Cycloartane Glycosides from the Rhizomes of *Cimicifuga racemosa* and Their Cytotoxic Activities

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Phytochemical analysis of the rhizomes of *Cimicifuga racemosa* (Ranunculaceae) resulted in the isolation of twelve cycloartane glycosides (1—12), including four new ones (4—6, 12). The structures of the new compounds were determined by spectroscopic analysis, including two-dimensional (2D) NMR data, and chemical methods. The isolated compounds were evaluated for their cytotoxic activities against human oral squamous cell carcinoma (HSC-2) cells and normal human gingival fibroblasts (HGF).

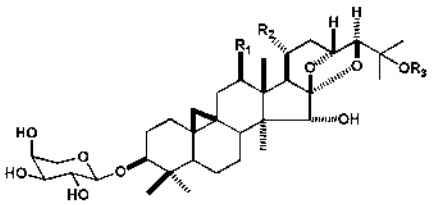
Key words *Cimicifuga racemosa*; Ranunculaceae; cycloartane glycoside; cytotoxic activity; HSC-2 cell

Cimicifuga racemosa NUTT., commonly going by the name of black cohosh, is a herb indigenous to North America and Europe, and its rhizomes have long been used for the treatment of a variety of ailments such as diarrhea, sore throat, and rheumatism by Native Americans.[1)] Now, black cohosh has become a well-known alternative herbal medicine with health benefits in treating painful menstrual periods and menopausal disorders not only in the United States but also in European countries.[2)] Black cohosh has been revealed to contain triterpene monoglycosides with the cycloartane skeleton,[3)] isoflavones,[4)] alkaloids,[5)] and phenylpropanoids,[6)] among which the cycloartane glycosides are its main secondary metabolites and are considered to partially contribute to the pharmacological effects of this herbal medicine.[7)] The present investigation is part of a series of studies on the chemical constituents of the herbal medicines.[8)] As a result, a total of twelve cycloartane glycosides (**1**—**12**), including four new compounds (**4**—**6**, **12**), were isolated from the MeOH extract of *C. racemosa.* This paper deals with the structural assignments of the new glycosides on the basis of spectroscopic analysis, including two-dimensional (2D) NMR data, and chemical methods. The cytotoxic activities of the isolated compounds against human oral squamous cell carcinoma (HSC-2) cells and normal human gingival fibroblasts (HGF) are also reported.

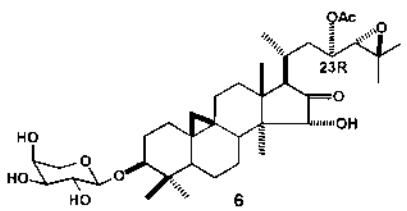
Results and Discussion

The rhizomes of *C. racemosa* (dry weight of 5.2 kg) was extracted with hot MeOH. The MeOH extract was subjected to column chromatography over porous-polymer polystyrene resin (Diaion HP-20), silica gel, and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, to give compounds **1**—**12**. Compounds **1**—**3** and **7**—**11** were identified as cimigenol 3-*O*-α-L-arabinopyranoside (**1**),[9)] 25-*O*-methoxycimigenol 3-*O*-α-L-arabinopyranoside (**2**),[10)] 12β-hydroxycimigenol 3-*O*-α-L-arabinopyranoside (**3**),[11)] 27-deoxyactein (**7**),[12)] actein (**8**),[13)] cimiracemoside F (**9**),[14)] cimiracemoside G (**10**),[14)] and cimiracemoside H (**11**),[14)] respectively.

Compound **4** was isolated as an amorphous solid. Its molecular formula was derived as $C_{37}H_{58}O_{11}$ by data from the positive-ion FAB-MS, which showed an $[M+Na]^+$ ion at *m/z* 701. The ^{13}C-NMR spectrum, with a total of 37 carbon signals, and the results of elemental analysis were consistent with the deduced formula. The IR spectrum showed an ab-



	R_1	R_2	R_3
1	H	CH_3	H
2	H	CH_3	CH_3
3	OH	CH_3	H
4	OH	CH_3	Ac
5	OH	CH_2OH	H



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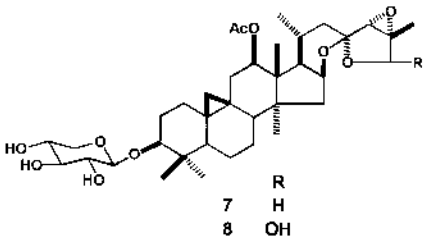
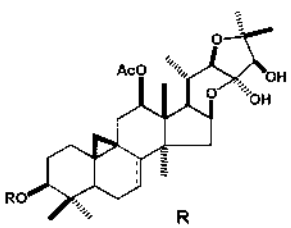


	R
7	H
8	OH



	R	
9	β-D-Xylp	Δ^7
10	α-L-Arap	Δ^7
11	β-D-Xylp	
12	α-L-Arap	

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Table 1. ^{1}H-NMR Chemical Shift Assignments of **4**—**6**, **5a**, **12**, and **12a** in Pyridine-d_5

H	4	5	5a	6	12	12a
1	1.58	1.59 ddd (13.6, 13.3, 3.1)	1.52	1.61 ddd (13.7, 13.5, 3.1)	1.49	1.47
	1.24	1.28	1.23	1.25	1.10	1.07
2	2.32	2.33	2.06	2.40	2.27	1.91
	1.90	1.91	1.80	1.97	1.85	1.80
3	3.48 dd (11.7, 4.4)	3.49 dd (11.7, 4.3)	3.48 dd (11.6, 4.4)	3.52 dd (11.7, 4.3)	3.44 dd (11.7, 4.3)	3.48 dd (11.5, 4.4)
5	1.32	1.32 dd (12.4, 4.1)	1.24 dd (12.4, 5.5)	1.39	1.23 dd (12.3, 4.2)	1.23 dd (12.6, 4.3)
6	1.54	1.53	1.52	1.60 dd (13.0, 1.6)	1.45	1.50
	0.77 qd-like (12.7, 1.9)	0.78 qd-like (12.5, 1.8)	0.77 qd-like (12.5, 2.1)	0.76 qd-like (13.0, 1.6)	0.68 qd-like (12.3, 1.8)	0.75 qd-like (12.7, 2.0)
7	2.14	2.09	2.07	2.09	1.26	1.26
	1.27	1.22	1.19	1.28	0.92	0.94
8	1.82	2.08	1.85 dd (12.0, 5.0)	1.87 dd (12.5, 4.5)	1.56 dd (11.8, 5.3)	1.58 dd (12.1, 5.2)
11	2.79 dd (15.6, 9.0)	2.69 dd (15.5, 9.0)	2.68 dd (15.5, 9.1)	2.13	2.71 dd (16.0, 9.0)	2.76 dd (16.0, 9.0)
	1.45 dd (15.6, 2.6)	1.47 dd (15.5, 3.8)	1.46 dd (15.5, 3.7)	1.15	1.15 dd (16.0, 3.7)	1.15 dd (16.0, 3.5)
12	4.13 dd (9.0, 2.6)	4.36 dd (9.0, 3.8)	4.36 dd (9.1, 3.7)	1.79	5.13 dd (9.0, 3.7)	5.15 dd (9.0, 3.5)
15	4.44 s	4.49 s	4.46 s	4.36 s	1.90 dd (12.3, 7.9)	1.82 dd (10.7, 8.5)
					1.71	1.75
16	—	—	—	—	5.00 q-like (7.9)	5.01 q-like (7.9)
17	1.83	2.41 d (9.0)	2.38 d (9.0)	2.34 br d (6.5)	1.81 dd (10.6, 7.9)	1.82 dd (10.7, 8.5)
18	1.42 s	1.52 s	1.49 s	1.37 s	1.35 s	1.38 s
19	0.62 d (4.1)	0.65 d (4.1)	0.63 d (4.1)	0.58 d (4.0)	0.53 d (4.1)	0.57 d (4.2)
	0.38 d (4.1)	0.34 d (4.1)	0.35 d (4.1)	0.32 d (4.0)	0.19 d (4.1)	0.23 d (4.2)
20	1.83	2.23	2.18	2.13	2.23	2.25
21	1.39 d (5.8)	4.11 dd (10.6, 4.5)	4.13 dd (10.7, 4.5)	1.26 d (6.6)	1.32 d (6.3)	1.33 d (6.3)
		4.00 dd (10.6, 5.9)	3.98 dd (10.7, 5.8)			
22	2.39	2.46 ddd (13.5, 8.5, 8.4)	2.41 ddd (13.6, 8.4, 8.4)	2.65	3.88 d (10.5)	3.89 d (10.5)
	1.10	1.46	1.50	1.75		
23	4.62 br d (9.0)	4.83 br d (8.3)	4.79 br d (8.6)	5.40 ddd (10.7, 8.5, 2.5)		
24	4.17 br s	3.92 d (0.6)	3.92 d (0.8)	3.03 d (8.5)	4.22 s	4.22 s
26	1.69 s	1.49 s	1.45 s	1.25 s	1.68 s	1.19 s
27	1.71 s	1.49 s	1.47 s	1.40 s	1.75 s	1.75 s
28	1.21 s	1.23 s	1.21 s	1.21 s	0.85 s	0.87 s
29	1.27 s	1.28 s	1.14 s	1.30 s	1.28 s	1.68 s
30	1.00 s	1.00 s	1.01 s	1.05 s	0.96 s	1.03 s
Ara 1′	4.79 d (6.9)	4.78 d (7.0)		4.82 d (7.0)	4.77 d (7.0)	
2′	4.44 dd (7.7, 6.9)	4.43 dd (8.7, 7.0)		4.47 dd (8.8, 7.0)	4.43 dd (8.8, 7.0)	
3′	4.17 br d (6.9)	4.16 dd (8.7, 3.4)		4.19 dd (8.8, 3.3)	4.16 dd (8.8, 3.3)	
4′	4.32 br s	4.32 br s		4.34 br s	4.31 br s	
5′a	4.29 dd (12.4, 2.3)	4.29 dd (11.8, 2.8)		4.33 dd (12.9, 2.8)	4.29 dd (11.7, 2.7)	
b	3.79 br d (12.4)	3.79 dd (11.8, 1.1)		3.82 br d (10.6)	3.78 br d (10.9)	
Ac	1.98 s			2.06 s	2.07 s	2.09 s

sorption band for hydroxyl groups at 3419 cm^{-1}. The ^{1}H-NMR spectrum of **4** showed signals for a cyclopropane methylene group at δ 0.62 and 0.38 (each 1H, d, J=4.1 Hz), six tertiary methyl groups at δ 1.71, 1.69, 1.42, 1.27, 1.21, and 1.00 (each 3H, s), a secondary methyl group at δ 1.39 (3H, d, J=5.8 Hz), and an anomeric proton at δ 4.79 (1H, d, J=6.9 Hz). These ^{1}H-NMR data and ^{13}C-NMR spectral features of **4** were quite similar to those of **3**. In addition, the presence of an acetyl group in **4** was shown by the IR (1734 cm^{-1}), ^{1}H-NMR [δ 1.98 (3H, s)], and ^{13}C-NMR [δ 170.2 (C=O), 22.3 (Me)] spectra. Alkaline hydrolysis of **4** with Na_2CO_3 solution yielded **3**, indicating that **4** was a monoacetate of **3**. When the ^{13}C-NMR spectrum of **4** was compared with that of **3**, the signal due to C-25 was shifted to a lower field by 12.1 ppm, whereas the signals assignable to C-24, C-26, and C-27 occurred, respectively, at higher fields by 3.3, 2.4, and 3.7 ppm. Thus, the acetyl moiety was revealed to be linked to the aglycon C-25 hydroxyl group, and the structure of **4** was formulated as 25-*O*-acetyl-12β-hydroxycimigenol 3-*O*-α-L-arabinopyranoside.

Compound **5** was shown to have the molecular formula $C_{35}H_{56}O_{11}$ on the basis of the positive-ion FAB-MS (*m*/*z* 675 $[M+Na]^+$), ^{13}C-NMR spectrum, and elemental analysis. The ^{1}H-NMR spectrum of **5** contained signals for a cyclopropane methylene group at δ 0.65 and 0.34 (each 1H, d, J=4.1 Hz), six tertiary methyl groups at δ 1.52, 1.49×2, 1.28, 1.23, and 1.00 (each 3H, s), and an anomeric proton at δ 4.78 (1H, d, J=7.0 Hz). Enzymatic hydrolysis of **5** with naringinase furnished the genuine aglycon ($C_{30}H_{48}O_7$: **5a**) and L-arabinose. Comparsion of the ^{1}H- and ^{13}C-NMR spectra of **5a** with those of 12β-hydroxycimigenol[11] showed their considerable structural similarity. However, the molecular formula of **5a** was higher by one oxygen atom than that of 12β-hydroxycimigenol, and complete acetylation of **5a** gave the corresponding pentaacetate (**5b**). This indicated the presence of one more hydroxyl group in addition to the C-3β, C-12β, C-15α, and C-25 hydroxyl groups. When the ^{1}H-NMR spectrum of **5a** was compared with that of 12β-hydroxy-

cimigenol, the three-proton doublet signal observed at δ 1.39 (d, J=6.0 Hz) in 12β-hydroxycimigenol, was displaced by the hydroxymethylene signals at δ 4.13 (dd, J=10.7, 4.5 Hz) and 3.98 (dd, J=10.7, 5.8 Hz). All other signals appeared at almost the same positions between the two compounds. Furthermore, tracing out the spin-coupling correlations, starting from H-17 at δ 2.38 in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum of **5a**, revealed the structural fragment of –$C_{(17)}$H–$C_{(20)}$H–($C_{(21)}H_2O$–)–$C_{(22)}H_2$–$C_{(23)}$H(–O–)–$C_{(24)}$H(–O–)– constituting **5a**. Thus, the presence of a C-21 hydroxyl group was evident. The C-20α configuration was ascertained by nuclear Overhauser effect (NOE) correlations from H-20 (δ 2.18) to Me-18 (δ 1.49) and H-22β (δ 2.41) in the phase-sensitive NOE correlation spectroscopy (NOESY) spectrum. In the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum of **5**, a long-range correlation was observed from the anomeric proton of the α-L-arabinopyranosyl group at δ 4.78 to the aglycon C-3 carbon at δ 88.4. Accordingly, the structure of **5a**, a new triterpene sapogenin, was shown to be (20*R*,23*R*,24*S*)-16α,23:16α,24-diepoxy-9,19-cyclolanostane-3β,12β,15α,21,25-pentol (12β,21-dihydroxycimigenol), and consequently, the structure of **5** was established as 12β,21-dihydroxycimigenol 3-*O*-α-L-arabinopyranoside.

Compound **6** was analyzed for $C_{37}H_{58}O_{10}$ by combined positive-ion FAB-MS (*m*/*z* 685 $[M+Na]^+$), ^{13}C-NMR spectrum with distortionless enhancement by polarization transfer (DEPT) data, and elemental analysis. The ^{1}H-NMR spectrum of **6** showed a pair of cyclopropane methylene proton signals at δ 0.58 and 0.32 (each 1H, d, J=4.0 Hz), six tertiary methyl proton signals at δ 1.40, 1.37, 1.30, 1.25, 1.21, and 1.05 (each 3H, s), a secondary methyl proton signal at δ 1.26 (3H, d, J=6.6 Hz). In addition, a secondary hydroxyl group, a carbonyl group, an acetyloxy group, and an epoxy ring, as well as an α-L-arabinopyranosyl moiety, were revealed to be included in the structure of **6** by analysis of its ^{1}H- and ^{13}C-NMR spectra. Thus, **6** was presumed to be an α-L-arabinopyranoside of a shengmanol derivative. Chemical conversion of **6** to the corresponding cimigenol saponin was carried out according to the method reported by Kusano *et al.*[15] After deacetylation of **6** with Na_2CO_3 solution, the hydrolysate was treated with 2.5% AcOH at 95 °C to furnish **1**. This chemical evidence allowed the structural determination of **6** as 23-*O*-acetylshengmanol 3-*O*-α-L-arabinopyranoside.

Compound **12** was deduced as $C_{37}H_{58}O_{11}$ from its FAB-MS, ^{13}C-NMR spectral, and elemental analysis data. The ^{1}H-NMR spectrum showed signals characteristic of the 9,19-cycloartane derivative. Analysis of the ^{13}C-NMR spectrum of **12** and comparison with that of **11** implied that the aglycon of **12** was identical to that of **11**, but differed from **11** in terms of the monosaccharide constituent. Instead of the signals for a xylose moiety, five signals assignable to an α-L-arabinopyranosyl residue were observed at δ 107.3 (CH), 72.9 (CH), 74.6 (CH), 69.5 (CH), and 66.7 (CH_2). Hydrolysis of **12** with naringinase gave L-arabinose and an aglycon (**12a**), which was identical to the product obtained by enzymatic hydrolysis of **11**. The glycosidic linkage of the arabinosyl group to C-3 of the aglycon was ascertained by an HMBC correlation from the H-1 arabinose proton at δ 4.77 (d, J=7.0 Hz) to the C-3 aglycon carbon at δ 88.1. Thus, the structure of **12** was shown to be (22*R*,23*R*,24*R*)-12β-acetyloxy-16β,23:22,25-diepoxy-23,24-dihydroxy-9,19-cyclolanostan-3β-yl α-L-arabinopyranoside.

Table 2. ^{13}C-NMR Chemical Shift Assignments of **4**—**6**, **5a**, **12**, and **12a** in Pyridine-d_5

C	**4**	**5**	**5a**	**6**	**12**	**12a**
1	32.5	32.4	32.4	32.2	31.9	32.2
2	30.0	29.9	31.0	30.0	29.8	31.0
3	88.5	88.4	77.8	88.4	88.1	77.7
4	41.3	41.2	40.8	41.3	41.1	40.9
5	47.3	47.2	46.9	47.5	47.0	46.9
6	20.9	20.9	20.8	21.0	20.4	20.8
7	26.1	25.9	25.9	26.7	25.7	25.9
8	47.3	47.0	47.0	48.2	45.6	45.9
9	20.7	20.7	20.8	20.1	19.9	19.8
10	26.6	26.9	27.0	26.8	26.7	26.9
11	40.8	39.4	39.4	26.0	36.7	36.9
12	72.7	73.2	73.0	33.0	76.9	77.1
13	47.8	47.9	47.8	41.5	49.3	49.4
14	48.2	48.6	48.4	46.1	48.1	48.1
15	79.8	80.0	79.9	82.9	43.0	43.0
16	112.8	112.4	112.3	220.0	72.1	72.1
17	59.6	54.1	53.8	60.0	52.4	52.3
18	12.0	12.1	11.9	19.8	13.7	13.7
19	30.7	30.5	30.7	30.5	29.6	30.0
20	23.9	31.6	31.4	28.0	34.4	34.4
21	21.0	66.3	66.0	20.3	17.4	17.4
22	38.5	32.8	32.8	37.0	86.7	86.7
23	71.6	71.9	71.8	72.1	105.5	105.5
24	86.7	88.8	88.7	65.2	83.3	83.3
25	83.1	70.9	70.9	58.5	83.5	83.5
26	21.6	25.3	25.3	24.7	24.7	26.1
27	23.3	27.1	26.7	19.3	27.8	27.8
28	11.8	11.9	12.0	12.0	19.6	19.7
29	25.7	25.7	26.0	25.7	25.7	24.8
30	15.3	15.3	14.6	15.4	15.2	14.7
Ara 1′	107.4	107.4		107.4	107.3	
2′	72.9	72.9		72.9	72.9	
3′	74.6	74.6		74.6	74.6	
4′	69.5	69.5		69.5	69.5	
5′	66.7	66.7		66.7	66.7	
Ac	170.2			170.6	170.5	170.5
	22.3			21.0	21.6	21.6

Table 3. Cytotoxic Activities of Compounds **1**—**12** and Etoposide against HSC-2 Cells and HGF

Compounds	IC_{50} (μM)	
	HSC-2	HGF
1	>400	>400
2	30	54
3	74	352
4	142	271
5	222	265
6	63	267
7	211	276
8	44	141
9	80	275
10	18	280
11	171	294
12	170	261
Etoposide	24	>400

The isolated compounds were evaluated for their cytotoxic activities against HSC-2 cells and HGF. The results are shown in Table 3. It is suggested that slight differences in the

aglycon structures exerted some effects on the cytotoxic activities. Although **1** did not exhibit any apparent cytotoxicity against HSC-2 cells even at the sample concentration of 400 μM, the 25-*O*-methyl derivative (**2**) of **1** dose-dependently reduced the viable cell number and its IC_{50} value was calculated to be 30 μM. The C-27 hydroxy derivative (**8**) of **7**, which is the main secondary metabolite of *C. racemosa*, was more cytotoxic than **7**. In the cimiacerogenin derivatives (**9**—**12**), the cytotoxicity of the 7(8)-dehydro saponins (**9**, **10**) was more potent than that of the corresponding saturated saponins (**11**, **12**). It is notable that **10** showed about 15-fold higher cytotoxic activity against HSC-2 tumor cells than against normal HGF.

Experimental

NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), silica gel (Fuji-silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. 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 Capcell Pak C_{18} UG80 column (10 mm i.d.×250 mm, ODS, 5 μm, Shiseido, 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 plant material defined as the rhizomes of *C. racemosa* was provided by Tokiwa Phytochemical Co., Ltd., Chiba, Japan. A small amount of the sample is preserved in our laboratory (00-CR-011).

Extraction and Isolation The plant material (dry weight, 5.2 kg) was extracted with hot MeOH (21 l) for 3 h twice. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (445 g) was passed through a Diaion HP-20 column, successively eluting with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. Column chromatography of the 50% MeOH eluate portion (14 g) on silica gel and elution with stepwise gradient mixtures of $CHCl_3$–MeOH–H_2O (19 : 1 : 0; 9 : 1 : 0; 40 : 10 : 1; 20 : 10 : 1), and finally with MeOH alone, gave 7 fractions (frs. I—VII). Fr. IV was subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH (30 : 1; 10 : 1; 5 : 1) and ODS silica gel with MeOH–H_2O (8 : 5) to give **4** (12.5 mg). Fr. V was refined using ODS silica gel column chromatography eluting with MeOH–H_2O (8 : 5) to give **3** (67.0 mg). Fr. VII was chromatographed on silica gel eluting with $CHCl_3$–MeOH (9 : 1) and ODS silica gel with MeOH–H_2O (4 : 3) to give **5** (55.3 mg). The MeOH eluate portion (181 g) was chromatographed on silica gel and elution with stepwise gradient mixtures of $CHCl_3$–MeOH (19 : 1; 9 : 1; 4 : 1; 2 : 1), and finally with MeOH alone, gave 4 fractions (frs. VIII—XI). Fr. IX was subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH (19 : 1) and further divided into two fractions (frs. IXa, IXb). Fr. IXa was suspended in MeOH and the insoluble solid was filtered off. The filtrate was subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH (30 : 1; 19 : 1), ODS silica gel with MeCN–H_2O (1 : 1) and MeOH–H_2O (8 : 3), and on Sephadex LH-20 with MeOH to give **1** (39.0 mg), **2** (102 mg), **6** (9.8 mg), **7** (100 mg), and **8** (810 mg). Fr. IXb was separated by subjecting it to a silica gel column eluting with $CHCl_3$–MeOH (19 : 1; 9 : 1; 4 : 1), an ODS silica gel column with MeCN–MeOH–H_2O (2 : 2 : 3; 1 : 1 : 1; 8 : 8 : 5) and MeOH–H_2O (8 : 5), and to preparative HPLC using MeCN–MeOH–H_2O (8 : 8 : 11) to yield **9** (39.8 mg), **10** (10.2 mg), **11** (45.8 mg), and **12** (17.3 mg).

Compound **4**: Amorphous solid, $[\alpha]_D^{26}$ +26.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 701 $[M+Na]^+$. *Anal.* Calcd for $C_{37}H_{58}O_{11}\cdot 2H_2O$: C, 62.16; H, 8.74. Found: C, 62.44; H, 8.47. IR ν_{max} (film) cm^{-1}: 3419 (OH), 2936 and 2869 (CH), 2869 (CH), 1734 (C=O), 1456, 1372, 1249, 1141, 1088, 1044, 1024, 988, 947. ^{1}H-NMR, see Table 1. ^{13}C-NMR, see Table 2.

Alkaline Hydrolysis of 4 Compound **4** (2.5 mg) was dissolved in MeOH (0.5 ml) and added to 2% Na_2CO_3 solution (2.0 ml), which was stirred at room temperature for 24 h. The reaction mixture was neutralized by passing it through an Amberlite IR-120B (Organo, Tokyo, Japan) column and purified by silica gel column chromatography eluting with $CHCl_3$–MeOH (9 : 1) to afford **3** (1.3 mg).

Compound **5**: Amorphous solid, $[\alpha]_D^{27}$ +10.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 675 $[M+Na]^+$. *Anal.* Calcd for $C_{35}H_{56}O_{11}\cdot 1/2H_2O$: C, 63.52; H, 8.68. Found: C, 63.65; H, 8.36. IR ν_{max} (film) cm^{-1}: 3384 (OH), 2946 and 2924 (CH), 1404, 1365, 1236, 1141, 1077, 1037, 1021, 1006, 977, 947. ^{1}H-NMR, see Table 1. ^{13}C-NMR, see Table 2.

Enzymatic Hydrolysis of 5 Compound **5** (20.0 mg) was treated with naringinase (Sigma, EC 232-962-4) (80 mg) in AcOH/AcOK buffer (pH 4.3, 10 ml) at room temperature for 72 h. The reaction mixture was passed through a combination of Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) and Toyopak IC-SP M cartridge (Tosoh) eluting with 20% MeOH followed by MeOH. The MeOH eluate fraction was purified by silica gel column chromatography eluting with $CHCl_3$–MeOH (19 : 1) to afford **5a** (8.6 mg). The 20% MeOH eluate fraction was 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, 1.0 ml/min; detection, reflactive index (RI) and optical rotation (OR). Identification of L-arabinose was carried out by comparison of its retention time and OR with those of an authentic sample; t_R (min): 9.93 (positive optical rotation).

Compound **5a**: Amorphous solid, $[\alpha]_D^{26}$ +6.0° (MeOH, c=0.10). High-resolution electron impact (HR-EI)-MS m/z: 520.3397 $[M]^+$ ($C_{30}H_{48}O_7$, Calcd for 520.3400). IR ν_{max} (film) cm^{-1}: 3358 (OH), 2935 and 2889 (CH), 1454, 1384, 1288, 1169, 1071, 1026, 984, 948. ^{1}H-NMR, see Table 1. ^{13}C-NMR, see Table 2.

Complete Acetylation of 5a Compound **5a** (3.3 mg) was acetylated with a mixture of Ac_2O (1.0 ml) and pyridine (1.0 ml) in the presence of 4-(dimethylamino)pyridine (1.8 mg) as catalyst, and the crude acetate was chromatographed on silica gel eluting with hexane–Me_2CO (4 : 1) to afford the corresponding pentaacetate (**5b**) (1.6 mg).

Compound **5b**: Amorphous solid, IR ν_{max} (film) cm^{-1}: 2917 and 2849 (CH), 1736 (C=O), 1367, 1242, 1139, 1025, 985. ^{1}H-NMR (pyridine-d_5) δ: 5.90 (1H, s, H-15), 5.24 (1H, dd, J=11.6, 3.4 Hz, H-12), 4.76 (1H, dd, J=14.5, 5.6 Hz, H-3), 4.66 (1H, d, J=10.7 Hz, H-23), 4.53 (1H, dd, J=13.6, 4.7 Hz, H-21a), 4.17 (1H, d, J=10.7 Hz, H-24), 3.99 (1H, dd, J=13.6, 8.7 Hz, H-21b), 2.27, 2.25, 2.09, 2.07, 2.00 (each 3H, s, Ac), 1.69, 1.58, 1.42, 1.28, 0.91, 0.88 (each 3H, Me), 0.56 (1H, d, J=5.3 Hz, H-19a), 0.30 (1H, d, J=5.3 Hz, H-19b).

Compound **6**: Amorphous solid, $[\alpha]_D^{26}$ −26.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 685 $[M+Na]^+$. *Anal.* Calcd for $C_{37}H_{58}O_{10}\cdot 3/2H_2O$: C, 64.42; H, 8.91. Found: C, 64.75; H, 8.91. IR ν_{max} (film) cm^{-1} 3408 (OH), 2935 and 2889 (CH), 1739 (C=O), 1456, 1381, 1241, 1068, 991, 953. ^{1}H-NMR, see Table 1. ^{13}C-NMR, see Table 2.

Transformation of 6 into 1 Compound **6** (1.5 mg) was dissolved in MeOH (0.2 ml) and added to 1% Na_2CO_3 solution (1.0 ml), which was stirred at room temperature for 12 h. The reaction mixture was neutralized by addition of 5% AcOH (0.6 ml) and extracted with EtOAc (3.0 ml×3). After removal of EtOAc, the residue was dissolved in a mixture of 1,4-dioxane (0.6 ml) and 5% AcOH (0.6 ml), and was heated at 95 °C for 2 h under an Ar atmosphere. The reaction mixture was concentrated and subjected to column chromatography on silica gel eluting with $CHCl_3$–MeOH (19 : 1) to give **1** (0.6 mg).

Compound **12**: Amorphous solid, $[\alpha]_D^{26}$ −20.0° (MeOH, c=0.10). FAB-MS (positive mode) m/z: 701 $[M+Na]^+$. *Anal.* Calcd for $C_{37}H_{58}O_{11}\cdot H_2O$: C, 63.77; H, 8.68. Found: C, 63.74; H, 8.80. IR ν_{max} (film) cm^{-1}: 3417 (OH), 2966 and 2936 (CH), 1731 (C=O), 1456, 1381, 1246, 1145, 1060, 986, 950. ^{1}H-NMR, see Table 1. ^{13}C-NMR, see Table 2.

Enzymatic Hydrolysis of 12 Compound **12** (7.6 mg) was subjected to enzymatic hydrolysis using naringinase as described for **5** to give an aglycon (**12a**) (4.3 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **5a** showed the presence of L-arabinose. Compound **12a** was identical to the aglycon obtained by enzymatic hydrolysis of **11**. Since the ^{1}H- and ^{13}C-NMR spectral data for **12a** have not been reported previously, they are shown in Tables 1 and 2.

Cell Culture HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. HGF were isolated, as described previously.[16] 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

flask. 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 trypsinizaition 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, U.S.A.), 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 supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 ml dimethyl sulfoxide (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 IC_{50} value, the concentration that reduces the viable cell number by 50%, was determined from the dose–response curve.

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