26.8	26.8	26.2
7	26.8	26.4	26.4	26.4	26.4	26.7
8	35.4	34.7	34.7	34.7	34.7	35.4
9	40.1	41.9	41.9	41.9	41.9	41.3
10	35.1	35.7	35.7	35.7	35.7	37.0
11	21.0	37.7	37.7	37.7	37.7	21.2
12	40.1	213.0	213.1	213.1	213.0	40.2
13	40.8	55.6	55.6	55.6	55.6	41.1
14	56.3	56.0	56.0	56.0	56.0	56.2
15	32.0	31.4	31.4	31.5	31.4	32.2
16	81.5	80.1	80.1	79.8	80.1	81.2
17	63.2	54.3	54.3	54.3	53.9	63.8
18	16.4	16.0	16.0	16.0	16.0	16.6
19	23.9	23.1	23.1	23.1	23.1	23.8
20	41.7	42.5	42.5	42.6	42.9	40.6
21	14.9	13.9	13.8	13.9	13.8	16.3
22	109.4	109.5	109.5	109.3	111.9	110.3
23	28.8	33.2	33.2	31.8	41.8	37.9
24	33.1	28.9	28.9	29.2	70.5	28.3
25	144.3	144.2	144.2	30.5	39.9	147.2
26	65.0	65.1	65.0	66.9	65.4	72.0
27	108.7	108.8	108.8	17.3	13.6	110.7

δ_C 102.2, 81.2, 75.1, 69.7, 76.5, and 62.0] and a β-D-glucopyranosyl ($^4C_1$) unit (Glc) [δ_H 5.28 (1H, *d*, *J* = 7.7 Hz); δ_C 105.7, 76.7, 77.9, 71.6, 78.3, and 62.7] (Agrawal et al., 1985). The absence of a glycosylation shift in the carbon resonances suggested that the glucosyl residue was a terminal unit (Table 2). In the HMBC spectrum of **1**, the anomeric proton (H-1) of Glc at δ_H 5.28 showed a long-range correlation with C-2 of Gal at δ_C 81.2, and H-1 of Gal at δ_H 4.89 showed a correlation with C-3 of the aglycone at δ_C 75.2. Thus, **1** was assigned as 5β-spirost-25(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside.

Compound **2** was obtained as an amorphous solid with a molecular formula $C_{39}H_{60}O_{14}$ as determined by HRESI–TOFMS (*m*/*z*: 753.4061 [M+H]$^+$) data. Comparison of the ^{13}C NMR spectrum of **2** indicated that it possessed a diglycoside moiety identical to that of **1**, but that the aglycone structure was slightly different. The IR (1705 cm^{-1}) and ^{13}C NMR (δ_C 213.0) spectra indicated that the aglycone contained a carbonyl group. The HMBC correlations between C-12 at δ_C 213.0 and H_2-11 at δ_H 2.36 (*dd*, *J* = 13.6, 13.6 Hz), and δ_H 2.19 (*dd*, *J* = 13.6, 4.8 Hz), H-17 at δ_H 2.82 (dd, *J* = 8.2, 7.1 Hz), and Me-18 at δ_H 1.09 (*s*) showed that the carbonyl was located at C-12. Acid hydrolysis of **2** gave 3β-hydroxy-5β-spirost-25(27)-en-12-one (**2a**) (Miyakoshi et al., 2000), together with D-galactose and D-glucose. Thus, **2** was assigned as 3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl)oxy]-5β-spirost-25(27)-en-12-one.

Compound **3** had a molecular formula $C_{44}H_{68}O_{18}$ as determined by HRESI–TOFMS (*m*/*z*: 907.4250 [M+Na]$^+$). Its molecular formula was larger than that of **2** by $C_5H_8O_4$, and the ^{1}H NMR spectrum showed signals for three anomeric protons at δ_H 5.57 (1H, *d*, *J* = 7.9 Hz), 5.23 (1H, *d*, *J* = 7.7 Hz), and 4.88 (1H, *d*, *J* = 7.7 Hz). Acid hydrolysis of **3** yielded **2a**, D-galactose, D-glucose, and D-xylose. When the ^{13}C NMR spectrum of **3** was compared with that of **2**, a set of five additional signals corresponding to a terminal β-D-xylopyranosyl moiety (Xyl, see Table 1) was observed. The signals assigned to C-3 of the glucosyl moiety linked to C-3 of the aglycone and its neighboring carbons varied, whereas all other resonances were mainly unaffected. In the HMBC spectrum of **3**, long-range correlations were observed between H-1 of Glc at δ_H 5.57 and C-2 of Gal at δ_C 77.6; between H-1 of Xyl at δ_H 5.23 and C-3 of Gal at δ_C 84.2; and between H-1 of Gal at δ_H 4.88 and C-3 of the aglycone at δ_C 74.8. Thus, **3** was assigned as 3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranosyl)oxy]-5β-spirost-25(27)-en-12-one.

Compound **4** had a molecular formula of $C_{44}H_{70}O_{18}$ as determined by HRESI–TOFMS data (*m*/*z*: 909.4509 [M+Na]$^+$). Its ^{13}C NMR spectrum was very similar to **3**, with the exception of the aglycone F-ring (C-22–C-27) signals. Comparing the ^{1}H and ^{13}C NMR spectra of **4** with those of **3** showed that the resonances from the C-25(27)-exomethylene group in **3** were replaced by those from a Me-CH group at δ_H 0.70 (3H, *d*, *J* = 5.6 Hz) and 1.57 (1H, *m*), and δ_C 17.3 (Me) and 30.5 (CH) in **4**. Acid hydrolysis of **4** gave (25*R*)-3β-hydroxy-5β-spirostan-12-one (**4a**) (Nakano et al., 1991), D-galactose, D-glucose, and D-xylose. Accordingly, **4** was assigned as (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranosyl)oxy]-5β-spirostan-12-one.

Compound **5** had a molecular formula of $C_{44}H_{70}O_{19}$, as determined by HRESI–TOFMS (*m*/*z*: 925.4352 [M+Na]$^+$). The molecular formula was higher than that of **4** by one oxygen atom. Acid hydrolysis of **5** gave D-galactose, D-glucose, and D-xylose, and the aglycone decomposed under acidic conditions. Comparison of the ^{1}H and ^{13}C NMR assignments of **5** with those of **4** established that portions A–E (C-1–C-21) of the aglycone and the sugar moiety attached to C-3 of the aglycone were the same as those of **4**. However, differences were observed in the signals from the F-ring (C-22–C-27). In the ^{1}H-^{1}H COSY spectrum of **5**, an oxymethine proton at δ_H 4.03 exhibited spin-coupling correlations with methylene protons at δ_H 2.33 and 2.02 (H_2-23), and methine protons δ_H 1.85 (H-25). The methine proton was coupled with oxymethylene protons at δ_H 3.71 and 3.60 (H_2-26) and methyl protons at δ_H 1.10 (Me-27). This showed the presence of a hydroxy group at C-24. The 24*R* and 25*S* configurations were revealed by ROE correlations from H-24 at δ_H 4.03 to H-26ax at δ_H 3.60 and Me-27 at δ_H 1.10 observed in the ROESY spectrum of **5** (Fig. 2). Thus, **5** was assigned as (24*R*,25*S*)-3β-[(*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranosyl)oxy]-24-hydroxy-5β-spirostan-12-one.

Compound **13** had a molecular formula of $C_{45}H_{74}O_{20}$ as determined by HRESI–TOFMS (*m*/*z*: 957.4672 [M+H]$^+$). Its ^{1}H NMR spectrum showed signals for three steroid methyl groups at δ_H 1.30 (*d*, *J* = 6.9 Hz), 0.94 (*s*), and 0.83 (*s*), an exomethylene group at δ_H 5.33 and 5.04 (each 1H, *br s*), as well as resonances for three anomeric protons at δ_H 5.28 (1H, *d*, *J* = 7.7 Hz), 4.98 (1H, *d*, *J* = 7.6 Hz), and 4.89 (1H, *d*, *J* = 7.7 Hz). The ^{1}H NMR data, the acetalic carbon signal at δ_C 110.3 in the ^{13}C NMR spectrum, and a positive color reaction in Ehrlich's test, indicated that **13** was a 22-hydroxyfurostanol saponin with three monosaccharides (Kiyosawa and Hutoh, 1968; Nohara et al., 1975). Enzymatic hydrolysis of **13** with β-D-glucosidase gave **9** and D-glucose. The HMBC spectrum of **13** showed a long-range correlation between H-1 of Glc (II) at δ_H 4.98 and C-26 of the aglycone at δ_C 72.0, showing that a glucosyl group was linked to the C-26 hydroxy group of the aglycone, which is typical of naturally occurring furostanol glycosides (Table 2). Accordingly, **13** was assigned as 26-[(β-D-glucopyranosyl)oxy]-2β,22ζ-dihydroxy-5β-furostan-25(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside.

Cytotoxic activity

The isolated compounds (**1**–**20**) were evaluated for their cytotoxic activities against HL-60 cells using a modified MTT assay (Table 3). The 5β-spirostanol glycosides (**1**, **6**, and **9**–**12**) and 5β-furostanol glycosides (**13**–**18** and **20**) showed cytotoxic activity against HL-60 cells with IC_{50} values from 2.5 to 17.8 μM. These compounds were also subjected to a cytotoxic screening test using A549 cells. Compounds **1**, **6**, **11**, **12**, and **16**–**18** exhibited cytotoxic activity against HL-60 and A549 cells. Compounds **9**, **10**, and **13**–**15**, which were the 2β-hydroxy derivatives of **1**, **6**,

Table 2
^{1}H and ^{13}C NMR chemical shift assignments for the sugar moieties of **1**–**5**, and **13** in C_5D_5N.[a]

1				**2**				**3**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Gal	1	4.89 d (7.5)	102.2	Gal	1	4.88 d (7.7)	102.4	Gal	1	4.88 d (7.7)	101.9
	2	4.66 dd (8.5, 7.7)	81.2		2	4.66 dd (8.6, 7.7)	81.7		2	4.82 dd (9.0, 7.7)	77.6
	3	4.25 dd (8.5, 2.7)	75.1		3	4.26 dd (8.6, 3.1)	75.2		3	4.28 dd (9.0, 3.1)	84.2
	4	4.54 br s	69.7		4	4.56 br s	69.8		4	4.77 br d (3.1)	69.8
	5	4.11 m	76.5		5	4.01 m	76.6		5	4.04 m	76.4
	6 (2H)	4.39 m	62.0		6 (2H)	4.42 m	62.1		6 (2H)	4.39 m	62.0
Glc	1	5.28 d (7.7)	105.7	Glc	1	5.29 d (7.7)	106.1	Glc	1	5.57 d (7.9)	104.4
	2	4.06 dd (8.9, 7.7)	76.7		2	4.08 d (9.1, 7.7)	76.9		2	4.02 dd (9.0, 7.9)	76.4
	3	4.18 dd (8.9, 8.9)	77.9		3	4.21 dd (9.1, 9.1)	78.0		3	4.23 dd (9.0, 9.0)	78.5
	4	4.26 dd (8.9, 8.9)	71.6		4	4.31 dd (9.1, 9.1)	71.7		4	4.16 dd (9.0, 9.0)	72.6
	5	3.83 m	78.3		5	3.85 m	78.4		5	3.78 m	77.9
	6a	4.49 dd (11.8, 2.7)	62.7		6a	4.51 dd (11.6, 2.5)	62.8		6a	4.47 dd (11.4, 3.3)	63.4
	b	4.42 dd (11.8, 4.5)			b	4.47 m			b	4.34 dd (11.4, 5.4)	
								Xyl	1	5.23 d (7.7)	106.2
									2	3.95 dd (8.6, 7.7)	75.1
									3	4.10 dd (8.6, 8.6)	78.5
									4	4.13 m	71.0
									5a	4.19 m	67.1
									b	3.60 dd (10.3, 10.3)	

4				**5**				**13**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Gal	1	4.88 d (7.7)	101.9	Gal	1	4.88 d (7.7)	101.9	Gal	1	4.89 d (7.7)	103.2
	2	4.82 dd (9.4, 7.7)	77.7		2	4.82 dd (9.5, 7.7)	77.7		2	4.73 dd (9.3, 7.7)	81.5
	3	4.28 dd (9.4, 3.1)	84.2		3	4.28 dd (9.5, 3.1)	84.2		3	4.28 dd (9.3, 3.1)	75.1
	4	4.78 br d (3.1)	69.8		4	4.77 br d (3.1)	69.8		4	4.51 br s	69.7
	5	4.03 m	76.4		5	4.03 m	76.4		5	4.11 m	76.9
	6 (2H)	4.39 m	62.1		6 (2H)	4.39 m	62.1		6a	4.46 m	62.0
									b	4.38 m	
Glc	1	5.57 d (7.9)	104.4	Glc	1	5.57 d (7.9)	104.4	Glc (I)	1	5.28 d (7.7)	106.0
	2	4.02 dd (8.9, 7.9)	76.4		2	4.02 dd (8.9, 7.9)	76.4		2	4.09 d (9.0, 7.7)	76.9
	3	4.23 dd (8.9, 8.9)	78.5		3	4.23 dd (8.9, 8.9)	78.5		3	4.20 dd (9.0, 9.0)	77.9
	4	4.16 dd (8.9, 8.9)	72.7		4	4.16 dd (8.9, 8.9)	72.7		4	4.29 dd (9.0, 9.0)	71.6
	5	3.77 m	77.8		5	3.77 m	77.8		5	3.85 m	78.5
	6a	4.46 dd (11.4, 3.2)	63.4		6a	4.46 dd (11.4, 3.2)	63.4		6a	4.52 m	62.7
	b	4.34 dd (11.4, 5.3)			b	4.34 dd (11.4, 5.3)			b	4.43 m	
Xyl	1	5.23 d (7.7)	106.2	Xyl	1	5.23 d (7.7)	106.2	Glc (II)	1	4.98 d (7.6)	103.8
	2	3.95 dd (8.6, 7.7)	75.1		2	3.95 dd (8.5, 7.7)	75.1		2	4.06 d (8.9, 7.6)	75.1
	3	4.10 dd (8.6, 8.6)	78.5		3	4.10 dd (8.5, 8.5)	78.5		3	4.25 dd (8.9, 8.9)	78.5
	4	4.13 m	71.0		4	4.13 m	71.0		4	4.23 dd (8.9, 8.9)	71.6
	5a	4.19 m	67.1		5a	4.19 m	67.2		5	3.93 m	78.4
	b	3.60 dd (10.5, 10.5)			b	3.60 dd (10.5, 10.5)			6a	4.54 dd (11.4, 2.0)	62.7
									b	4.36 m	

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

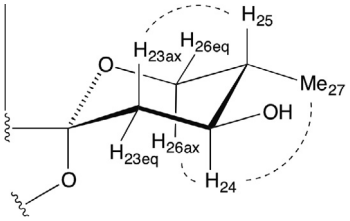

Fig. 2. Important ROE correlations in **5**.

and **16**–**18**, were only cytotoxic to HL-60 cells. The 5β-spirostanol glycosides with the C-12 carbonyl group (**2**–**5** and **8**) and C-12 hydroxy group (**7**) were not cytotoxic to the HL-60 and A549 cells.

Apoptosis induction activity

Compounds **1**, **6**, **11**, **12**, and **16**–**18** showed cytotoxicity against HL-60 and A549 cells. Morphological observations of the cultured HL-60 cells stained with DAPI suggested that HL-60 cell death caused by **1**, **6**, **11**, **12**, and **16**–**18** was mediated partially through induction of apoptosis, because nuclear chromatin condensation and cell shrinkage were observed (Fig. 3). Caspases are cysteine proteases and play a crucial role in the apoptotic signaling pathways. Caspace-3 has been shown to be the executing enzyme for apoptosis. Compound **1** is a new 5β-spirostanol glycoside with cytotoxicity against HL-60 cells, and **16** is the corresponding 5β-furostanol glycoside of **1**. When HL-60 cells were treated with **1** (20 μg/ml) for 6 h, the cell viability was reduced to 5.4% and caspase-3 was markedly activated (Figs. 4 and 5). However, **16** (20 μg/ml) failed to induce apoptotic cell death in HL-60 cells after 6 h, and required 16 h for caspase-3 activation and induction of apoptosis in HL-60 cells.

Conclusion

The underground parts of *Y. glauca* yielded 20 steroidal glycosides, including five new 5β-spirostanol glycosides (**1**–**5**) and a new 5β-furostanol glycoside (**13**). 5β-Steroidal glycosides are

Table 3
Cytotoxic activities of the isolated compounds **1**–**20**, cisplatin, and etoposide against HL-60 and A549 cells.

Compounds	IC_{50} (μM)[a]	
	HL-60	A549
1	2.5 ± 0.47	7.3 ± 0.63
2	>20	–[b]
3	>20	–
4	>20	–
5	>20	–
6	3.1 ± 0.35	8.4 ± 0.34
7	>20	–
8	>20	–
9	5.0 ± 0.09	>20
10	11.3 ± 1.42	>20
11	4.9 ± 0.43	6.0 ± 0.65
12	4.2 ± 0.37	5.9 ± 0.43
13	13.3 ± 0.09	>20
14	17.8 ± 2.47	>20
15	9.2 ± 1.21	>20
16	4.4 ± 0.10	11.9 ± 0.70
17	3.7 ± 0.55	7.0 ± 0.29
18	3.3 ± 0.15	9.3 ± 2.07
19	>20	–
20	14.3 ± 0.07	>20
Cisplatin	1.7 ± 0.06	2.1 ± 1.10
Etoposide	0.39 ± 0.08	–

[a] Data are represent the mean value ± S.E.M. of three experiments performed in triplicate.
[b] Not determined.

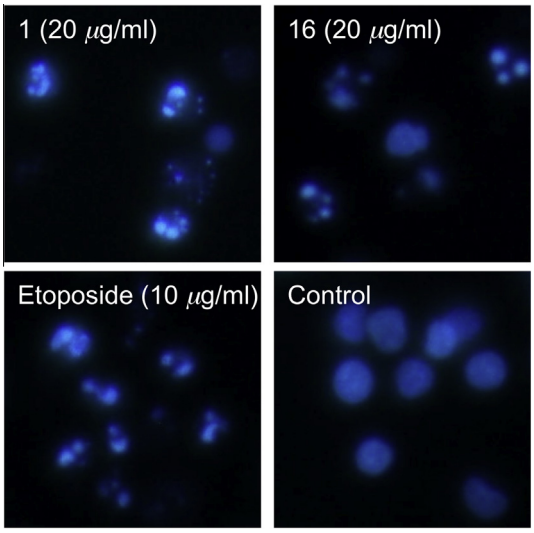

Fig. 3. Morphology of representative fields of HL-60 cells stained with DAPI after treatment with **1**, **16**, or etoposide for 16 h to evaluate fragmented and condensed nuclear chromatins.

a small group of naturally occurring steroidal glycosides compared to 5α- and 5(6)-ene steroidal glycosides, and are known to occur only in a limited number of higher plants belonging to the families Agavaceae and Liliaceae (Yokosuka et al., 2009b; Higano et al., 2007). Seven compounds (**1**, **6**, **11**, **12**, and **16**–**18**) showed cytotoxic activity against both HL-60 and A549 cells. Compound **1** induced apoptotic cell death in HL-60 cells and caspase-3 activation after 6 h. In contrast, **16** required 16 h for caspase-3 activation and induction of apoptosis in HL-60 cells.

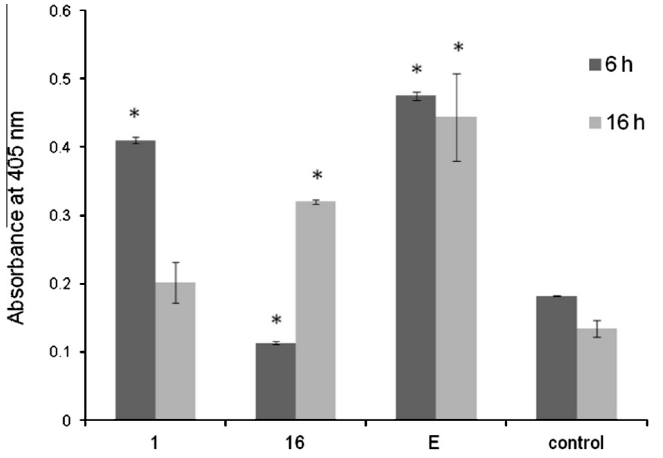

Fig. 4. Caspase-3 activity in the lysates of cells treated with **1**, **16**, or etoposide HL-60 cells were incubated at 37 °C for 6 h or 16 h with 20 μg/ml of **1** or **16**, or 10 μg/ml of etoposide (E). The data represent the mean ± S.E.M. of three experiments. Results with $p < 0.05$ were considered significantly different from the control group, and are marked with *.

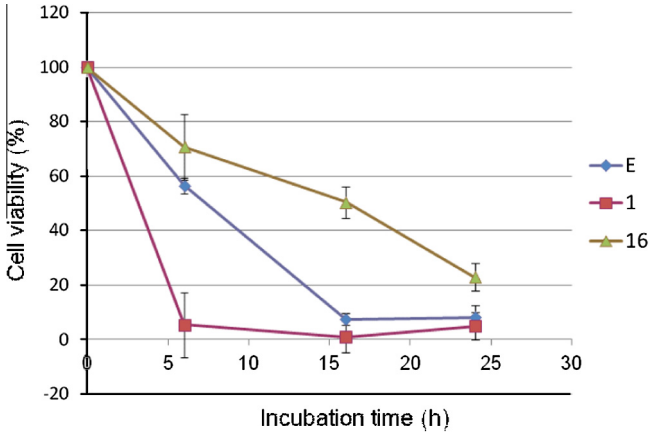

Fig. 5. Cell viability of HL-60 cells treated with **1**, **16**, or etoposide. Cell viability was determined by an MTT assay after incubation with 20 μg/ml of **1**, **16**, or 10 μg/ml of etoposide (E) at 6, 16, and 24 h. The data represent the mean ± S.E.M. of three experiments.

Experimental

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. NMR spectra were recorded on a Bruker DRX-500 spectrometer (Karlsruhe, Germany) at 500 MHz for ^{1}H NMR, using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESI–TOFMS was recorded on a Waters-Micromass LCT mass spectrometer (Manchester, UK). 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 silica gel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 aqueous solution, followed by heating. HPLC was performed on a system composed of a DP-8020 (Tosoh, Tokyo, Japan) pump, a RI-8021

(Tosoh) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. A Capcell pak C18 AQ column (10 mm i.d. × 250 mm, ODS 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC. The following reagents were used: RPMI 1640 medium, MEM and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma–Aldrich, St. Louis, MO, USA); fetal bovine serum (FBS) (Bio-Whittaker, Walkersville, MO, USA); penicillin G sodium salt and streptomycin sulfate (Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

Plant material

The plant material, defined as the underground parts of *Y. glauca* Nutt. ex J. Fraser, was obtained from a wholesale firm in Richters, Ontario, Canada in 1999. A voucher specimen has been deposited in our laboratory (voucher No. YG-99-001, Department of Medicinal Pharmacognosy).

Extraction and isolation

The plant material (dry weight 2.0 kg) was extracted with hot MeOH (2 × 5 l). The combined MeOH extract was concentrated under reduced pressure, and the viscous concentrate (435 g) was passed through a Diaion HP-20 column (90 mm i.d. × 300 mm), successively eluted with MeOH–H_2O (3:7, 5:5), MeOH, EtOH, and EtOAc (5 l each). Silica gel CC (75 mm i.d. × 220 mm) of the MeOH eluted fraction (50 g), and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (9:1:0, 40:10:1, 20:10:1, 1:1:0), and finally with MeOH alone, gave 12 fractions (I–XII). Fraction VI was applied to an ODS silica gel column eluted with MeCN–H_2O (1:2, 2:3, 1:1) to give **1** (43.5 mg), **6** (315 mg), and **8** (152 mg), respectively. Fraction VIII was separated by ODS silica gel CC eluted with MeOH–H_2O (6:4; 2:1; 7:3; 8:2) to give **2** (10.8 mg), **3** (11.0 mg), **4** (265 mg), **7** (379 mg), **9** (158 mg), **10** (94.7 mg), **11** (6.1 mg), and **12** (9.6 mg). Fraction IX was separated by ODS silica gel CC eluted with MeCN–H_2O (1:4, 1:3, 1:2) to give nine subfractions (IXa–IXi). Fraction IXb was purified on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (30:10:1, 20:10:1) to give **19** (59 mg). Fraction IXc was purified on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (20:10:1) to afford **5** (10.0 mg). Fraction IXd was purified by preparative HPLC using MeCN–H_2O (1:4) to yield **16** (13.5 mg), **17** (315 mg), and **18** (180 mg). Fraction IXg was purified on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (30:10:1) to afford **20** (9.0 mg). Fraction XI was purified on an ODS silica gel column eluted with MeCN–H_2O (1:4, 2:7, 2:5, 2:3) and by preparative HPLC using MeCN–H_2O (1:4) to yield **13** (22.0 mg), **14** (21.5 mg), and **15** (19.0 mg).

*Compound **1***

5*β*-Spirost-25(27)-en-3*β*-yl *O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-galactopyranoside; amorphous solid; $[\alpha]_D^{25}$ −4.4 (*c* 0.10; MeOH); IR (film) ν_{max} 3347 (OH), 2925 (CH), 1041 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) *δ* 4.81 and 4.77 (each 1H, *br s*, H_2-27), 4.61 (1H, *m*, H-16), 4.46 and 4.04 (each 1H, *d*, *J* = 12.3 Hz, H_2-26), 4.32 (1H, *br s*, H-3), 1.09 (3H, *d*, *J* = 6.8 Hz, Me-21), 0.95 (3H, *s*, Me-19), 0.81 (3H, *s*, Me-18), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESI–TOFMS *m*/*z*: 739.4244 $[M+H]^+$ (calcd for $C_{39}H_{63}O_{13}$, 739.4269).

*Acid hydrolysis of **1***

A solution of **1** (10.2 mg) in 1 M HCl (dioxane-H_2O, 1:1; 2 ml) was heated at 95 °C for 2 h under Ar. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column and was applied to silica gel column eluted with $CHCl_3$–MeOH (9:1) followed by MeOH to yield **1a** (2.8 mg) and a sugar fraction (3.0 mg). The sugar fraction was passed through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), and was then analyzed by HPLC under the following conditions: column, Capcell Pak NH2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido); solvent, MeCN–H_2O (17:3); flow rate, 0.8 ml/min; detection, RI and OR. Identification of D-galactose and D-glucose present in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min): 13.88 (D-galactose, positive optical rotation), 14.84 (D-glucose, positive optical rotation).

*Compound **2***

3*β*-[(*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-galactopyranosyl)oxy]-5*β*-spirost-25(27)-en-12-one; amorphous solid; $[\alpha]_D^{25}$ −1.6 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3363 (OH), 2925 (CH), 1705 (C=O), 1077, 1041; ^1H NMR (500 MHz, C_5D_5N): *δ* 4.82 and 4.79 (each 1H, *br s*, H_2-27), 4.55 (1H, *m*, H-16), 4.46 and 4.04 (each 1H, *d*, *J* = 12.3 Hz, H_2-26), 4.25 (1H, *br s*, H-3), 2.82 (dd, *J* = 8.2, 7.1 Hz, H-17), 2.36 (*dd*, *J* = 13.6, 13.6 Hz, H-11a), 2.19 (*dd*, *J* = 13.6, 4.8 Hz, H-11b), 1.32 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.09 (3H, *s*, Me-18), 0.99 (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 Table 2; HRESI–TOFMS *m*/*z*: 753.4061 $[M+H]^+$ (calculated for $C_{39}H_{61}O_{14}$, 753.4061).

*Compound **3***

3*β*-[(*O*-*β*-D-glucopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-galactopyranosyl)oxy]-5*β*-spirost-25(27)-en-12-one; amorphous solid; $[a]_D^{25}$ −6.8 (*c* 0.10; MeOH); IR (film) ν_{max} 3376 (OH), 2925 (CH), 1706 (C=O), 1040 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) *δ* 4.82 and 4.78 (each 1H, *br s*, H_2-27), 4.55 (1H, *m*, H-16), 4.46 and 4.04 (each 1H, *d*, *J* = 12.3 Hz, H_2-26), 4.24 (1H, *br s*, H-3), 1.32 (3H, *d*, *J* = 7.0 Hz, Me-21), 1.08 (3H, *s*, Me-18), 0.96 (3H, *s*, Me-19), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESI–TOFMS *m*/*z* 907.4250 $[M+Na]^+$ (calcd for $C_{44}H_{68}O_{18}Na$, 907.4303).

*Compound **4***

(25*R*)-3*β*-[(*O*-*β*-D-glucopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-galactopyranosyl)oxy]-5*β*-spirostan-12-one; amorphous solid; $[a]_D^{25}$ −8.9 (*c* 0.10; MeOH); IR (film) ν_{max} 3376 (OH), 2927 (CH), 1706 (C=O), 1074 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) *δ* 4.55 (1H, *m*, H-16), 4.25 (1H, *br s*, H-3), 3.59 (1H, *m*, H-26a), 3.49 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-26b), 1.57 (1H, *m*, H-25), 1.36 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.08 (3H, *s*, Me-18), 0.96 (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; HRESI–TOFMS *m*/*z* 909.4509 $[M+Na]^+$ (calcd for $C_{44}H_{70}O_{18}Na$, 909.4460).

*Compound **5***

(24*R*,25*S*)-3*β*-[(*O*-*β*-D-glucopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-galactopyranosyl)oxy]-24-hydroxy-5*β*-spirostan-12-one: amorphous solid; $[a]_D^{25}$ −13.6 (*c* 0.10; MeOH); IR (film) ν_{max} 3377 (OH), 2927 (CH), 1704 (C=O), 1073, 1037 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) *δ* 4.56 (1H, *m*, H-16), 4.25 (1H, *br s*, H-3), 4.03 (1H, *m*, H-24), 3.71 (1H, *dd*, *J* = 11.1, 4.7 Hz, H-26a), 3.60 (1H, *dd*, *J* = 11.1, 11.1 Hz, H-26b), 2.33 (1H, *dd*, *J* = 12.6, 4.6 Hz, H-23a), 2.02 (1H, *dd*, *J* = 12.6, 12.6 Hz, H-23b), 1.85 (1H, *m*, H-25), 1.39 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.10 (3H, *d*, *J* = 6.5 Hz, Me-27), 1.07 (3H, *s*, Me-18), 0.96 (3H, *s*, Me-19), signals for the

sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESI–TOFMS *m/z* 925.4352 $[M+Na]^+$ (calcd for $C_{44}H_{70}O_{19}Na$, 925.4409).

*Acid hydrolysis of **2–5***

Compounds **2** (6.3 mg), **3** (8.0 mg), **4** (9.7 mg), and **5** (8.3 mg) were independently subjected to the acid hydrolysis described for **1** to give the aglycones (**2a**: 3.5 mg and 2.9 mg from **2** and **3**, respectively, and **4a**: 3.0 mg from **4**) and sugar fractions (**2**: 2.7 mg, **3**: 1.0 mg, **4**: 3.0 mg, and **5**: 3.0 mg). HPLC analysis of the sugar fractions under the same conditions used for **1** showed the presence of D-xylose, D-galactose, and D-glucose in those of **2–5**. t_R (min): 9.39 (D-xylose, positive optical rotation), 13.88 (D-galactose, positive optical rotation), 14.84 (D-glucose, positive optical rotation).

*Compound **13***

26-[(β-D-glucopyranosyl)oxy]-2β,22ζ-dihydroxy-5β-furostan-25(27)-en-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside; amorphous solid; $[\alpha]_D^{25}$ −13.9 (*c* 0.10; MeOH); IR (film) ν_{max} 3377 (OH), 2928 (CH), 1075, 1043 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 5.33 and 5.04 (each 1H, *br s*, H_2-27), 4.97 (1H, *m*, H-16), 4.61 and 4.34 (each 1H, *d*, *J* = 12.3 Hz, H_2-26), 4.40 (1H, *br s*, H-3), 3.82 (1H, *m*, H-2), 1.30 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.94 (3H, *s*, Me-19), 0.83 (3H, *s*, Me-18), signals for the sugar moiety, see Table 1; ^{13}C NMR, see Table 2; HRESI–TOFMS *m/z* 957.4672 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{20}Na$, 957.4671).

*Enzymatic hydrolysis of **13***

Compound **13** (9.3 mg) was treated with β-D-glucosidase (Sigma–Aldrich, EC 3.2.1.2.1, 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 **9** (2.8 mg) and D-glucose (1.0 mg).

Cell culture assays

HL-60 cells (JCRB 0085) and A549 cells (JCRB 0076) were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan). HL-60 cells or A549 cells were cultured in RPMI 1640 medium or MEM containing heat-inactivated 10% (v/v) FBS supplemented with L-glutamine, 100 unit/ml penicillin G sodium salt, and 100 μg/ml streptomycin sulfate in a humidified incubator at 37 °C with 5% CO_2. The cell growth was measured with an MTT reduction assay as previously described (Yokosuka et al., 2013b). The cells (HL-60 cells: 4×10^4 cells/ml, A549 cells: 1×10^4 cells/ml) were continuously treated with each compound for 72 h, and cell viability was measured with an MTT reduction assay procedure. Dose–response curves were plotted for **1**, **6**, **9–18**, and **20**, and the concentrations giving 50% inhibition (IC_{50}) were calculated.

Assay for caspase-3 activation

The activity of caspase-3 was measured by using a commercially available kit (Appocyto Caspase-3 Colorimetric Assay Kit, MBL, Aichi, Japan). HL-60 cells (2×10^6) were treated with test samples for 6 h, and the cells were centrifuged and collected. Cell pellets were suspended in 60 μl of ice cold cell lysis buffer, and incubated on ice for 10 min. This cell pellet suspension was centrifuged at 10,000*g* for 5 min and the supernatant was collected. The cell lysate (50 μl, equivalent to 200 μg protein) was mixed with of reaction buffer (2×50 μl) containing the substrates for caspase-3 [DEVD-*p*NA (*p*-nitroanilide)]. After incubation for 2 h at 37 °C, the absorbance at 405 nm of the liberated chromophore *p*NA was measured using a microplate reader. The activity of caspase-3 was evaluated in triplicate.

Statistical analysis

For statistical analysis, one-way analysis of variance (ANOVA) followed by Dunnett's test was performed. A probability (*p*) value of less than 0.05 was considered to represent a statistically significant difference.

References

Agrawal, P.K., Jain, D.C., Gupta, R.K., Thakur, R.S., 1985. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry 24, 2479–2496.

Chen, H., Wang, G., Wang, N., Yang, M., Wang, Z., Wang, X., Yao, X., 2007. New furostanol saponins from the bulbs of *Allium macrostemon* Bunge and their cytotoxic activity. Pharmazie 62, 544–548.

Cheng, S., Du, Y., Ma, B., Li, L., Zhao, Y., 2009. Synthesis of oligosaccharide timosaponin BII from sarsasapogenin via borohydride stereoselective reductive cyclization reaction. PCT Int. Appl. (WO 2009133401 A1 20091105).

Higano, T., Kuroda, M., Sakagami, H., Mimaki, Y., 2007. Convallasaponin A, a new 5β-spirostanol triglycoside from the rhizomes of *Convallaria majalis*. Chem. Pharm. Bull. 55, 337–339.

Inoue, T., Mimaki, Y., Sashida, Y., Kobayashi, M., 1995. Structures of toxic steroidal saponins from *Narthecium asiaticum* Maxim. Chem. Pharm. Bull. 43, 1162–1166.

Kang, L., Ma, B., Shi, T., Zhang, J., Xiong, C., 2006. Two new furostanol saponins from the rhizomes of *Anemarrhena asphodeloides*. Yaoxue Xuebao 41, 527–532.

Kiyosawa, S., Hutoh, M., 1968. Detection of proto-type compounds of diosgenin and other spirostanol glycosides. Chem. Pharm. Bull. 16, 1162–1164.

Mahato, S.B., Ganguly, A.N., Sahu, N.P., 1982. Steroid saponins. Phytochemistry 21, 959–978.

Minaki, Y., Kuroda, M., Takaashi, Y., Sashida, Y., 1998. Steroidal saponins from the leaves of *Cordyline stricta*. Phytochemistry 47, 79–85.

Miyakoshi, M., Tamura, Y., Masuda, H., Mizutani, K., Tanaka, O., Ikeda, T., Ohtani, K., Kasai, R., Yamasaki, K., 2000. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave Yucca), a new anti-food-deteriorating agent. J. Nat. Prod. 63, 332–338.

Nagumo, S., Kishi, S., Inoue, T., Nagai, M., 1991. Saponins of *Anemarrhenae rhizoma*. Yakugaku Zasshi 111, 306–310.

Nakano, K., Yamasaki, T., Imamura, Y., Murakami, K., Takaishi, Y., Tomimatsu, T., 1989. The constituents of *Yucca gloriosa*. Part 2. The steroidal glycosides from the caudex of *Yucca gloriosa*. Phytochemistry 28, 1771–1772.

Nakano, K., Hara, Y., Murakami, K., Takaishi, Y., Tomimatsu, T., 1991. The constituents of *Yucca gloriosa*. Part 4. 12-Hydroxy steroidal glycosides from the caudex of *Yucca gloriosa*. Phytochemistry 30, 1993–1995.

Nohara, T., Miyahara, K., Kawasaki, T., 1975. Steroid saponins and sapogenins of aerial parts of *Trillium kamtschaticum* PALL. II. Pennogenin and kryptogenin 3-*O*-glycosides and related compounds. Chem. Pharm. Bull. 23, 872–885.

Pant, G., Sati, O.P., Miyahara, K., Kawasaki, T., 1986. Spirostanol glycosides from *Agave cantala*. Phytochemistry 25, 1491–1494.

Peng, J., Yao, X., Okada, Y., Okuyama, T., 1994. Further studies on new furostanol saponins from the bulbs of *Allium macrostemon*. Chem. Pharm. Bull. 42, 2180–2182.

Tsukamoto, Y. (Ed.), 1988. The Grand Dictionary of Horticulture, vol. 5. Shogakukan, Tokyo, pp. 180–181.

Yokosuka, A., Mimaki, Y., 2007. Steroidal glycosides from *Agave utahensis*. Chem. Pharm. Bull. 55, 145–149.

Yokosuka, A., Mimaki, Y., 2009. Steroidal saponins from the whole plants of *Agave utahensis* and their cytotoxic activity. Phytochemistry 70, 807–815.

Yokosuka, A., Jitsuno, M., Yui, S., Yamazaki, M., Mimaki, Y., 2009. Steroidal glycosides from *Agave utahensis* and their cytotoxic activity. J. Nat. Prod. 72, 1399–1404.

Yokosuka, A., Suzuki, T., Mimaki, Y., 2012. New cholestane glycosides from the leaves of *Cordyline terminalis*. Chem. Pharm. Bull. 60, 275–279.

Yokosuka, A., Sekiguchi, A., Mimaki, Y., 2013a. Chemical constituents of the leaves of *Dracaena thalioides*. Nat. Prod. Commun. 8, 315–318.

Yokosuka, A., Takagi, K., Mimaki, Y., 2013b. New cholestane glycosides and sterols from the underground parts of *Chamaelirium luteum* and their cytotoxic activity. J. Nat. Med. 67, 590–598.

Zhang, Y., Zhang, Y.J., Jacob, M.R., Li, X.C., Yang, C.R., 2007. Steroidal saponins from the stem of *Yucca elephantipes*. Phytochemistry 69, 264–270.