Triterpene Glycosides from the Tubers of *Anemone coronaria*

Yoshihiro MIMAKI,*[,a] Kazuki WATANABE,[b] Yukiko MATSUO,[a] and Hiroshi SAKAGAMI[c]

[a] *Laboratory of Medicinal Pharmacognosy, Tokyo University of Pharmacy and Life Sciences, School of Pharmacy; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan:* [b] *Faculty of Pharmaceutical Sciences, Aomori University; 2–3–1 Koubata, Aomori, Aomori 030–0943, Japan: and* [c] *Department of Dental Pharmacology, Meikai University School of Dentistry; 1–1 Keyaki-dai, Sakado, Saitama 350–0283, Japan.*

Received March 17, 2009; accepted April 11, 2009; published online April 14, 2009

Six new triterpene glycosides (1—6), together with 11 known ones (7—17), have been isolated from a glycoside-enriched fraction prepared from the tubers of *Anemone coronaria* L. (Ranunculaceae). On the basis of extensive spectroscopic analysis, including 2D NMR data, and the results of hydrolytic cleavage, the structures of 1—6 were determined to be 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-2β,23-dihydroxyolean-12-en-28-oic acid (1), 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (2), 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (3), 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-2β,23-dihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (4), 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-2β-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (5), and 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-18-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (6), respectively. Furthermore, the isolated compounds were evaluated for their cytotoxic activity against HSC-2 cells.

Key words *Anemone coronaria*; Ranunculaceae; triterpene glycoside; cytotoxic activity; HSC-2 cell

The genus *Anemone* (Ranunculaceae) is taxonomically related to the genus *Pulsatilla* and the underground parts of several *Anemone* species such as *Anemone tomentosa*, *A. japonica*, and *A. vitifolia* are used in traditional Chinese medicine as crude drug substitutes for Pulsatillae Radix (the roots of *Pulsatilla chinensis*).[1)] Previous phytochemical examinations of the roots of *P. chinensis* have resulted in the isolation of new triterpene glycosides based on the oleanane and lupane skeletons, some of which have showed cytotoxic activity against HL-60 human promyelocytic leukemia cells.[2,3)] *Anemone coronaria* L. is indigenous to the Mediterranean Sea coast and is cultivated for ornamental purposes around the world; it has no folkloric background. However, preliminary TLC analysis of the MeOH extract suggests that it contains numerous triterpene glycosides and a glycoside-enriched fraction showed cytotoxic activity against HSC-2 human oral squamous cell carcinoma cells, which prompted us to make a phytochemical examination of the MeOH extract of *A. coronaria* tubers. As a result, six new triterpene glycosides (**1**—**6**), together with 11 known ones (**7**—**17**), were isolated. This paper deals with the structural determination of **1**—**6** on the basis of extensive spectroscopic analysis, including two-dimensional (2D) NMR, and the results of hydrolytic cleavage. In addition, the cytotoxic activity of the isolated compounds against HSC-2 cells is also described.

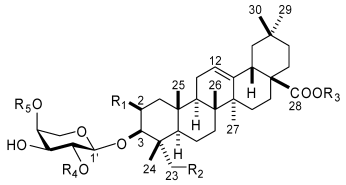
Results and Discussion

The glycoside-enriched fraction prepared from *A. coronaria* tubers (fresh weight, 3.3 kg) was subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, giving compounds **1** (18.5 mg), **2** (46.6 mg), **3** (15.3 mg), **4** (46.5 mg), **5** (10.4 mg), **6** (10.8 mg), **7** (7.4 mg), **8** (4.2 mg), **9** (33.7 mg), **10** (36.9 mg), **11** (1.20 g), **12** (15.3 mg), **13** (624 mg), **14** (1.11 g), **15** (70.5 mg), **16** (223 mg), and **17** (4.4 mg).

Compounds **7**—**17** were identified as 3β-[(α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (**7**),[4)] 3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**8**),[4)] 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**9**),[4)] 3β-[(*O*-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (**10**),[2)] 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (**11**),[2,5)] 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-β-D-glucopyranosyl ester (**12**),[6)] 3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**13**),[4,7)] 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**14**),[4)] 3β-[(*O*-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**15**),[8)] 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**16**),[9)] and 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**17**),[10)] respectively.

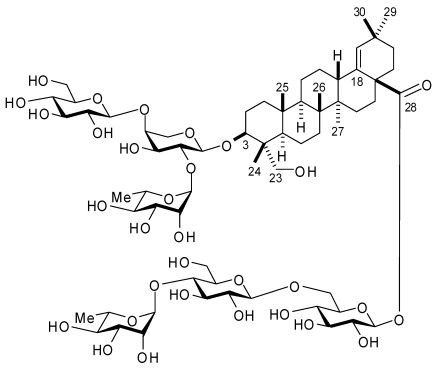
Compound **1**, isolated as an amorphous solid, exhibited an accurate $[M+Na]^+$ ion at *m/z* 951.4940 in the high-resolu-

* To whom correspondence should be addressed. e-mail: mimakiy@toyaku.ac.jp



	R_1	R_2	R_3	R_4	R_5
1	OH	OH	H	Rha	Glc
2	H	OH	H	Glc-(1→3)-Rha	Glc
3	H	OH	Glc-(1→6)-Glc	Rha	Glc
4	OH	OH	Rha-(1→4)-Glc-(1→6)-Glc	Rha	Glc
5	OH	H	Rha-(1→4)-Glc-(1→6)-Glc	Rha	Glc
7	H	OH	H	H	H
8	H	H	H	Rha	H
9	H	OH	H	Rha	H
10	H	OH	H	H	Glc
11	H	OH	H	Rha	Glc
12	H	OH	Glc	Rha	Glc
13	H	H	Rha-(1→4)-Glc-(1→6)-Glc	Rha	H
14	H	OH	Rha-(1→4)-Glc-(1→6)-Glc	Rha	H
15	H	OH	Rha-(1→4)-Glc-(1→6)-Glc	H	Glc
16	H	H	Rha-(1→4)-Glc-(1→6)-Glc	Rha	Glc
17	H	OH	Rha-(1→4)-Glc-(1→6)-Glc	Rha	Glc

Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl



6

tion electrospray-ionization time of flight mass spectrum (HR-ESI-TOF-MS), corresponding to the molecular formula $C_{47}H_{76}O_{18}$, which was also deduced by analysis of the ^{13}C-NMR spectrum combined with distortionless enhancement by polarization transfer (DEPT) spectral data. The IR spectrum of **1** was consistent with the presence of hydroxy groups (3398 cm^{-1}) and a carbonyl group (1691 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra showed signals for six tertiary methyl groups at δ_H 1.58/δ_C 17.2, δ_H 1.48/δ_C 15.1, δ_H 1.26/δ_C 26.2, δ_H 1.07/δ_C 17.5, δ_H 0.99/δ_C 23.7, and δ_H 0.92/δ_C 33.2, an olefinic group at δ_H 5.48 (t-like, J=3.3 Hz)/δ_C 144.8 (C) and 122.7 (CH), a hydroxymethyl group at δ_H 4.17 and 3.73 (ABq, J=10.7 Hz)/δ_C 64.7 (CH_2), and a carboxy group at δ_C 180.2 (C), which were characteristic of the oleanolic acid skeleton with one methyl group modified to be a hydroxymethyl group (Table 1). Furthermore, three anomeric protons and carbons were observed at δ_H 6.24 (br s)/δ_C 101.8 (CH), δ_H 5.12 (d, J=7.9 Hz)/δ_C 106.8 (CH), and δ_H 5.07 (d, J=6.8 Hz)/δ_C 104.6 (CH). These spectral features were essentially analogous to those of the known compound **11**; however, the molecular formula of **1** was higher by one oxygen atom than that of **11**. In addition, the oxymethine proton assignable to H-3 of the aglycone was observed at δ 4.26 (d, J=3.4 Hz) and was found to be coupled

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**—**6** in C_5D_5N

	1	**2**	**3**	**4**	**5**	**6**
1	44.5	39.0	39.0	44.6	44.4	39.3
2	71.4	26.4	26.3	71.4	70.8	26.3
3	81.8	81.3	81.0	81.8	89.0	81.0
4	42.9	43.6	43.5	42.9	39.0	43.5
5	48.0	47.6	47.7	48.0	56.3	48.0
6	17.9	18.1	18.1	17.9	18.3	17.9
7	33.2	33.2	32.8	32.8	33.2	34.5
8	39.8	39.8	39.9	40.0	40.0	40.9
9	48.6	48.2	48.2	48.6	48.5	51.4
10	37.0	36.9	36.9	36.9	37.0	36.9
11	24.0	23.9	23.8	23.9	23.9	21.2
12	122.7	122.6	123.1	123.1	123.1	26.4
13	144.8	144.8	144.1	144.1	144.1	41.2
14	42.3	42.2	42.1	42.2	42.3	42.9
15	28.2	28.3	28.3	28.2	28.2	29.8
16	23.6	23.7	23.3	23.3	23.3	33.9
17	46.6	46.7	47.0	47.0	47.0	48.7
18	41.9	42.0	41.7	41.7	41.7	137.8
19	46.4	46.4	46.2	46.1	46.2	132.8
20	30.9	30.9	30.7	30.7	30.7	32.2
21	34.2	34.2	34.0	33.9	33.9	33.6
22	32.9	32.9	32.5	32.5	32.5	33.4
23	64.7	63.9	63.9	64.6	29.5	63.8
24	15.1	14.1	14.0	15.1	18.5	13.7
25	17.2	16.1	16.2	17.3	16.6	17.4
26	17.5	17.5	17.5	17.6	17.5	16.3
27	26.2	26.2	26.0	26.1	26.1	15.2
28	180.2	180.2	176.5	176.5	176.5	175.4
29	33.2	33.3	33.1	33.1	33.1	30.6
30	23.7	23.8	23.6	23.6	23.6	29.0
	Ara	Ara	Ara	Ara	Ara	Ara
1	104.6	104.9	104.3	104.6	105.0	104.3
2	76.3	76.2	76.3	76.3	76.7	76.2
3	74.8	74.9	74.9	74.9	73.9	74.6
4	80.4	80.6	80.3	80.5	79.6	80.1
5	65.5	65.9	65.3	65.6	64.8	65.2
	Rha	Rha	Rha	Rha	Rha	Rha
1	101.8	101.6	101.7	101.8	102.0	101.6
2	72.2	71.5	72.2	72.2	72.2	72.1
3	72.4	83.0	72.5	72.5	72.5	72.5
4	74.1	72.8	74.1	74.1	74.0	74.0
5	69.7	69.9	69.6	69.7	69.9	69.6
6	18.5	18.6	18.6	18.5	18.5	18.6
	Glc	Glc	Glc	Glc	Glc	Glc
1	106.8	106.8	106.7	106.9	106.5	106.5
2	75.5	75.5	75.5	75.5	75.5	75.4
3	78.5	78.4	78.4	78.6	78.5	78.3
4	71.2	71.3	71.2	71.2	71.3	71.1
5	78.8	78.7	78.8	78.8	78.7	78.6
6	62.4	62.5	62.5	62.4	62.4	62.3
		Glc′	Glc′	Glc′	Glc′	Glc′
1		106.7	95.7	95.6	95.6	95.8
2		75.9	73.9	73.9	73.9	74.0
3		78.4	78.7	78.7	78.7	78.7
4		71.7	70.9	70.8	70.8	70.7
5		78.5	78.0	78.0	78.0	78.0
6		62.5	69.4	69.2	69.2	69.2
			Glc″	Glc″	Glc″	Glc″
1			105.3	104.9	104.8	104.9
2			75.2	75.3	75.3	75.2
3			78.5	76.5	76.5	76.4
4			71.5	78.1	78.1	78.1
5			78.4	77.1	77.1	77.0
6			62.6	61.2	61.2	61.2
				Rha″	Rha″	Rha″
1				102.7	102.6	102.6
2				72.4	72.4	72.4
3				72.7	72.7	72.7
4				74.0	74.0	73.9
5				70.3	70.2	70.2
6				18.5	18.5	18.5

with another oxymethine proton signal at δ 4.74 (m) in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum, suggesting the presence of a 2β-hydroxy group in the aglycone moiety of **1**. Acid hydrolysis of **1** with 1.0 M HCl yielded 2β,3β,23-trihydroxyolean-12-en-28-oic acid (**1a**) as the genuine aglycone,[11] and L-arabinose, D-glucose, and L-rhamnose as the carbohydrate moieties. Identification of the sugars was carried out by direct HPLC analysis of the hydrolysate. The structure of the triglycoside residue of **1**, including its linkage position to the aglycone, was indicated to be the same as that of **11** by the ^{1}H- and ^{13}C-NMR spectral data. This was confirmed by long-range correlations between the anomeric proton (H-1) of the α-L-rhamnopyranosyl unit (Rha) at δ 6.24 and C-2 of the α-L-arabinopyranosyl unit (Ara) at δ 76.3, H-1 of the β-D-glucopyranosyl unit (Glc) at δ 5.12 and C-4 of Ara at δ 80.4, and between H-1 of Ara at δ 5.07 and C-3 of the aglycone at δ 81.8 in the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum of **1**. Thus, the structure of **1** was established as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-2β,23-dihydroxyolean-12-en-28-oic acid.

Compound **2** was obtained as an amorphous solid with a molecular formula of $C_{53}H_{86}O_{22}$, as determined from its HR-ESI-TOF-MS data at *m/z* 1097.5436 $[M+Na]^+$. The deduced molecular formula was higher than that of **11** by $C_6H_{10}O_5$, corresponding to the presence of one more hexosyl group. The ^{1}H-NMR spectrum of **2** contained signals for four anomeric protons at δ 6.18 (br s), 5.47 (d, J=7.7 Hz), 5.10 (d, J=7.9 Hz), and 4.93 (d, J=7.1 Hz), together with signals for six tertiary methyl groups at δ 1.26, 1.11, 1.02, 1.00, and 0.93×2, an olefinic proton at δ 5.47 (t-like, J=3.3 Hz), a hydroxymethyl group δ 4.31 and 3.91 (ABq, J=11.5 Hz), and an oxymethine proton at δ 4.22 (m). Acid hydrolysis of **2** with 1.0 M HCl gave 3β,23-dihydroxyolean-12-en-28-oic acid (**2a**),[4] L-arabinose, D-glucose, and L-rhamnose. On comparison of the ^{13}C-NMR spectrum of **2** with that of **11**, a set of six signals corresponding to a terminal β-D-glucopyranosyl unit was observed at δ 106.7 (CH), 75.9 (CH), 78.4 (CH), 71.7 (CH), 78.5 (CH), and 62.5 (CH_2) in addition to the signals due to the glucosyl-(1→4)-*O*-[rhamnosyl-(1→2)]-α-L-arabinosyl unit. In the HMBC spectrum, H-1 of the additional β-D-glucosyl unit (Glc′) at δ 5.47 showed a long-range correlation with C-3 of Rha at δ 83.0. Furthermore, long-range correlations were observed between H-1 of Rha at δ 6.18 and C-2 of the Ara at δ 76.2, H-1 of Glc at δ 5.10 and C-4 of Ara at δ 80.6, and between H-1 of Ara at δ 4.93 and C-3 of the aglycone at δ 81.3. The structure of **2** was determined to be 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid.

Compound **3** was shown to have a molecular formula of $C_{59}H_{96}O_{27}$ on the basis of the HR-ESI-TOF-MS data at *m/z* 1259.6039 $[M+Na]^+$. The ^{1}H-NMR spectrum of **3** showed signals for five anomeric protons at δ 6.26 (d, J=8.2 Hz), 6.24 (br s), 5.12 (d, J=7.9 Hz), 5.04 (d, J=7.8 Hz), and 4.98 (d, J=6.7 Hz), as well as signals for six tertiary methyl groups at δ 1.17, 1.12, 1.09, 0.98, 0.87, and 0.85, an olefinic proton at δ_H 5.41 (t-like, J=3.7 Hz), a hydroxymethyl group at δ_H 4.13 and 3.72 (ABq, J=10.6 Hz), and an oxymethine proton at δ 4.21 (m). Acid hydrolysis of **3** with 1.0 M HCl gave **2a**, L-arabinose, D-glucose, and L-rhamnose, whereas alkaline hydrolysis of **3** with 6% KOH in EtOH yielded **11**. In the ^{13}C-NMR spectrum of **3**, the C-3 and C-28 carbons of the aglycone moiety were observed at δ 81.0 and 176.5, respectively, which suggest that **3** is a 3,28-bisdesmoside.[4,12] These chemical and spectral data, and comparison of the ^{13}C-NMR spectrum of **3** with that of **12** suggest that **3** is related to **12** with an additional sugar moiety composed of two β-D-glucospynosyl units (Glc′ and Glc″) at C-28 of the aglycone. In the HMBC spectrum of **3**, H-1 of the terminal β-D-glucopyranosyl unit (Glc″) at δ 5.04 showed a long-range correlation with C-6 of the inner β-D-glucopyranosyl unit (Glc′) at δ 69.4, of which H-1 exhibited a long-range correlation with C-28 of the aglycone at δ 176.5. The structure of **3** was formulated as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Compound **4** was found to have a molecular formula of $C_{65}H_{106}O_{32}$ from its HR-ESI-TOF-MS data at *m/z* 1421.6539 $[M+Na]^+$. The ^{1}H-NMR spectrum of **4** exhibited signals for six anomeric protons at δ 6.26 (br s), 6.25 (d, J=8.3 Hz), 5.87 (br s), 5.13 (d, J=7.9 Hz), 5.06 (d, J=6.8 Hz), and 5.00 (d, J=8.0 Hz), together with signals for six tertiary methyl groups at δ 1.62, 1.50, 1.19, 1.16, 0.86, and 0.85, an olefinic proton at δ 5.41 (t-like, J=3.6 Hz), and a hydroxymethyl group at δ 4.16 and 3.73 (ABq, J=10.5 Hz). Furthermore, the oxymethine proton due to H-3 of the aglycone at δ 4.26 was shown to be coupled with the H-2 proton (δ 4.75) geminally bearing a hydroxy group, with a J value of 4.4 Hz. Acid hydrolysis of **4** with 1.0 M HCl gave **1a**, L-arabinose, D-glucose, and L-rhamnose, whereas alkaline hydrolysis of **4** with 6% KOH in EtOH yielded **1**. These spectral and chemical data, and comparison of the spectral data of **4** with those of the concomitantly isolated known glycosides indicate that **4** is a bisdesmoside related to **1** with a triglycoside composed of a terminal α-L-rhamnopyranosyl unit (Rha′), a β-D-glucopyranosyl unit glycosylated at C-4 (Glc″), and a β-D-glucopyranosyl unit glycosylated at C-6 (Glc′) at C-28 of the aglycone. In the HMBC spectrum of **4**, long-range correlations were observed between H-1 of Rha′ at δ 5.87 and C-4 of Glc″ at δ 78.1, H-1 of Glc″ at δ 5.00 and C-6 of Glc′ at δ 69.2, and between H-1 of Glc′ at δ 6.25 and C-28 of the aglycone at δ 176.5. The structure of **4** was assigned as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-2β,23-dihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Compound **5** was determined to be $C_{65}H_{106}O_{31}$ by the HR-ESI-TOF-MS data at *m/z* 1405.6570 $[M+Na]^+$. All the spectral properties of **5** showed a close similarity to those of **4**. On comparison of the ^{1}H- and ^{13}C-NMR spectra of **5** with those of **4**, the C-23 hydroxymethyl signals observed in **4** at δ_H 4.16 and 3.73 (ABq, J=10.5 Hz)/δ_C 64.6 (CH_2) were replaced by the signals assignable to a tertiary methyl group at δ_H 1.24/δ_C 29.5 (Me) in **5**. All other signals appeared at almost the same positions between the two compounds. Acid hydrolysis of **5** with 1.0 M HCl gave 2β,3β-dihydroxyolean-12-en-28-oic acid (**5a**),[13] L-arabinose, D-glucose, and L-rhamnose. The structure of **5** was characterized as 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-

α-L-arabinopyranosyl)oxy]-2β-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Compound **6** exhibited a molecular formula of $C_{65}H_{106}O_{31}$ based on the HR-ESI-TOF-MS data at *m*/*z* 1383.6854 $[M+H]^+$, which was the same as that of **17**. The ^{1}H-NMR spectral properties of **6** were analogous to those of **17**, showing signals for six anomeric protons at δ 6.33 (d, *J*=8.2 Hz), 6.21 (br s), 5.83 (br s), 5.09 (d, *J*=7.9 Hz), 4.98 (d, *J*=6.6 Hz), and 4.94 (d, *J*=7.7 Hz), six tertiary methyl groups at δ 1.12, 1.03, 1.02, 0.95, 0.87, and 0.80, a hydroxymethyl group at δ 4.11 and 3.70 (ABq, *J*=10.6 Hz), and an oxymethine proton at δ 4.19 (m). Analysis of the ^{1}H- and ^{13}C-NMR spectra of **6** and comparison of those of **17** indicate that a branched triglycoside and a linear triglycoside attached to C-3 and C-28 of the aglycone, respectively, were identical to those of **17**. However, since an olefinic proton was observed at δ 5.20 as a singlet signal in the ^{1}H-NMR spectrum of **6**, **6** differed from **17** in terms of the locus of an olefinic group in the aglycone moiety. In the HMBC spectrum of **6**, the olefinic proton at δ 5.20 showed a long-range correlation with the quaternary carbon at δ 32.2 assignable to C-20, with which the methyl protons due to Me-29 at δ 1.02 and Me-30 at δ 0.95 in turn exhibited long-range correlations. These spectral data provide evidence for the presence of a double bond between C-18 and C-19. Acid hydrolysis of **6** with 1.0 M HCl gave 3β,23-dihydroxyolean-18-en-28-oic acid (**6a**),[14] L-arabinose, D-glucose, and L-rhamnose. Accordingly, the structure of **6** was determined to be 3β-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-18-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The isolated compounds were evaluated for their cytotoxic activity against HSC-2 cells. The monodesmosidic triterpene glycosides (**1**, **2**, **7**—**11**) were cytotoxic to HSC-2 cells, among which **8** showed the most potent cytotoxic activity with an IC_{50} value of 2.9 μg/ml (Table 2). The triterpene glycosides whose C-28 carboxy group are glycosylated (**3**—**6**, **12**—**17**) did not exhibit any cytotoxic activity against HSC-2 cells at a sample concentration of 100 μg/ml.

Table 2. Cytotoxic Activity of Compounds **1**—**17** against HSC-2 Cells

	IC_{50} (μg/ml)
1	21.0
2	14.0
3	>100
4	>100
5	>100
6	>100
7	18.0
8	2.9
9	6.9
10	18.0
11	7.6
12	>100
13	>100
14	>100
15	>100
16	>100
17	>100
Etoposide	10.1
Cisplatin	4.7
Doxorubicin	0.1

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 spectrometer (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-value with reference to tetramethylsilane (TMS) as an internal standard. ESI-TOF-MS data were obtained on a Waters-Micromass LCT mass spectrometer (Manchester, U.K.). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C18 UG120 column (10 mm i.d.×250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC and a Capcell Pak NH2 UG80 column (4.6 mm i.d.×250 mm, 5 μm, Shiseido) was employed for analytical HPLC. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, U.S.A.); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, U.S.A.); penicillin G, streptomycin sulfate, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material *Anemone coronaria* was purchased from a garden center in Heiwa-en, Japan, in December 2001 and identified by Dr. Yutaka Sashida, emeritus professor of medicinal pharmacognosy at Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher No. 01-12-03-AC, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation The plant material (fresh weight, 3.3 kg) was extracted with hot MeOH for 3 h twice (each 12 l). The extract was concentrated under residue pressure, and the viscous concentrate (835 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, 60% MeOH, MeOH, EtOH, and EtOAc (each 4 l). The MeOH and 60% MeOH eluate portions showed cytotoxic activity against HSC-2 cells with IC_{50} values of 21 μg/ml and 95 μg/ml, respectively. The MeOH eluate portion (55 g) was chromatographed on silica gel, eluted with of $CHCl_3$–MeOH–H_2O gradients (9 : 1 : 0; 20 : 10 : 1; 7 : 4 : 1) and finally with MeOH alone, to give five fractions (A—E). Fraction A was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (70 : 10 : 1; 40 : 10 : 1) and an ODS silica gel column with MeOH–H_2O (4 : 1) to furnish **7** (7.4 mg). Fraction B was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (6 : 1) and $CHCl_3$–MeOH–H_2O (40 : 10 : 1; 30 : 10 : 1; 20 : 10 : 1), and ODS silica gel column chromatography with MeOH–H_2O (7 : 3; 4 : 1) to yield **8** (4.2 mg), **9** (33.7 mg), and **10** (36.9 mg). Fraction D was subjected to column chromatography on ODS silica gel eluted with MeCN–H_2O (2 : 5) to yield **11** (1.20 g). The 60% MeOH eluate portion (320 g) was chromatographed on silica gel, eluted with $CHCl_3$–MeOH gradients (3 : 1; 2 : 1; 1 : 1) and finally with MeOH alone, to give seven fractions (I—VII). Fraction II was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and an ODS silica gel column with MeOH–H_2O (8 : 3) to furnish **1** (18.5 mg). Fraction III was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and ODS silica gel column chromatography with MeOH–H_2O (2 : 1) to yield **2** (46.6 mg) and **12** (15.3 mg). Fraction IV was subjected to column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and ODS silica gel with MeOH–H_2O (7 : 4; 7 : 3; 3 : 1) to yield **3** (15.3 mg), **13** (624 mg), and **14** (1.11 g). Fraction V was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and ODS silica gel with MeOH–H_2O (8 : 3) to give two subfractions (Va and Vb). Fraction Va was subjected to preparative HPLC using MeOH–H_2O (4 : 9) to furnish **15** (70.5 mg). Fraction Vb was also purified by preparative HPLC using MeCN–H_2O (2 : 5) to afford **16** (223 mg). Fraction VI was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to give **5** and **17** with few impurities, which were purified by preparative HPLC using MeCN–H_2O (4 : 11; 4 : 9) to furnish **5** (10.4 mg) and **17** (4.4 mg) in a pure form. Fraction VII was divided into six subfractions (VIIa—VIIf) by subjecting it to column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (7 : 4 : 1; 6 : 4 : 1) and ODS

silica gel with MeCN–H_2O (2 : 7; 2 : 5) and MeOH–H_2O (4 : 3; 8 : 5). Fraction VIIb was subjected to preparative HPLC using MeOH–H_2O (4 : 3) to furnish **4** (46.5 mg). Fraction VIIe was also purified by preparative HPLC using MeOH–H_2O (4 : 3) to afford **6** (10.8 mg).

Compound **1**: Amorphous solid, $[\alpha]_D^{26}$ +14.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 951.4940 $[M+Na]^+$ (Calcd for $C_{47}H_{76}O_{18}Na$: 951.4929). IR (film) ν_{max} cm^{-1}: 3398 (OH), 2938 (CH), 1691 (C=O), 1057. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.24 (1H, br s, H-1 of Rha), 5.48 (1H, t-like, J=3.3 Hz, H-12), 5.12 (1H, d, J=7.9 Hz, H-1 of Glc), 5.07 (1H, d, J=6.8 Hz, H-1 of Ara), 4.74 (1H, m, H-2), 4.26 (1H, d, J=3.4 Hz, H-3), 4.17 (1H, d, J=10.7 Hz, H-23a), 3.73 (1H, d, J=10.7 Hz, H-23b), 3.29 (1H, dd, J=13.8, 4.1 Hz, H-18), 1.58 (3H, d, J=6.2 Hz, Me-6 of Rha), 1.58 (3H, s, Me-25), 1.48 (3H, s, Me-24), 1.26 (3H, s, Me-27), 1.07 (3H, s, Me-26), 0.99 (3H, s, Me-30), 0.92 (3H, s, Me-29). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 1 A solution of **1** (4.1 mg) in 1.0 M HCl (dioxane–H_2O, 1 : 1, 3 ml) was heated at 95 °C for 1 h under an Ar atmosphere. The reaction mixture was diluted with H_2O (2 ml) and extracted with EtOAc (5 ml×3). The EtOAc extract was chromatographed on silca gel eluted with $CHCl_3$–MeOH (19 : 1) to give **1a** (1.5 mg). The H_2O residue was neutralized by passage through an Amberlite IA-93ZU (Organo, Tokyo, Japan) column and then passed through a Sep-Pak C18 cartridge (Waters, Milford, MA, U.S.A.), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH2 UG80; solvent, MeCN–H_2O (17 : 3); flow rate, 1.0 ml/min; detection, RI and OR. Identification of L-arabinose, D-glucose, and L-rhamnose in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples; t_R (min) 6.33 (L-rhamnose, negative optical rotation), 7.14 (L-arabinose, positive optical rotation), and 11.62 (D-glucose, positive optical rotation).

Compound **2**: Amorphous solid, $[\alpha]_D^{26}$ +12.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 1097.5436 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{22}Na$: 1097.5508). IR (film) ν_{max} cm^{-1}: 3417 (OH), 2936 (CH), 1690 (C=O), 1073, 1039. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.18 (1H, br s, H-1 of Rha), 5.47 (1H, d, J=7.7 Hz, H-1 of Glc′), 5.47 (1H, t-like, J=3.3 Hz, H-12), 5.10 (1H, d, J=7.9 Hz, H-1 of Glc), 4.93 (1H, d, J=7.1 Hz, H-1 of Ara), 4.31 (1H, d, J=11.5 Hz, H-23a), 4.22 (1H, m, H-3), 3.91 (1H, d, J=11.5 Hz, H-23b), 3.29 (1H, dd, J=13.7, 4.0 Hz, H-18), 1.58 (3H, d, J=6.1 Hz, Me-6 of Rha), 1.26 (3H, s, Me-27), 1.11 (3H, s, Me-24), 1.02 (3H, s, Me-26), 1.00 (3H, s, Me-30), 0.93 (3H×2, s, Me-25 and Me-29). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 2 A solution of **2** (5.5 mg) was subjected to acid hydrolysis as described for **1** to give **2a** (2.7 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-arabinose, D-glucose, and L-rhamnose.

Compound **3**: Amorphous solid, $[\alpha]_D^{26}$ +2.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 1259.6039 $[M+Na]^+$ (Calcd for $C_{59}H_{96}O_{27}Na$: 1259.6037). IR (film) ν_{max} cm^{-1}: 3388 (OH), 2926 and 2856 (CH), 1731 (C=O), 1070. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.26 (1H, d, J=8.2 Hz, H-1 of Glc′), 6.24 (1H, br s, H-1 of Rha), 5.41 (1H, t-like, J=3.7 Hz, H-12), 5.12 (1H, d, J=7.9 Hz, H-1 of Glc), 5.04 (1H, d, J=7.8 Hz, H-1 of Glc′), 4.98 (1H, d, J=6.7 Hz, H-1 of Ara), 4.21 (1H, m, H-3), 4.13 (1H, d, J=10.6 Hz, H-23a), 3.72 (1H, d, J=10.6 Hz, H-23b), 3.18 (1H, dd, J=13.9, 3.9 Hz, H-18), 1.65 (3H, d, J=6.2 Hz, Me-6 of Rha), 1.17 (3H, s, Me-27), 1.12 (3H, s, Me-26), 1.09 (3H, s, Me-24), 0.98 (3H, s, Me-25), 0.87 (3H, s, Me-30), 0.85 (3H, s, Me-29). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 3 A solution of **3** (3.7 mg) was subjected to acid hydrolysis as described for **1** to give **2a** (1.8 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-arabinose, D-glucose, and L-rhamnose.

Alkaline Hydrolysis of 3 Compound **3** (3.5 mg) was treated with 6% KOH in EtOH (3 ml) at 80 °C for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo) column and then chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to afford **11** (1.8 mg).

Compound **4**: Amorphous solid, $[\alpha]_D^{26}$ −10.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 1421.6539 $[M+Na]^+$ (Calcd for $C_{65}H_{106}O_{32}Na$: 1421.6565). IR (film) ν_{max} cm^{-1}: 3385 (OH), 2925 (CH), 1730 (C=O), 1064. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.26 (1H, br s, H-1 of Rha), 6.25 (1H, d, J=8.3 Hz, H-1 of Glc′), 5.87 (1H, br s, H-1 of Rha′), 5.41 (1H, t-like, J=3.6 Hz, H-12), 5.13 (1H, d, J=7.9 Hz, H-1 of Glc), 5.06 (1H, d, J=6.8 Hz, H-1 of Ara), 5.00 (1H, d, J=8.0 Hz, H-1 of Glc″), 4.75 (1H, m, H-2), 4.26 (1H, d, J=4.4 Hz, H-3), 4.16 (1H, d, J=10.5 Hz, H-23a), 3.73 (1H, d, J=10.5 Hz, H-23b), 3.16 (1H, dd, J=13.6, 3.7 Hz, H-18), 1.70 (3H, d, J=6.1 Hz, Me-6 of Rha′), 1.62 (3H, s, Me-25), 1.59 (3H, d, J=6.2 Hz, Me-6 of Rha), 1.50 (3H, s, Me-24), 1.19 (3H, s, Me-27), 1.16 (3H, s, Me-26), 0.86 (3H, s, Me-30), 0.85 (3H, s, Me-29). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 4 A solution of **4** (6.5 mg) was subjected to acid hydrolysis as described for **1** to give **1a** (2.3 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-arabinose, D-glucose, and L-rhamnose.

Alkaline Hydrolysis of 4 Compound **4** (21.1 mg) was treated with 6% KOH in EtOH (3 ml) at 80 °C for 2 h to afford **1** (15.3 mg).

Compound **5**: Amorphous solid, $[\alpha]_D^{26}$ −24.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 1405.6570 $[M+Na]^+$ (Calcd for $C_{65}H_{106}O_{31}Na$: 1405.6616). IR (film) ν_{max} cm^{-1}: 3409 (OH), 2932 (CH), 1734 (C=O), 1066. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.23 (1H, d, J=8.2 Hz, H-1 of Glc′), 6.13 (1H, br s, H-1 of Rha), 5.85 (1H, br s, H-1 of Rha′), 5.42 (1H, t-like, J=3.4 Hz, H-12), 5.12 (1H, d, J=7.9 Hz, H-1 of Glc), 4.99 (1H, d, J=7.8 Hz, H-1 of Glc″), 4.83 (1H, d, J=6.8 Hz, H-1 of Ara), 4.54 (1H, m, H-2), 3.27 (1H, d, J=3.3 Hz, H-3), 3.17 (1H, dd, J=13.5, 4.0 Hz, H-18), 1.69 (3H, d, J=6.2 Hz, Me-6 of Rha′), 1.58 (3H, d, J=6.2 Hz, Me-6 of Rha), 1.50 (3H, s, Me-25), 1.47 (3H, s, Me-24), 1.26 (3H, s, Me-27), 1.24 (3H, s, Me-23), 1.13 (3H, s, Me-26), 0.88 (3H×2, s, Me-29 and Me-30). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 5 A solution of **5** (6.1 mg) was subjected to acid hydrolysis as described for **1** to give **5a** (2.6 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-arabinose, D-glucose, and L-rhamnose.

Compound **6**: Amorphous solid, $[\alpha]_D^{26}$ −26.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) m/z: 1383.6854 $[M+H]^+$ (Calcd for $C_{65}H_{107}O_{31}$: 1383.6796). IR (film) ν_{max} cm^{-1}: 3388 (OH), 2934 (CH), 1727 (C=O), 1068. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.33 (1H, d, J=8.2 Hz, H-1 of Glc′), 6.21 (1H, br s, H-1 of Rha), 5.83 (1H, br s, H-1 of Rha′), 5.20 (1H, s, H-19), 5.09 (1H, d, J=7.9 Hz, H-1 of Glc), 4.98 (1H, d, J=6.6 Hz, H-1 of Ara), 4.94 (1H, d, J=7.7 Hz, H-1 of Glc″), 4.19 (1H, m, H-3), 4.11 (1H, d, J=10.6 Hz, H-23a), 3.70 (1H, d, J=10.6 Hz, H-23b), 2.59 (1H, br d, J=11.1 Hz, H-13), 1.67 (3H, d, J=6.2 Hz, Me-6 of Rha), 1.62 (3H, d, J=6.2 Hz, Me-6 of Rha′), 1.12 (3H, s, Me-26), 1.03 (3H, s, Me-24), 1.02 (3H, s, Me-29), 0.95 (3H, s, Me-30), 0.87 (3H, s, Me-25), 0.80 (3H, s, Me-27). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 6 A solution of **6** (5.1 mg) was subjected to acid hydrolysis as described for **1** to give **6a** (1.8 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-arabinose, D-glucose, and L-rhamnose.

Cell Culture and Assay for Cytotoxic Activity HSC-2 cells were provided through the courtesy of Prof. M. Nagumo (Showa University, Tokyo, Japan) and were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate in a humidified 5% CO_2 atmosphere. Cells were trypsinized and inoculated at 6×10^3 to 1.2×10^4 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 phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4), they were treated for 24 h without or with test compounds. They were then 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-10.5).[15,16] The IC_{50} value, the concentration that reduces the vial cell number by 50%, was determined from the dose–response curve.

References

1) "Dictionary of Chinese Medicinal Materials," Vol. 1, Jiang Su New Medical College, ed. by Shanghai Scientific and Technological Press, Shanghai, 1977, pp. 704—706.
2) Mimaki Y., Kuroda M., Asano T., Sashida Y., *J. Nat. Prod.*, **62**, 1279—1283 (1999).
3) Mimaki Y., Yokosuka A., Kuroda M., Hamanaka M., Sakuma C., Sashida Y., *J. Nat. Prod.*, **64**, 1226—1229 (2001).
4) Mimaki Y., Doi S., Kuroda M., Yokosuka A., *Chem. Pharm. Bull.*, **55**, 1319—1324 (2007).
5) Shimizu M., Shingyouchi K., Morita N., Kizu H., Tomimori T., *Chem. Pharm. Bull.*, **26**, 1666—1671 (1978).
6) Zhang Q., Ye W., Che C., Zhao S., *Acta Pharmaceutica Sinica*, **35**, 756—759 (2000).
7) Sano K., Sanada S., Ida Y., Shoji J., *Chem. Pharm. Bull.*, **39**, 865—870 (1991).

8) Li X. C., Wang D. Z., Wu S. G., Yang C. R., *Phytochemistry*, **29**, 595—599 (1990).
9) Dekanosidze G. E., Pkheidz T. A., Gorovits T. T., Kemertelidze E. P., *Khim. Prir. Soedin.*, **6**, 484—485 (1970).
10) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **20**, 2143—2149 (1972).
11) Fujioka T., Iwamoto M., Iwase Y., Hachiyama S., Okabe H., Yamauchi T., Mihashi K., *Chem. Pharm. Bull.*, **37**, 2355—2360 (1989).
12) Mimaki Y., Kuroda M., Yokosuka A., Harada H., Fukushima M., Sashida Y., *Chem. Pharm. Bull.*, **51**, 960—965 (2003).
13) Kohda H., Tanaka S., Yamaoka Y., Ohhara Y., *Chem. Pharm. Bull.*, **39**, 2609—2612 (1991).
14) Melek F. R., Miyase T., Abdel-Khalik S. M., Hetta M. H., Mahmoud I. I., *Phytochemistry*, **60**, 185—195 (2002).
15) Furuya S., Takayama F., Mimaki Y., Sashida Y., Satoh K., Sakagami H., *Anticancer Res.*, **20**, 4189—4194 (2000).
16) Furuya S., Takayama F., Mimaki Y., Sashida Y., Satoh K., Sakagami H., *Anticancer Res.*, **21**, 959—964 (2001).