Saponins from the Flower Buds of *Buddleja officinalis*

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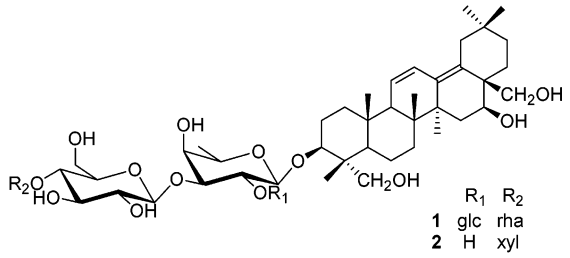
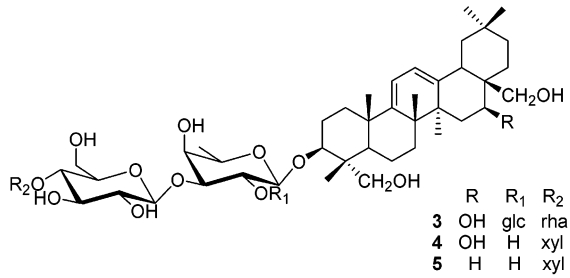
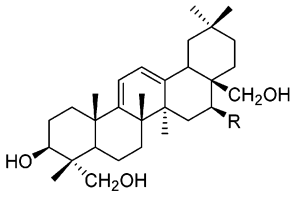
Five new saponins, mimengosides C−G (**1**−**5**), were isolated from the flower buds of *Buddleja officinalis* along with five known compounds, namely, songaroside A, acteoside, phenylethyl 2-glucoside, echinacoside, and phenylethyl alcohol 8-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside. The structures of **1**−**5** were elucidated using spectroscopic and chemical methods, and these compounds were evaluated for their inhibitory effects against HL-60 leukemia cells.

The flower buds of *Buddleja officinalis* Maxim. (Loganiaceae) are a commonly used herb in traditional Chinese medicine (Chinese name "Mimenghua") and is officially listed in the Pharmacopoeia of People's Republic of China. It is an anti-inflammatory and antimicrobial herb that is used in the treatment of ailments of the eyes.[1]

Buddleja species have been investigated extensively, resulting in the isolation of terpenoids,[2] flavonoids,[3] iridoids,[4] phenylethanoids,[5] and saponins (e.g., mimengosides A and B).[6] Several of these compounds, have shown leukocyte eicosanoid generation[7] and antihepatotoxic,[8] antifungal,[9] and antiprotozoal activities.[10] As part of our current interest in plant saponins, we have isolated 10 compounds from the flower buds of *B. officinalis*, including five new saponins (**1**−**5**) along with one known saponin and four known phenylethanoids. The activity of the new saponins against the HL-60 leukemia cell line has also been evaluated.







Results and Discussion

The crude flower buds of *Buddleja officinalis* (20 kg) were refluxed with 95% ethanol at 80 °C, and the crude extract was extracted with petroleum ether, ethyl acetate, and *n*-butanol, respectively. The *n*-butanol part was subjected to passage over a Diaion HP-20 column eluted with water with increasing amounts of methanol (20−100%). The eluate produced with 80% methanol was subjected to silica gel and ODS open column chromatography and preparative HPLC, and furnished six saponins.

Mimengoside C (**1**), an amorphous powder, gave a sodiated molecular ion at *m*/*z* 1111.9 $[M + Na]^+$ in the positive MADLI-TOFMS, which suggested the molecular formula $C_{54}H_{88}O_{22}$. The ^{1}H NMR spectrum (Table 1) showed two double-bond proton signals, at *δ* 5.65 (1H, d, *J* = 10.6 Hz) and 6.48 (1H, dd, *J* = 10.6, 2.1 Hz), four sugar anomeric proton signals, at *δ* 4.93 (d, *J* = 8.3 Hz), 5.24 (d, *J* = 7.8 Hz), 5.57 (d, *J* = 7.8 Hz), and 5.80 (s), and six tertiary methyl signals, at *δ* 0.81, 0.83, 0.92, 1.02, and 1.04. The coupling constants of the two double bonds suggested that they are linked as a diene, which was confirmed from the ^{13}C NMR data (*δ* 126.9, 125.5 and 136.2, 133.1). After appropriate 2D NMR experiments, the aglycon of **1** was identified as 3*β*,16*β*,23,28-tetrahydroxyoleana-11,13(18)-diene, also known as saikogenin A.[11] The ^{13}C NMR spectrum of **1** (Table 2) indicated 54 carbons, and among them there were eight methyls, 12 methylenes, 26 methines, and eight quarternary carbons. Besides 30 carbons assigned to the aglycon, 24 carbons were assigned to four sugars. Acid hydrolysis of **1** yielded D-glucopyranose, D-fucopyranose, and L-rhamnopyranose, in a ratio of 2:1:1. On the basis of combined analysis of the DQF-COSY, HETCOR, and HMBC spectra, the linkage of the four sugar chains was established from the following HMBC correlations: H-1 (*δ* 5.24) of Glc with C-3 (*δ* 84.5) of Fuc; H-1 (*δ* 5.80) of Rha with C-4 (*δ* 78.1) of Glc; H-1 (*δ* 5.57) of Glc′ with C-2 (*δ* 76.9) of Fuc. The attachment of the sugar moieties was confirmed from the H−C long-range correlation between H-1 (*δ* 4.93) of fucopyranose and C-3 (*δ* 83.4) observed in the HMBC spectrum, and from the NOESY spectrum. The anomeric configurations for the sugar moieties were defined as *β* for glucose and fucose from their coupling constants of 7.8 and 8.3 Hz, respectively. The ^{13}C NMR data of rhamnose were compared with those of methyl-α-L- and

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Table 1. ^{13}C NMR Data for the Aglycons of **1**−**5** and for **5a** (125 MHz, C_5D_5N)

position	1	2	3	4	5	5a
1	38.2	38.3	37.7	37.5	37.5	37.3
2	25.8	25.9	26.3	26.7	26.6	26.0
3	82.3	81.6	82.5	81.4	82.6	72.8
4	43.6	43.5	44.3	43.6	43.6	43.2
5	47.5	47.3	44.0	43.4	44.0	44.5
6	18.1	18.1	17.4	17.8	18.0	18.4
7	32.2	32.3	32.3	31.9	32.0	32.1
8	40.3	40.3	43.3	43.0	41.0	41.0
9	54.3	54.4	154.9	154.8	155.0	155.1
10	36.3	36.4	38.9	38.5	38.5	39.0
11	126.9	126.9	116.0	115.9	115.7	116.0
12	125.5	125.5	121.1	121.0	121.2	121.4
13	136.2	136.5	145.2	145.2	146.6	146.8
14	44.1	44.1	43.5	43.1	43.0	43.2
15	34.9	34.8	36.4	36.0	25.4	25.5
16	76.4	76.1	66.9	66.6	23.0	23.1
17	44.2	44.3	40.7	40.4	38.5	37.6
18	133.1	133.5	42.9	42.5	40.9	41.0
19	38.1	38.2	47.1	46.8	46.9	47.0
20	32.1	32.5	31.2	30.8	31.0	31.2
21	34.7	35.0	34.3	33.9	34.4	34.5
22	29.7	29.8	27.0	26.0	31.6	31.8
23	64.2	64.0	65.1	64.1	65.1	67.6
24	12.6	12.9	13.6	13.5	13.1	13.2
25	18.5	18.6	26.3	26.0	25.9	26.0
26	16.8	16.9	21.5	21.1	21.0	21.1
27	21.7	21.8	21.3	20.9	20.2	20.3
28	63.8	63.8	69.4	69.2	69.0	69.1
29	24.6	24.7	24.2	23.9	23.6	23.8
30	32.1	32.1	33.4	33.0	33.0	33.2

methyl β-L-rhamnopyranosides. They agreed well with values for methyl α-L-rhamnopyranoside. Thus, the structure of **1** (mimengoside C) was elucidated as 3β,16β,23,-28-tetrahydroxyoleana-11,13(18)-diene 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside.

Mimengoside D (**2**), an amorphous powder, gave a protonated molecular ion at *m*/*z* 913.4 in the positive ESIMS, corresponding to the molecular formula $C_{47}H_{76}O_{17}$, or 176 mass units less than that of **1**. When the ^1H and ^{13}C NMR data of **2** were compared with those of **1** (Table 1, 2), it was apparent that both compounds are based on the same aglycon. Acid hydrolysis of **2** furnished D-fucopyranose, D-glucopyranose, and D-xylopyranose in a ratio of 1:1:1. The configuration of the anomeric carbons was defined as β for fucose, glucose, and xylose from their coupling constants of 7.8, 7.8, and 7.8 Hz, respectively. On the combined analysis of the HMBC, HETCOR, and NOESY spectra, the sugar chain was elucidated as β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranose, and the linkage to the aglycon was also determined at the C-3 position with the fucopyranose unit, as revealed in the HMBC and NOESY spectra, respectively. Thus, the structure of **2** (mimengoside D) was determined as 3β,16β,-23,28-tetrahydroxyoleana-11,13(18)-diene 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside.

Mimengoside E (**3**), an amorphous powder, gave a protonated molecular ion at *m*/*z* 1111.9 in the positive ESIMS, corresponding to the molecular formula $C_{54}H_{88}O_{22}$. The ^1H NMR spectrum (Table 1) showed different signals from **1** and **2**, with double-bond protons at δ 5.70 (d, J = 5.7 Hz) and 5.59 (d, J = 6.0 Hz), which suggested the presence of a homoannular diene in the aglycon. The ^{13}C NMR data (Table 2) indicated 54 carbons, and among them there were eight methyls, 12 methylenes, 26 methines, and eight quarternary carbons. Of those, two double bonds were linked as a homoannular diene on the basis of signals at δ_C 154.9, 116.0, 121.1, and 145.2. A combination of the ^1H NMR, ^{13}C NMR, and DEPT spectra confirmed the aglycon of **3** was 3β,16β,23,28-tetrahydroxyoleana-9(11),12-diene, which is also known as saikogenin H.[11] The β-orientation of OH-16 was determined by the ^{13}C NMR data (δ 66.9) and further confirmed by the correlation between Hα-16 (δ 4.56) and H-27 (δ 1.23) which was observed in the NOESY spectrum. Acid hydrolysis of **3** yielded D-gluco-

Table 2. ^1H NMR Data for the Sugar Moieties of **1**−**5** (C_5D_5N, δ ppm, J in Hz)[a]

position	1	2	3	4	5
Fuc					
1	4.93 d (8.3)[b]	4.94 d (7.8)	4.90 d (7.8)	4.95 d (7.8)	4.99 d (7.6)
2	4.64	4.49	4.56	4.50	4.62
3	4.05	3.98	3.98	3.99	4.06
4	4.15	4.09	4.05	4.11	4.15
5	3.60	4.15	4.15	4.14	3.61
6	1.38 d (6.2)	1.43 d (6.4)	1.39 d (6.2)	1.45 d (6.4)	1.39 d (6.2)
Glc					
1	5.24 d (7.8)	5.10 d (7.8)	5.26 d (7.8)	5.08 d (7.8)	5.20 d (7.8)
2	3.90	4.01	3.94	4.00	3.91
3	4.11	4.25	4.17	4.21	4.12
4	4.35	4.30	4.42	4.28	4.32
5	3.69	3.92	3.74	3.92	3.72
6	4.16, 4.06	4.46, 4.55	4.10, 4.08	4.44, 4.54	4.07, 4.16
	Glc′	Xyl	Glc′	Xyl	Glc′
1	5.57 d (7.8)	5.25 d (7.8)	5.58 d (7.8)	5.24 d (7.8)	5.55 d (7.8)
2	4.06	4.02	4.07	4.02	4.05
3	4.18	4.08	4.22	4.07	4.17
4	3.62	3.68	3.65	3.70	3.64
5	4.13	3.65, 4.22	4.13	3.64, 4.23	4.16
6	4.23, 4.33		4.30, 4.35		4.24, 4.32
Rha					
1	5.80 (s)		5.85 d (1.1)		5.78 (s)
2	4.65		4.67		4.63
3	4.50		4.55		4.48
4	4.32		4.35		4.28
5	4.90		4.87		4.87
6	1.69 d (6.2)		1.69 d (6.2)		1.69 d (6.2)

[a] The assignments are based on the DQFCOSY, TOCSY, NOESY, HMBC, and DEPT experiments. [b] Signal multiplicity and $^1J_{C1,H1}$ values (Hz) are shown in parentheses.

pyranose, D-fucopyranose, and L-rhamnopyranose in a ratio of 2:1:1. The configuration of the anomeric carbons was defined as β for fucose and glucose and α for rhamnose, the same as those of **1**. On the basis of combined analysis of the DQF-COSY, HETCOR, HMBC, and NOESY spectra, the sugar chain was shown to be the same as that of **1**, so the sequence was determined as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranose. The glycosidic linkage of the fucopyranose in the sugar chain to the C-3 position of the aglycon was revealed from the H−C long-range correlation between the fucopyranose anomeric proton (δ 4.90) and C-3 (δ 82.5) observed in the HMBC spectrum and confirmed in the NOESY spectrum. Thus, the structure of **3** (mimengoside E) was elucidated as 3β,16β,23,28-tetrahydroxyoleana-9(11),12-diene 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside.

Mimengoside F (**4**), an amorphous powder, gave a molecular formula of $C_{47}H_{76}O_{17}$ on the basis of the protonated molecular ion at *m*/*z* 913.3 in the positive ESIMS. Detailed analysis of the ^{1}H and ^{13}C NMR spectra of **4** suggested the same aglycon as **3** and the same sugar chain as **2**. Acid hydrolysis of **4** furnished D-fucopyranose, D-glucopyranose, and D-xylopyranose in a ratio of 1:1:1. On the basis of the DQF-COSY, HETCOR, HMBC, and NOESY spectra, the sequence and linkage positions of the sugar chain were determined as β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranose, and the position of glycosylation was C-3. Thus, the structure of **4** (mimengoside F) was determined to be 3β,16β,23,28-tetrahydroxyoleana-9(11),12-diene 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside.

Mimengoside G (**5**), an amorphous powder, gave a sodiated molecular ion at *m*/*z* 1095.7 $[M + Na]^+$ in the positive ESIMS, corresponding to the molecular formula $C_{54}H_{88}O_{21}$. Compared with **3**, there were 16 mass units less in the molecular weight of **5**, suggesting one oxygen atom less in the molecule. The detailed analysis of its ^{1}H NMR, ^{13}C NMR, and DEPT data indicated **5** had an aglycon similar to **3** except for a 16-hydroxy moiety. After several combined NMR experiments, including the HETCOR, DQF-COSY, TOCSY, HMBC, and NOESY techniques, the aglycon was elucidated as 3β,23,28-trihydroxyoleana-9(11),-12-diene. Acid hydrolysis gave the aglycon, **5a**, for which the structure was confirmed by a ^{13}C NMR experiment (Table 2). To the best of our knowledge, 3β,23,28-trihydroxyoleana-9(11),12-diene (**5a**) is a new sapogenin. Acid hydrolysis of **5** also furnished D-fucopyranose, D-glucopyranose, and L-rhamnopyranose in a ratio of 1:2:1. On the combined analysis of the DQF-COSY, HETCOR, HMBC, and NOESY spectra, the linkage sequence and positions of the sugars were determined as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranose, and the glycosidic linkage of the fucopyranose to the C-3 position of aglycon was also determined. Thus, the structure of **5** (mimengoside G) was established as 3β,23,28-trihydroxyoleana-9(11),12-diene 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside.

A known saponin, songaroside A, was also isolated from the *n*-butanol extract and was identified by spectral data comparison with the literature.[12] Four known phenylethanoids, acteoside,[13] phenylethyl 2-glucoside,[14] echinacoside, and phenylethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside,[15,16] have also been isolated in the investigation and again identified by comparison with the relevant literature.

The five new saponins were tested against HL-60 leukemia cells, and the results indicated these compounds have weak cytotoxic activity, with IC_{50} values of 23.2 (**1**), 26.7 (**2**), 16.8 (**3**), 22.7 (**4**), and 29.4 μM (**5**) being obtained. Etoposide (IC_{50} 0.2 μM) was used as the positive control.

Experimental Section

General Experimental Procedures. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. IR spectra were carried out on a JASCO D-300 FT-IR spectrometer. The NMR experiments were carried out on a JEOL ECP500 instrument using standard JEOL sequences for 1D and 2D NMR experiments in pyridine-d_5 solution. MALDI-TOFMS was conducted using PerSeptive Biosystems Voyager DESTR mass spectrometer. ESIMS was conducted on a LCQ mass spectrometer.

Diaion HP-20 (Mitsubishi Chemical Co.), Si gel (Silica gel 60, Merck), and ODS (Chromatorex, 100−200 mesh, Fujisylisia) were used for open column chromatography. Analytical HPLC was performed on a Waters 600 pump system connected with a Shimadzu SPD M10AvP diode array detector, using an Inertsil ODS-3 column (4.6 mm i.d. × 250 mm, 5 μm). Preparative HPLC was performed on a JASCO PU 980 pump connected with a JASCO UV 970 detector (at 254 nm), using a Senshu Pak PEGASIL ODS II column (20 mm i.d. × 250 mm, 5 μm).

Plant Material. *Buddleja officinalis* (flower buds) used in this study was purchased from a market place at Bozhou, Anhui Province, People's Republic of China, in May 2000, and identified by one of us (D.G.). A voucher specimen has been deposited in the Division of Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Peking University, People's Republic of China (voucher #PBP 00412).

Extraction and Isolation. The powdered flower buds (20 kg) of *B. officinalis* were refluxed with 95% EtOH three times for 2 h each at 80 °C. The alcoholic extract was concentrated (2.5 kg), suspended in water, and then partitioned successively with petroleum ether (4000 mL), EtOAc (4000 mL), and *n*-BuOH (5000 mL). The *n*-BuOH-soluble part (120 g) was subjected to passage over a column of Diaion HP-20 (2500 mL) and washed with water and 20, 40, 60, 80, and 100% MeOH. The 80% MeOH fraction (16 g) was chromatographed over Si gel and ODS columns and then subjected to preparative HPLC purification (MeOH/H_2O, 60%), to afford six saponins: **1** (30 mg), **2** (25 mg), **3** (16 mg), **4** (20 mg), **5** (20 mg), and songaroside A (80 mg). The 60% MeOH fraction (13 g) was subjected to Si gel and ODS open column chromatography, in turn. Fractions containing crude phenylethanyl glycosides were purified using repeated preparative HPLC (MeOH/H_2O, 40%) to afford acteoside (30 mg), phenylethyl 2-glucoside (13 mg), echinacoside (12 mg), and phenylethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (24 mg).

Mimengoside C (1): amorphous powder; $[\alpha]^{25}_D$ +86.8° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3403, 2940, 1635, 1454, 1384, 1068 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.48 (1H, dd, J = 10.6, 2.1 Hz, H-12), 5.65 (1H, d, J = 10.6 Hz, H-11), 1.04, 1.02, 0.94, 0.92, 0.83, 0.81 (3H each, s, H_3-27, -24, -30, -25, -29, -26); for other NMR data, see Tables 1−3; MALDI-TOFMS (positive-ion mode) *m*/*z* 1111.9 $[M + Na]^+$.

Mimengoside D (2): amorphous powder; $[\alpha]^{25}_D$ +88.2° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3423, 2931, 1628, 1365, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.53 (1H, dd, J = 10.6, 2.8 Hz, H-12), 5.70 (1H, d, J = 10.6 Hz, H-11), 1.08, 0.98, 0.97, 0.91, 0.87, 0.87 (3H each, s, H_3-27, -25, -30, -24, -29, -26); for other NMR data, see Tables 1−3; ESIMS (positive-ion mode) *m*/*z* 913.4 $[M + H]^+$.

Mimengoside E (3): amorphous powder; $[\alpha]^{25}_D$ +72.5° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3402, 2930, 1633, 1384, 1069 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.71 (1H, d, J = 6.0 Hz, H-11), 5.61 (1H, d, J = 6.0 Hz, H-12), 1.27, 1.25, 1.23, 1.10, 0.98, 0.87 (3H each, s, H_3-26, -25, -27, -24, -30, -29); for other

Table 3. ^{13}C NMR Data for the Sugar Moieties of **1**−**5** (125 MHz, C_5D_5N)

position	1	2	3	4	5
Fuc					
1	104.0	105.79	104.1	105.8	103.8
2	76.9	71.64	77.2	71.6	76.9
3	84.5	85.47	84.7	85.3	84.7
4	71.8	72.03	72.1	72.0	71.8
5	70.3	70.65	70.6	70.6	70.3
6	17.0	17.07	17.4	17.0	17.0
Glc					
1	104.8	105.44	105.0	105.4	104.7
2	75.3	74.39	75.6	74.8	75.3
3	76.2	76.39	76.5	76.1	76.3
4	78.1	80.76	78.3	80.6	78.3
5	77.0	76.65	77.3	76.6	77.0
6	61.1	61.67	61.4	61.4	61.2
	Glc′	Xyl	Glc′	Xyl	Glc′
1	103.7	106.28	104.0	106.2	103.7
2	76.1	75.36	76.3	75.3	76.0
3	78.6	78.20	78.9	78.2	78.6
4	77.4	70.92	77.6	70.8	77.3
5	72.0	67.25	72.2	67.2	72.0
6	63.0		63.2		62.9
Rha					
1	102.6		102.8		102.6
2	72.4		72.7		72.3
3	72.6		72.9		72.6
4	73.7		74.1		73.7
5	70.2		70.5		70.2
6	18.3		18.8		18.3

NMR data, see Tables 1−3; ESIMS (positive-ion mode) *m*/*z* 1111.9 $[M + H]^+$.

Mimengoside F (4): amorphous powder; $[\alpha]^{25}_D$ −49.0° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3123, 2926, 2859, 1637, 1386, 1071 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.72 (1H, d, J = 5.7 Hz, H-11), 5.63 (1H, d, J = 6.2 Hz, H-12), 1.28, 1.27, 1.26, 0.98, 0.96, 0.87 (each 3H, s, H_3-26, -25, -27, -30, -24, -29); for other NMR data, see Tables 1−3; ESIMS (positive-ion mode) *m*/*z* 913.3 $[M + H]^+$.

Mimengoside G (5): amorphous powder; $[\alpha]^{25}_D$ −42.2° (*c* 0.6, MeOH); IR(KBr) ν_{max} 3401, 2937, 1635, 1457, 1365, 1068 cm^{-1}; ^1H NMR (pyirdine-d_5, 500 MHz) δ 5.70 (1H, d, J = 5.7 Hz, H-11), 5.59 (1H, d, J = 6.0 Hz, H-12), 1.24, 1.20, 1.18, 1.09, 0.98, 0.89 (3H each, s, H_3-25, -26, -27, -24, -30, -29); for other NMR data, see Tables 1−3; ESIMS (positive ion mode) *m*/*z* 1095.7 $[M + Na]^+$.

Acid Hydrolysis of Mimengosides C−G (1−5). A solution of each saponin (8 mg) in 1 M HCl (dioxane/H_2O, 1:1, 5 mL) was heated at 80 °C for 2 h. After the dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extract was washed with H_2O and then concentrated to give a residue, which was purified by HPLC to give an aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column and concentrated under reduced pressure to dryness. The residue was dissolved in H_2O (1 mL), to which (*S*)-(−)-phenylethylamine (7 mg) and $NaBH_3CN$ (10 mg) in EtOH (1 mL) were added. After being set aside at 40 °C for 4 h followed by addition of glacial HOAc (0.2 mL) and evaporating to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) and pyridine (0.3 mL) overnight. The reaction mixture was evaporated to dryness and dissolved in 1 mL of H_2O/MeCN (4:1). The solution was passed through a Sep-Pak C_{18} cartridge (Waters) eluted with H_2O/MeCN (4:1, 1:1, each 10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[17] which was analyzed by HPLC under the following conditions: solvent, MeCN−H_2O (2:3); flow rate, 0.8 mL/min; detection, 230 nm. The retention times of derivatives of monosacchrides were as follows: D-fucopyranose, 24.63 min; D-glucopyranose, 27.68 min; l-rhamnopyranose, 31.64 min; D-xylopyranose, 21.10 min.

3*β*,23,28-Trihydroxyoleana-9(11),12-diene (5a): amorphous powder, $[\alpha]^{25}_D$ +20.0° (*c* 0.2, $CHCl_3$); IR ν_{max} (KBr) 3400, 2923, 1614, 1457, 1365, 1068 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.75 (1H, d, J = 5.6 Hz, H-11), 5.64 (1H, d, J = 5.6 Hz, H-12), 1.24, 1.19, 1.18, 1.09, 0.96, 0.89 (each 3H, s, H_3-25, 26, 27, 24, 30, 29); for other NMR data, see Tables 1; ESIMS (positive ion mode) *m*/*z* 479.2 $[M + Na]^+$.

Cytotoxicity Assay. HL-60 leukemia cells (RIKEN Cell Bank, Tsukuba, Japan) were maintained in RPMI 1640 medium (GIBCO RBL Co., Grand Island, NY) containing 10% fetal bovine serum supplemented with L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cell growth was evaluated by a MTT assay procedure as previously reported.[18]

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