Steroidal Glycosides from the Bulbs of *Camassia leichtlinii* and Their Cytotoxic Activities

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Phytochemical analysis of the bulbs of *Camassia leichtlinii* (Liliaceae) resulted in the isolation of six new spirostanol saponins, a new furostanol saponin, a cholestane glucoside, and four known steroidal saponins. The structures of the new saponins were determined by detailed analysis of their spectral data, including two-dimensional NMR spectroscopy, and by the results of hydrolytic cleavage. Cytotoxic activities of the isolated compounds against human oral squamous cell carcinoma (HSC-2) cells and normal human gingival fibroblasts (HGF) are also reported.

Key words *Camassia leichtlinii*; Liliaceae; steroidal saponin; spirostanol saponin; furostanol saponin; cytotoxic activity; HSC-2 cell

The genus *Camassia* belongs to the subfamily Schilloideae in the Liliaceae and is found in North America. The fresh and preserved bulbs of some *Camassia* species have been used as a nourishing food and a feed for domestic animals by American Indians. Among the *Camassia* species, the bulbs of *Camassia cusickii* have a strongly bitter taste and are not edible. We previously made a phytochemical screening of *C. cusickii* bulbs and isolated fourteen new steroidal saponins,[1] twelve of which were based upon (25*R*)-spirostane-3β,6α-diol (chlorogenin) as the aglycon. The chlorogenin glycosides were obtained in good yield and were shown to contribute to the bitter taste of the bulbs of *C. cusickii*. Following further chemical investigation of the *Camassia* species, we focused on the steroidal glycoside constituents of *C. leichtlinii* (BAK.) S. WATS. It is distributed from British Columbia in Canada to California in the United States and grows in grasslands on damp soils. The bulbs are commercially available through importation in Japan and slightly bitter to the taste. From the *n*-BuOH-soluble portion of a MeOH extract of *C. leichtlinii* bulbs, we have isolated six new spirostanol saponins, a new furostanol saponin, a cholestane glucoside, and four known steroidal saponins. This paper deals with the structural determination of the new saponins and the cytotoxicity of the isolated compounds against human oral squamous cell (HSC-2) cells and normal human gingival fibroblasts (HGF).

Fresh bulbs of *C. leichtlinii* were extracted with hot MeOH. After removal of solvent, the crude extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble portion was repeatedly subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, yielding compounds **1**—**12**. Compounds **1**, **4**, **9**, **10**, and **12** were identified as (25*R*)-5α-spirostan-3β-yl *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 (**1**),[2] (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 (**4**),[3] (25*R*)-3β-hydroxy-5α-spirostan-6α-yl *O*-β-D-glucopyranoside (**9**),[1a] (25*R*)-3β-hydroxy-5α-spirostan-6α-yl *O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**10**),[1a] and 16β-[(β-D-glucopyranosyl)-oxy]-3β,7β-dihydroxycholest-5-en-23-one (**12**),[1b] respectively. Although **12** was previously obtained through partial enzymatic hydrolysis of a bisdesmosidic cholestane glycoside from *C. cusickii*,[1b] this is the first isolation from a natural source.

Compound **2** was obtained as an amorphous solid. Its molecular formula was derived as $C_{57}H_{92}O_{28}$ by data of the positive- and negative-ion FAB-MS, which showed an $[M+Na]^+$ ion at *m*/*z* 1247 and an $[M-H]^-$ ion at *m*/*z* 1223. The ^{13}C-NMR spectrum with a total of 57 carbon signals and elemental analysis were consistent with the deduced formula. The ^{1}H-NMR spectrum contained signals for four steroid methyl groups at δ 1.36 (d, J=6.9 Hz), 1.08 (s), 0.66 (s), and 0.65 (d, J=5.7 Hz), and five anomeric protons at δ 5.77 (d, J=1.3 Hz), 5.59 (d, J=7.8 Hz), 5.28 (d, J=7.9 Hz), 5.13 (d, J=7.9 Hz), and 4.86 (d, J=7.7 Hz). Acid hydrolysis of **2** with 1 M HCl in dioxane–H_2O (1 : 1) gave a steroidal sapogenin, identified as (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin),[4] and D-glucose, D-galactose, and L-rhamnose as the carbohydrate moieties. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded silica gel column using MeCN–H_2O (17 : 3) as solvent system, with detection being carried out by using a combination of refractive index (RI) and optical rotation (OR) detectors. The ^{13}C-NMR signals due to the sugar moiety were almost superimposable on those of **1**, suggesting that the pentaglycoside sequence of **2** was the same as that of **1**. This was confirmed by ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) correlations from δ 5.77 (H-1‴″) to δ 78.4 (C-4‴″), δ 5.28 (H-1‴″) to δ 88.1 (C-3″), δ 5.59 (H-1‴) to δ 81.4 (C-2″), δ 5.13 (H-1″) to δ 80.2 (C-4′), and δ 4.86 (H-1′) to δ 77.1 (C-3 of aglycon). Thus, **2** was shown to be a new combination of a known steroidal sapogenin and sugar, and its structure was formulated as (25*R*)-3β-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyra-

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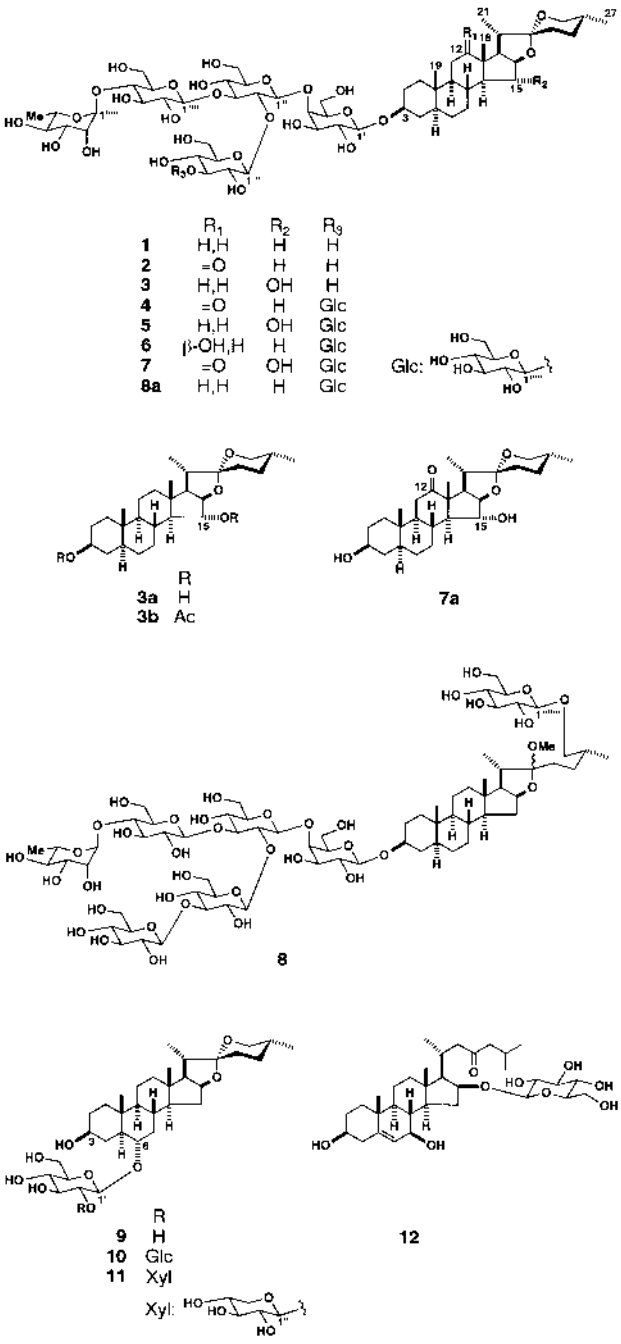
nosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α-spirostan-12-one.

The ^{1}H-NMR spectrum of compound **3** ($C_{57}H_{94}O_{28}$) showed signals for four steroid methyl groups at δ 1.16 (d, J=6.7 Hz), 0.90 (s), 0.68 (s), and 0.64 (d, J=5.5 Hz), as well as signals for five anomeric protons at δ 5.76 (br s), 5.58 (d, J=7.7 Hz), 5.28 (d, J=7.9 Hz), 5.12 (d, J=7.8 Hz), and 4.86 (d, J=7.7 Hz). Analysis of the ^{13}C-NMR spectrum of **3** and comparison with that of **2** revealed that **3** possessed a pentaglycoside moiety identical to that of **2**, but differed slightly from **2** in terms of the aglycon structure. Acid hydrolysis of **3** with 1 M HCl liberated a new steroidal sapogenin (**3a**), D-glucose, D-galactose, and L-rhamnose. The NMR spectral properties of **3a** were essentially analogous to those of (25*R*)-5α-spirostan-3β-ol (tigogenin).[4] However, the molecular formula of **3a**, $C_{27}H_{44}O_4$, which was determined by high-resolution (HR) EI-MS data (*m/z* 432.3230, Δ −1.0 mmu of calcd), was greater by one oxygen atom than that of tigogenin, and treatment of **3a** with Ac_2O in pyridine gave a diacetate (**3b**). These data implied that **3a** had one more hydroxyl group in addition to the C-3β hydroxyl group [$\delta_{H\text{-}3}$ 3.86 (br m, $W_{1/2}$=24.2 Hz); $\delta_{C\text{-}3}$ 70.6 (CH)]. The locus and configuration of the additional hydroxyl group was assigned by the following spectral analysis. In the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum, the downfield-shifted oxymethine carbon signal at δ 91.4 was correlated to a proton signal at δ 4.68, which showed an HMBC correlation with the quaternary carbon signal at δ 40.9 (C-13) and was assigned to H-16. By the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) experiment, the H-16 proton was shown to have spin-coupling links with the resonances at δ 4.41 (dd, J=10.8, 3.8 Hz) and 2.07 (dd, J=8.7, 6.6 Hz), the former was assignable to H-15 and the latter to H-17. Thus, the presence of the C-15 hydroxyl group was evident. The configuration of the C-15 hydroxyl group was determined to be α, as confirmed by NOE correlations from H-15 to H-8 [δ 1.89 (dddd, J=10.8, 10.8, 10.8, 3.8 Hz)], Me-18 [δ 0.95 (s)], and H-20 [δ 2.02 (qd, J=6.9, 6.6 Hz)] observed in the phase-sensitive NOE correlation spectroscopy (PHNOESY) spectrum of **3a**. Accordingly, the structure of **3a**, a new steroidal sapogenin, was shown to be (25*R*)-5α-spirostane-3β,15α-diol, and consequently, the full structure of **3** was established as (25*R*)-15α-hydroxy-5α-spirostan-3β-yl *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.

The ^{1}H-NMR spectrum of compound **5** ($C_{63}H_{104}O_{33}$) showed six anomeric proton signals at δ 5.77 (br s), 5.54 (d, J=7.6 Hz), 5.19 (d, J=7.8 Hz), 5.13 (d, J=7.8 Hz), 5.09 (d, J=7.7 Hz), and 4.85 (d, J=7.3 Hz), along with four steroid methyl proton signals. Acid hydrolysis of **5** yielded **3a**, D-glucose, D-galactose, and L-rhamnose. When the ^{13}C-NMR spectrum of **5** was compared with that of **4**, the ^{13}C-NMR shifts arising from the hexaglycoside moiety were almost identical between the two compounds. HMBC correlations from each anomeric proton across the glycosidic bond to the carbon of the other substituted monosaccharide or the aglycon confirmed the glycoside sequence of **5** being the same as that of **4**. The structure of **5** was defined as (25*R*)-15α-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.



The ^{1}H- and ^{13}C-NMR spectra of compound **6** ($C_{63}H_{104}O_{33}$) and **7** ($C_{63}H_{102}O_{34}$) indicated that the structure of the sugar moiety was identical to that of **4** and **5**. Acid hydrolysis of **6** resulted in the production of a known steroidal sapogenin, identified as (25*R*)-5α-spirostane-3β,12β-diol (rocogenin),[5] together with D-glucose, D-galactose, and L-rhamnose. Compound **6** was characterized as (25*R*)-12β-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. Acid hydrolysis of **7** afforded a new steroidal sapogenin (**7a**) with a molecular formula $C_{27}H_{42}O_5$ (HR EI-MS *m/z* 446.3037, Δ +0.5 mmu of calcd), as well as D-glucose, D-galactose, and L-rhamnose. The ^{1}H-NMR reso-

Table 1. ^{13}C-NMR Spectral Data for Compounds **2**, **3**, **3a**, **5**—**7**, **7a**, **8**, and **11** in Pyridine-d_5

C	**2**	**3**	**3a**	**5**	**6**	**7**	**7a**	**8**	**11**
1	36.6	37.3	37.7	37.3	37.1	36.8	37.1	37.2	37.9
2	29.9	29.9	32.6	29.9	29.8	29.7	32.2	29.9	32.5
3	77.1	77.4	70.6	77.3	77.3	77.1	70.3	77.4	71.0
4	34.6	34.8	39.4	34.8	34.7	34.6	39.1	34.8	33.0
5	44.4	44.6	45.2	44.6	44.6	44.3	44.8	44.6	51.2
6	28.6	29.1	29.3	29.1	28.9	28.8	28.9	28.9	79.6
7	31.7	32.8	33.0	32.8	32.1	32.0	32.1	32.4	40.8
8	34.3	36.0	36.3	36.0	34.3	35.2	35.3	35.2	34.0
9	55.5	54.5	54.7	54.5	53.5	55.4	55.6	54.4	54.0
10	36.2	36.1	36.1	36.1	35.8	36.4	36.4	35.8	36.6
11	38.0	21.2	21.4	21.3	31.6	37.7	37.9	21.2	21.3
12	212.7	40.8	41.0	40.8	79.2	212.6	212.7	40.0	40.1
13	55.3	40.8	40.9	40.8	46.5	54.7	54.8	41.1	40.8
14	55.9	60.9	61.1	60.9	55.1	60.2	60.4	56.3	56.5
15	31.4	78.8	78.8	78.8	31.9	77.8	77.9	32.1	32.0
16	79.7	91.3	91.4	91.3	81.2	89.8	89.8	81.3	81.0
17	54.3	60.5	60.6	60.5	62.9	51.7	51.7	64.3	63.0
18	16.1	17.9	18.0	17.9	12.2	17.3	17.3	16.3	16.6
19	11.7	12.4	12.7	12.4	11.2	11.8	12.0	12.3	13.7
20	42.6	42.0	42.0	42.0	43.0	42.7	42.7	40.5	42.0
21	13.9	15.0	15.1	15.0	14.3	13.9	13.9	16.5	15.0
22	109.3	108.9	108.9	108.8	109.5	109.0	109.0	112.6	109.1
23	31.8	31.8	31.8	31.8	31.8	31.7	31.8	30.8	31.8
24	29.2	29.2	29.3	29.3	29.3	29.2	29.2	28.2	29.3
25	30.5	30.5	30.5	30.5	30.6	30.4	30.5	34.2	30.6
26	66.9	66.8	66.8	66.8	66.8	66.9	66.9	75.2	66.8
27	17.3	17.2	17.3	17.3	17.3	17.4	17.4	17.1	17.3
OMe								47.2	
1′	102.4	102.4		102.3	102.4	102.4		102.4	103.4
2′	73.2	73.2		73.1	73.0	73.1		73.1	83.7
3′	75.6	75.5		75.6	75.5	75.6		75.6	78.5
4′	80.2	80.2		79.9	80.0	80.0		80.0	71.5
5′	75.3	75.2		75.3	75.3	75.3		75.3	77.9
6′	60.6	60.6		60.6	60.6	60.6		60.7	62.7
1″	105.0	105.0		104.7	104.7	104.7		104.7	106.6
2″	81.4	81.4		80.8	80.7	80.8		80.8	76.2
3″	88.1	88.0		88.1	88.1	88.1		88.1	77.8
4″	70.6	70.6		70.6	70.5	70.6		70.5	71.0
5″	77.4	77.3		77.4	77.4	77.4		77.4	67.5
6″	63.0	62.9		62.9	62.9	62.9		62.9	
1‴	104.8	104.7		104.0	103.9	104.0		104.0	
2‴	76.2	76.2		74.6	74.6	74.7		74.6	
3‴	78.0	77.9		88.1	88.1	88.1		88.1	
4‴	70.9	70.9		69.3	69.2	69.3		69.3	
5‴	78.7	78.6		78.0	77.9	78.0		78.0	
6‴	62.3	62.3		62.0	62.0	62.0		62.0	
1⁗	104.2	104.2		104.2	104.1	104.2		104.2	
2⁗	75.4	75.4		75.4	75.4	75.5		75.4	
3⁗	76.7	76.6		76.5	76.4	76.5		76.5	
4⁗	78.4	78.4		77.9	77.9	78.0		77.9	
5⁗	77.3	77.2		77.2	77.2	77.2		77.2	
6⁗	61.1	61.1		61.1	61.1	61.1		61.1	
1⁗′	102.8	102.8		102.6	102.6	102.6		102.6	
2⁗′	72.5	72.5		72.5	72.5	72.5		72.5	
3⁗′	72.7	72.7		72.7	72.6	72.7		72.7	
4⁗′	73.9	73.9		73.9	73.9	73.9		73.9	
5⁗′	70.4	70.4		70.3	70.3	70.4		70.3	
6⁗′	18.5	18.5		18.5	18.5	18.5		18.5	
1⁗″				105.6	105.5	105.5		105.6	
2⁗″				75.5	75.5	75.6		75.6	
3⁗″				78.0	77.9	78.0		77.9	
4⁗″				71.5	71.5	71.5		71.5	
5⁗″				78.4	78.4	78.5		78.4	
6⁗″				62.5	62.4	62.5		62.5	
1⁗‴								105.0	
2⁗‴								75.2	
3⁗‴								78.6	
4⁗‴								71.7	
5⁗‴								78.5	
6⁗‴								63.0	

nances at δ 4.57 (dd, J=10.6, 4.0 Hz) and 3.81 (br m, $W_{1/2}$=22.0 Hz) were readily assigned to the H-15β and H-3α proton by analysis of the ^{1}H–^{1}H COSY spectrum of **7a**, indicating the presence of two hydroxyl groups at C-3β and C-15α as in the aglycon of **3** and **4**. Furthermore, the IR (1699 cm^{-1}) and ^{13}C-NMR (δ 212.7) spectra demonstrated the presence of a carbonyl group. It was determined to be located at C-12 by HMBC correlations from the δ 212.7 resonance to H_2-11 at δ 2.48 (dd, J=13.9, 11.0 Hz) and 2.35 (dd, J=13.9, 5.1 Hz), H-14 at δ 1.84 (dd, J=10.6, 10.6 Hz), H-17 at δ 3.04 (dd, J=9.0, 6.6 Hz), and Me-18 at δ 1.21 (s). Thus, the structure of **7a** was revealed to be (25*R*)-3β,15α-dihydroxy-5α-spirostan-12-one, and the full structure of **7** to be (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]-15α-hydroxy-5α-spirostan-12-one.

Compound **8** ($C_{70}H_{118}O_{38}$) gave a positive Ehrlich's reaction[6] and its ^{1}H-NMR spectrum showed signals for seven anomeric protons at δ 5.77 (br s), 5.54 (d, J=7.7 Hz), 5.19 (d, J=7.7 Hz), 5.13 (d, J=7.8 Hz), 5.08 (d, J=7.6 Hz), and 4.85×2 (d, J=7.7 Hz), together with signals for four steroid methyls at δ 1.19 (d, J=6.8 Hz), 1.00 (d, J=6.6 Hz), 0.80 (s), and 0.65 (s), which were suggestive of a furostanol saponin with up to seven monosaccharides. Enzymatic hydrolysis of **8** with β-D-glucosidase furnished a known spirostanol saponin (**8a**) previously isolated by us from *C. cusickii*.[1c] The structure of **8** was shown to be (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]-22ξ-methoxy-5α-furostan-26-yl β-D-glucopyranoside.

Acid hydrolysis of compound **11** ($C_{38}H_{62}O_{13}$) gave a known steroidal sapogenin, identified as (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin),[1a] and D-glucose and D-xylose as the carbohydrate compounds. The ^{13}C-NMR spectrum of **11** showed the presence of a terminal β-D-xylopyranosyl unit and a C-2 substituted β-D-glucopyranosyl unit. HMBC correlations from δ 5.34 (d, J=6.7 Hz, anomer of xylose) to δ 83.7 (C-2 of glucose), and δ 4.98 (d, J=7.7 Hz, anomer of glucose) to δ 79.6 (C-6 of aglycon) allowed the assignment of the structure of **11** as (25*R*)-3β-hydroxy-5α-spirostan-6α-yl *O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside.

Compounds **2**, **3**, **5**—**8**, and **11** are new steroidal saponins, and the aglycons (**3a** and **7a**) of **3**, **5**, and **7** are newly described steroidal sapogenins.

The isolated compounds (**1**—**12**) and one derivative (**8a**) were evaluated for their cytotoxic activities against HSC-2 cells and HGF (Table 2).[7] The spirostanol penta- and hexaglycosides (**1**—**7**, **8a**), and the bisdesmosidic furostanol with up to seven monosaccharides (**8**) exhibited considerable cytotoxicity against HSC-2 cells with LD_{50} values ranging from 1.0 μg/ml to 35 μg/ml. Introduction of a hydroxyl group into the aglycon tended to reduce the cytotoxicity. Compounds **1**—**3** showed 5—10-fold higher cytotoxic activities against HSC-2 cells than against normal HGF. In contrast, **4**, **5**, and **8a**, which are the corresponding C-3‴-glucosylated saponins of **2**, **3**, and **1**, were cytotoxic against both HSC-2 and HGF. Thus, the structure of the sugar portion appears to play an important role in the tumor-specific cytotoxicity of these steroidal saponins. The chlorogenin 6-*O*-monoglycosides (**9**—**11**), and cholestane glucoside (**12**) were less cytotoxic and showed no tumor-specificity. As a result of our preliminary study on the cytotoxic mechanism of the saponins, we have found that **1** and **8** induced internucleosome DNA fragmentation in HL-60 leukemia cells. More details concerning the mechanism will be reported in the near future.[8]

Table 2. Cytotoxic Activities of Compounds **1**—**8**, **8a**, and **9**—**12** against HSC-2 Cells and HGF

Compounds	LD_{50} (μg/ml)	
	HSC-2	HGF
1	1.9	20
2	2.4	18
3	7.7	43
4	2.2	2.4
5	8.8	12
6	17	19
7	35	34
8	4.7	34
8a	1.0	3.1
9	70	148
10	102	183
11	120	135
12	68	83

Experimental

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA, U.S.A.) mass spectrometer, using a dithiothreitol and dithioerythritol (3 : 1) matrix. Elemental analysis was carried out using an Elemental Vario EL elemental analyzer (Hanau, Germany). Diaion HP-20 (Mitsubishi-Kasei, 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, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh RI-8010 detector, a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and Rheodyne injection port. A Kaseisorb LC ODS-120-5 column (10 mm i.d.×250 mm, ODS, 5 μm, Tokyo-Kasei, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, U.S.A.); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, U.S.A.); penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), and α-minimum essential medium (α-MEM) (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material The bulbs of *C. leichtlinii* were purchased from a nursery in Heiwaen, Nara prefecture, Japan. The bulbs were cultivated and a voucher of the plant is on file in our laboratory.

Extraction and Isolation The plant material (fresh weight, 8.9 kg) was extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure and partitioned between *n*-BuOH and H_2O. Column chromatography of the *n*-BuOH-soluble portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (90 : 10 : 1; 40 : 10 : 1; 20 : 10 : 1), and finally with MeOH alone, gave five fractions (I—V). Fraction III was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (70 : 10 : 1) and ODS silica gel with MeOH–H_2O (4 : 1) to give **9** (19.9 mg) and **12** (29.1 mg). Fraction IV was subjected to a silica gel column eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and an ODS column with MeOH–H_2O (4 : 1) and MeCN–H_2O (1 : 1) to yield **10** with a few impurities and **11** (190 mg). Purification of **10** was established by preparative HPLC using MeOH–

H_2O (4 : 1) to furnish **10** (9.6 mg) in a pure form. Fraction V was dissolved in MeOH and the resulting precipitate was filtered off. The filtrate was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and ODS silica gel column chromatography with MeOH–H_2O (2 : 1) and MeCN–H_2O (2 : 3; 1 : 2; 2 : 5; 1 : 3) to afford **1** (16.4 mg), **2** (86.0 mg), **3** (31.2 mg), **4** (600 mg), **5** (30.4 mg), **6** (23.8 mg), **7** (86.0 mg), and **8** (50.1 mg). The furostanol saponin (**8**) was obtained as a mixture of the C-22 hydroxyl and C-22 methoxyl forms. The C-22 hydroxyl form present in the mixture was completely converted to the C-22 methoxyl form by treatment with hot MeOH, and the structural elucidation of **8** was carried out with the C-22 methoxyl form.

Compound **2**: Amorphous solid, $[\alpha]_D^{25}$ −36.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1247 $[M+Na]^+$. FAB-MS (negative mode) m/z: 1223 $[M-H]^-$, 1077 $[M-H-$rhamnosyl$]^-$, 1061 $[M-H-$glucosyl$]^-$, 915 $[M-H-$rhamnosyl−glucosyl$]^-$, 753 $[M-H-$rhamnosyl−glucosyl×2$]^-$, 591 $[M-H-$rhamnosyl−glucosyl×3$]^-$. *Anal.* Calcd for $C_{57}H_{92}O_{28}\cdot 7/2H_2O$: C, 53.14; H, 7.75. Found: C, 53.08; H, 7.71. IR ν_{max} (film) cm^{-1}: 3367 (OH), 2925 and 2860 (CH), 1702 (C=O), 1453, 1369, 1252, 1066. ^{1}H-NMR (pyridine-d_5) δ: 5.77 (1H, d, J=1.3 Hz, H-1‴″), 5.59 (1H, d, J=7.8 Hz, H-1‴), 5.28 (1H, d, J=7.9 Hz, H-1‴″), 5.13 (1H, d, J=7.9 Hz, H-1″), 4.86 (1H, d, J=7.7 Hz, H-1′), 3.87 (1H, br m, $W_{1/2}$=21.8 Hz, H-3), 3.59 (1H, dd, J=10.6, 3.3 Hz, H-26eq), 3.49 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 1.71 (3H, d, J=6.2 Hz, Me-6‴″), 1.36 (3H, d, J=6.9 Hz, Me-21), 1.08 (3H, s, Me-18), 0.66 (3H, s, Me-19), 0.65 (3H, d, J=5.7 Hz, Me-27).

Acid Hydrolysis of 2 A solution of **2** (11.3 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 3 ml) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Diaion HP-20 eluting with H_2O–MeOH (3 : 2) followed by Me_2CO–EtOH (1 : 1) to give an aglycon fraction and a sugar fraction (4.9 mg). The aglycon fraction was chromatographed on silica gel eluting with $CHCl_3$–MeOH (12 : 1) to give (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) (2.9 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) and a Toyopak IC-SP M cartridge (Tosoh, Tokyo, Japan), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d.×250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN–H_2O (17 : 3); flow rate, 0.9 ml/min; detection, RI and OR. Identification of D-glucose, D-galactose, and L-rhamnose 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): 7.79 (L-rhamnose, negative optical rotation); 14.65 (D-galactose, positive optical rotation), 15.37 (D-glucose, positive optical rotation).

Compound **3**: Amorphous solid, $[\alpha]_D^{25}$ −36.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1249 $[M+Na]^+$. HR-FAB-MS (positive mode) m/z: 1249.5829 $[M+Na]^+$ ($C_{57}H_{94}O_{28}\cdot$Na, Calcd for 1249.5948). FAB-MS (negative mode) m/z: 1225 $[M-H]^-$, 1079 $[M-H-$rhamnosyl$]^-$, 1063 $[M-H-$glucosyl$]^-$, 917 $[M-H-$rhamnosyl−glucosyl$]^-$, 755 $[M-H-$rhamnosyl−glucosyl×2$]^-$, 593 $[M-H-$rhamnosyl−glucosyl×3$]^-$. IR ν_{max} (film) cm^{-1}: 3385 (OH), 2926 and 2856 (CH), 1452, 1375, 1260, 1154, 1069, 1039. ^{1}H-NMR (pyridine-d_5) δ: 5.76 (1H, br s, H-1‴″), 5.58 (1H, d, J=7.7 Hz, H-1‴), 5.28 (1H, d, J=7.9 Hz, H-1‴″), 5.12 (1H, d, J=7.8 Hz, H-1″), 4.86 (1H, d, J=7.7 Hz, H-1′), 4.66 (1H, dd, J=7.9, 3.3 Hz, H-16), 4.38 (1H, dd, J=10.7, 3.3 Hz, H-15), 3.89 (1H, br m, $W_{1/2}$=26.5 Hz, H-3), 3.49 (1H, dd, J=10.4, 3.7 Hz, H-26eq), 3.37 (1H, dd, J=10.4, 10.4 Hz, H-26ax), 1.70 (3H, d, J=6.1 Hz, Me-6‴″), 1.16 (3H, d, J=6.7 Hz, Me-21), 0.90 (3H, s, Me-18), 0.68 (3H, s, Me-19), 0.64 (3H, d, J=5.5 Hz, Me-27).

Acid Hydrolysis of 3 Compound **3** (10.0 mg) was subjected to acid hydrolysis as described for **2** to give an aglycon (**3a**) (3.2 mg) and a sugar fraction (4.8 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2** showed the presence of D-glucose, D-galactose, and L-rhamnose.

Compound **3a**: Amorphous solid, $[\alpha]_D^{25}$ −50.0° (MeOH, c=0.10). HR-EI-MS m/z: 432.3230 $[M]^+$ ($C_{27}H_{44}O_4$, Calcd for 432.3240). IR ν_{max} (film) cm^{-1}: 3377 (OH), 2926 and 2853 (CH), 1451, 1378, 1260, 1242, 1177, 1160, 1081, 1045, 1008, 981, 966, 920, 896, 864. ^{1}H-NMR (pyridine-d_5) δ: 4.68 (1H, dd, J=8.7, 3.8 Hz, H-16), 4.41 (1H, dd, J=10.8, 3.8 Hz, H-15), 3.86 (1H, br m, $W_{1/2}$=24.2 Hz, H-3), 3.50 (1H, dd, J=10.5, 3.5 Hz, H-26eq), 3.38 (1H, dd, J=10.5, 10.5 Hz, H-26ax), 2.07 (1H, dd, J=8.7, 6.6 Hz, H-17), 2.02 (1H, qd, J=6.9, 6.6 Hz, H-20), 1.89 (1H, dddd, J=10.8, 10.8, 10.8, 3.8 Hz, H-8), 1.52 (1H, dd, J=10.8, 10.8 Hz, H-14), 1.15 (3H, d, J=6.9 Hz, Me-21), 0.95 (3H, s, Me-18), 0.87 (3H, s, Me-19), 0.66 (3H, d, J=5.7 Hz, Me-27).

Acetylation of 3a Compound **3a** (3.0 mg) was treated with Ac_2O (2 ml) in pyridine (1 ml) at room temperature for 13 h. After addition of H_2O, the reaction mixture was extracted with Et_2O and the Et_2O-soluble phase was chromatographed on silica gel using hexane–Me_2CO (9 : 1) to afford the corresponding diacetate (**3b**) (2.6 mg).

Compound **3b**: Amorphous solid. IR ν_{max} (film) cm^{-1}: 2958, 2928 and 2855 (CH), 1739 (C=O), 1454, 1368, 1258, 1178, 1096, 1087, 1032, 984, 919, 896, 865. ^{1}H-NMR (pyridine-d_5) δ: 5.55 (1H, dd, J=11.1, 3.6 Hz, H-15), 4.84 (1H, br m, $W_{1/2}$=22.8 Hz, H-3), 4.50 (1H, dd, J=8.2, 3.6 Hz, H-16), 3.51 (2H, H_2-26), 2.07 and 2.06 (each 3H, s, Ac×2), 1.13 (3H, d, J=6.6 Hz, Me-21), 0.91 (3H, s, Me-18), 0.72 (3H, s, Me-19), 0.59 (3H, d, J=5.5 Hz, Me-27).

Compound **5**: Amorphous solid, $[\alpha]_D^{25}$ −40.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1411 $[M+Na]^+$. HR-FAB-MS (positive mode) m/z: 1411.6592 $[M+Na]^+$ ($C_{63}H_{104}O_{33}\cdot$Na, Calcd for 1411.6358). FAB-MS (negative mode) m/z: 1387 $[M-H]^-$, 1241 $[M-H-$rhamnosyl$]^-$, 1225 $[M-H-$glucosyl$]^-$, 1079 $[M-H-$rhamnosyl−glucosyl$]^-$, 1063 $[M-H-$glucosyl×2$]^-$, 917 $[M-H-$rhamnosyl−glucosyl×2$]^-$, 755 $[M-H-$rhamnosyl−glucosyl×3$]^-$, 593 $[M-H-$rhamnosyl−glucosyl×4$]^-$. IR ν_{max} (film) cm^{-1}: 3334 (OH), 2928 (CH), 1453, 1375, 1261, 1071. ^{1}H-NMR (pyridine-d_5) δ: 5.77 (1H, br s, H-1‴‴), 5.54 (1H, d, J=7.6 Hz, H-1‴), 5.19 (1H, d, J=7.8 Hz, H-1‴‴), 5.13 (1H, d, J=7.8 Hz, H-1″), 5.09 (1H, d, J=7.7 Hz, H-1‴″), 4.85 (1H, d, J=7.3 Hz, H-1′), 4.66 (1H, dd, J=8.4, 3.7 Hz, H-16), 4.38 (1H, dd, J=10.7, 3.7 Hz, H-15), 3.89 (1H, br m, overlapping, H-3), 3.49 (1H, dd, J=10.6, 3.2 Hz, H-26eq), 3.37 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 1.69 (3H, d, J=6.1 Hz, Me-6‴″), 1.13 (3H, d, J=6.7 Hz, Me-21), 0.90 (3H, s, Me-18), 0.69 (3H, s, Me-19), 0.63 (3H, d, J=5.7 Hz, Me-27).

Acid Hydrolysis of 5 Compound **5** (10.5 mg) was subjected to acid hydrolysis as described for **2** to give an aglycon (**3a**) (2.5 mg) and a sugar fraction (4.8 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2** showed the presence of D-glucose, D-galactose, and L-rhamnose.

Compound **6**: Amorphous solid, $[\alpha]_D^{25}$ −44.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1411 $[M+Na]^+$. FAB-MS (negative mode) m/z: 1387 $[M-H]^-$, 1241 $[M-H-$rhamnosyl$]^-$, 1225 $[M-H-$glucosyl$]^-$, 1079 $[M-H-$rhamnosyl−glucosyl$]^-$, 1063 $[M-H-$glucosyl×2$]^-$, 917 $[M-H-$rhamnosyl−glucosyl×2$]^-$, 755 $[M-H-$rhamnosyl−glucosyl×3$]^-$, 593 $[M-H-$rhamnosyl−glucosyl×4$]^-$. *Anal.* Calcd for $C_{63}H_{104}O_{33}\cdot 4H_2O$: C, 51.49; H, 7.72. Found: C, 51.61; H, 8.05. IR ν_{max} (film) cm^{-1}: 3376 (OH), 2927 (CH), 1447, 1368, 1263, 1065. ^{1}H-NMR (pyridine-d_5) δ: 5.77 (1H, br s, H-1‴‴), 5.54 (1H, d, J=7.6 Hz, H-1‴), 5.19 (1H, d, J=7.8 Hz, H-1‴‴), 5.12 (1H, d, J=7.8 Hz, H-1″), 5.08 (1H, d, J=7.9 Hz, H-1‴″), 4.82 (1H, d, J=7.2 Hz, H-1′), 3.88 (1H, br m, overlapping, H-3), 3.60 (1H, dd, J=10.4, 3.3 Hz, H-26eq), 3.54 (1H, dd, J=10.4, 10.4 Hz, H-26ax), 3.50 (1H, dd, J=11.6, 6.1 Hz, H-12), 1.68 (3H, d, J=6.1 Hz, Me-6‴″), 1.43 (3H, d, J=6.5 Hz, Me-21), 1.08 (3H, s, Me-18), 0.70 (3H, d, J=5.0 Hz, Me-27), 0.66 (3H, s, Me-19).

Acid Hydrolysis of 6 Compound **6** (10.3 mg) was subjected to acid hydrolysis as described for **2** to give (25*R*)-5α-spirostane-3β,12β-diol (rocogenin) (1.6 mg) and a sugar fraction (3.8 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2** showed the presence of D-glucose, D-galactose, and L-rhamnose.

Compound **7**: Amorphous solid, $[\alpha]_D^{25}$ −34.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1425 $[M+Na]^+$. FAB-MS (negative mode) m/z: 1401 $[M-H]^-$, 1255 $[M-H-$rhamnosyl$]^-$, 1239 $[M-H-$glucosyl$]^-$, 1093 $[M-H-$rhamnosyl−glucosyl$]^-$, 1077 $[M-H-$glucosyl×2$]^-$, 931 $[M-H-$rhamnosyl−glucosyl×2$]^-$, 769 $[M-H-$rhamnosyl−glucosyl×3$]^-$, 607 $[M-H-$rhamnosyl−glucosyl×4$]^-$. *Anal.* Calcd for $C_{63}H_{102}O_{34}\cdot 9H_2O$: C, 48.33; H, 7.72. Found: C, 48.14; H, 7.63. IR ν_{max} (film) cm^{-1}: 3290 (OH), 2923 and 2860 (CH), 1690 (C=O), 1445, 1424, 1356, 1266, 1255, 1239, 1093, 1077, 1061, 931. ^{1}H-NMR (pyridine-d_5) δ: 5.77 (1H, br s, H-1‴‴), 5.54 (1H, d, J=7.6 Hz, H-1‴), 5.18 (1H, d, J=7.8 Hz, H-1‴‴), 5.13 (1H, d, J=7.9 Hz, H-1″), 5.08 (1H, d, J=7.7 Hz, H-1‴″), 4.84 (1H, d, J=6.7 Hz, H-1′), 4.59 (1H, dd, J=8.9, 4.0 Hz, H-16), 4.54 (1H, dd, J=10.8, 4.0 Hz, H-15), 3.85 (1H, br m, overlapping, H-3), 3.49 (1H, br d, J=10.6 Hz, H-26eq), 3.34 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 1.69 (3H, d, J=6.2 Hz, Me-6‴″), 1.36 (3H, d, J=6.9 Hz, Me-21), 1.18 (3H, s, Me-18), 0.71 (3H, s, Me-19), 0.65 (3H, d, J=5.7 Hz, Me-27).

Acid Hydrolysis of 7 Compound **7** (40.0 mg) was subjected to acid hydrolysis as described for **2** to give an aglycon (**7a**) (8.2 mg) and a sugar fraction (10.6 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2** showed the presence of D-glucose, D-galactose, and L-rhamnose.

Compound **7a**: Amorphous solid, $[\alpha]_D^{25}$ −20.0° (MeOH, c=0.10). HR-EI-MS m/z: 446.3037 $[M]^+$ ($C_{27}H_{42}O_5$, Calcd for 446.3032). IR ν_{max} (film) cm^{-1}: 3538, 3303 and 3256 (OH), 2922 and 2854 (CH), 1699 (C=O), 1456,

1376, 1259, 1175, 1075, 1037, 980, 917, 896, 863. ^{1}H-NMR (pyridine-d_5) δ: 4.62 (1H, dd, J=9.0, 4.0 Hz, H-16), 4.57 (1H, dd, J=10.6, 4.0 Hz, H-15), 3.81 (1H, br m, $W_{1/2}$=22.0 Hz, H-3), 3.50 (1H, dd, J=10.6, 3.4 Hz, H-26eq), 3.34 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 3.04 (1H, dd, J=9.0, 6.6 Hz, H-17), 2.48 (1H, dd, J=13.9, 11.0 Hz, H-11ax), 2.35 (1H, dd, J=13.9, 5.1 Hz, H-11eq), 2.00 (1H, qd, J=7.0, 6.6 Hz, H-20), 1.84 (1H, dd, J=10.6, 10.6 Hz, H-14), 1.37 (3H, d, J=6.9 Hz, Me-21), 1.21 (3H, s, Me-18), 0.89 (3H, s, Me-19), 0.65 (3H, d, J=5.8 Hz, Me-27).

Compound **8**: Amorphous solid, $[\alpha]_D^{25}$ −44.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 1558 $[M+Na-OMe]^+$. FAB-MS (negative mode) m/z: 1565 $[M-H]^-$, 1419 [M−H−rhamnosyl]$^-$, 1403 [M−H−glucosyl]$^-$, 1257 [M−H−rhamnosyl−glucosyl]$^-$, 1241 [M−H−glucosyl×2]$^-$, 1095 [M−H−rhamnosyl−glucosyl−2]$^-$, 1079 [M−H−glucosyl×3]$^-$, 933 [M−H−glucosy×4]$^-$. *Anal.* Calcd for $C_{70}H_{118}O_{38}\cdot 9/2H_2O$: C, 50.99; H, 7.76. Found: C, 50.75; H, 7.93. IR ν_{max} (film) cm^{-1}: 3483 and 3311 (OH), 2931 (CH), 1449, 1419, 1376, 1261, 1155, 1072, 1039, 894. ^{1}H-NMR (pyridine-d_5) δ: 5.77 (1H, br s, H-1‴″), 5.54 (1H, d, J=7.7 Hz, H-1‴), 5.19 (1H, d, J=7.7 Hz, H-1‴″), 5.13 (1H, d, J=7.8 Hz, H-1″), 5.08 (1H, d, J=7.6 Hz, H-1⁗), 4.85 (1H×2, d, J=7.7 Hz, H-1′ and H-1⁗″), 3.27 (3H, s, OMe), 1.68 (3H, d, J=6.2 Hz, Me-6‴″), 1.19 (3H, d, J=6.8 Hz, Me-21), 1.00 (3H, d, J=6.6 Hz, Me-27), 0.80 (3H, s, Me-19), 0.65 (3H, s, Me-18).

Enzymatic Hydrolysis of 8 Compound **8** (5.0 mg) was dissolved in an AcOH/AcONa buffer (pH 5, 2.5 ml) with β-D-glucosidase (Sigma, EC 3.2.1.21) (10 mg) and incubated at room temperature for 15 h. The crude mixture was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to yield **8a** (3.9 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample. *Rf* 0.42 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Compound **11**: Amorphous solid, $[\alpha]_D^{25}$ −28.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 749 $[M+Na]^+$. FAB-MS (negative mode) m/z: 725 $[M-H]^-$, 593 [M−H−xylosyl]$^-$. *Anal.* Calcd for $C_{38}H_{62}O_{13}\cdot 3H_2O$: C, 58.44; H, 8.78. Found: C, 58.44; H, 8.71. IR ν_{max} (film) cm^{-1}: 3331 (OH), 2930 and 2874 (CH), 1451, 1376, 1243, 1171, 1074, 1052, 983, 956, 919, 897, 865. ^{1}H-NMR (pyridine-d_5) δ: 5.34 (1H, d, J=6.7 Hz, H-1″), 4.98 (1H, d, J=7.7 Hz, H-1′), 3.93 (1H, br m, $W_{1/2}$=22.3 Hz, H-3), 3.75 (1H, ddd, J=10.9, 10.9, 4.5 Hz, H-6), 3.55 (1H, dd, J=10.6, 2.9 Hz, H-26eq), 3.46 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 1.13 (3H, d, J=6.9 Hz, Me-21), 0.84 (3H, s, Me-19), 0.81 (3H, s, Me-18), 0.71 (3H, d, J=5.5 Hz, Me-27).

Acid Hydrolysis of 11 Compound **11** (40.0 mg) was subjected to acid hydrolysis as described for **2** to give (25*R*)-5α-spirostane-3β,6α-diol (chlorogenin) (13.1 mg) and a sugar fraction (12.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **2**, except for the flow rate (1.0 ml/min), showed the presence of D-glucose and D-xylose. t_R (min): 8.33 (D-xylose, positive optical rotation), 12.81 (D-glucose, positive optical rotation).

Cell Culture HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM medium supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. HGF were isolated, as described previously.[9] Briefly, gingival tissues were obtained from healthy gingival biopsies from a 10-year-old girl, undergoing periodontal surgery. The tissue was cut into 1 to 2 mm^3 pieces, washed twice with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, and placed into 25 cm^2 tissue culture flasks. The explants were incubated in α-MEM supplemented with 30% FBS and antibiotics. When outgrowth of the cells was observed, the medium was replaced twice until the cells reached confluence. The cells were detached from the monolayer by trypsinization and recultured in 100 cm^2 tissue culture flasks until confluent monolayers were again obtained. Cells between the fifth and seventh passages were used.

Assay for Cytotoxic Activity Cells were trypsinized and inoculated at 6×10^3 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA), and incubated for 24 h. After washing once with PBS, they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/ml MTT in DMEM medium supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 ml DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan® (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10. The LD_{50} value, which reduces the viable cell number by 50%, was determined from the dose-response curve.

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