Medicinal Flowers. XXXII.[1] Structures of Oleanane-Type Triterpene Saponins, Perennisosides VIII, IX, X, XI, and XII, from the Flowers of *Bellis perennis*

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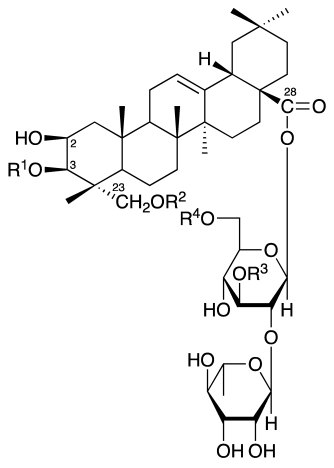
Five new triterpene saponins perennisosides VIII (1), IX (2), X (3), XI (4), and XII (5) were isolated from the MeOH-eluated fraction of the methanolic extract from the flowers of *Bellis perennis*. The MeOH-eluted fraction of the methanolic extract from the flowers of *B. perennis* was found to inhibit gastric emptying in olive oil-loaded mice at a dose of 200 mg/kg, *per os* (*p.o.*). The stereostructures of 1—5 were elucidated on the basis of chemical and spectroscopic evidence.

Key words *Bellis perennis*; Asteraceae; triterpene saponin; perennisoside; Daisy flower

An Asteraceae plant, *Bellis perennis*, is widely distributed in Europe and North Africa. The whole flowering plant of *B. perennis* has been used for bruises, bleeding, muscular pain, purulent skin diseases, and rheumatism in European folk medicine.[2] During the course of our studies on bioactive constituents from medicinal flowers,[1,3—12] we found that the methanolic extract and its saponin constituents were found to show inhibitory effects on plasma triglyceride elevation in olive oil-loaded mice[5] and pancreatic lipase inhibitory activity.[11] From the methanolic extract, 20 acylated triterpene saponin constituents, perennisosides I—VII[5] and perennisaponins A—M,[6,11] were isolated together with eight saponins, nine flavonoids, and two glycosides.[5,6] Our continuing search led to the isolation of five new oleanane-type triterpene saponins named perennisosides VIII—XII (**1**—**5**), which were obtained from the flowers of *B. perennis*. Here, we describe the isolation and structure elucidation of five new saponins (**1**—**5**).

The flowers of *B. perennis* cultivated in Albania were extracted with methanol to give a methanolic extract (25.8% from the dried flowers). The methanolic extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (6.7%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (H_2O→MeOH) to give H_2O- and MeOH-eluted fractions (12.5% and 6.4%, respectively), as described previously.[5] The MeOH-eluted fraction, which was found to inhibit gastric emptying in olive oil-loaded mice at a dose of 200 mg/kg *per os* (*p.o.*) (Table 1), was subjected to normal- and reversed-phase column chromatographies, and finally HPLC to give **1** (0.0124%), **2** (0.0076%), **3** (0.0125%), **4** (0.0132%), and **5** (0.0111%).

Structures of Perennisosides VIII (1), IX (2), X (3), XI (4), and XII (5) Perennisoside VIII (**1**) was obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{27}$ +11.9° in MeOH). The IR spectrum of **1** showed absorption bands at 1744 and 1655 cm^{-1} ascribable to ester carbonyl and olefin functions, and broad bands at 3440 and 1069 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1211 $(M+Na)^+$ and 1187 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{58}H_{92}O_{25}$. Treatment of **1** with 0.5% sodium methoxide (NaOMe)–MeOH provided a desacyl derivative, desacyl-perennisoside VIII (**1a**). Acid hydrolysis of **1a** with 5% sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) liberated bayogenin[5,13,14] together with L-rhamnose, D-fucose, and D-glucose, which were iden-



	R^1	R^2	R^3	R^4
Perennisoside VIII (**1**):	Fuc	Ac	Glc	Ac
Desacyl-perennisoside VIII (**1a**):	Fuc	H	Glc	H
Perennisoside IX (**2**):	Fuc	Ac	Gal	Ac
Desacyl-perennisoside IX (**2a**):	Fuc	H	Gal	H
Perennisoside X (**3**):	$Glc^1—{}^3Glc$	Ac	Glc	Ac
Desacyl-perennisoside X (**3a**):	$Glc^1—{}^3Glc$	H	Glc	H
Perennisoside XI (**4**):	$Glc^1—{}^3Glc$	Ac	Gal	Ac
Desacyl-perennisoside XI (**4a**):	$Glc^1—{}^3Glc$	H	Gal	H
Perennisoside XII (**5**):	H	H	Gal	Glc

Fuc: β-D-fucopyranosyl
Glc: β-D-glucopyranosyl
Gal: β-D-galactopyranosyl

Chart 1

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Table 1. Inhibitory Effect of the MeOH-Eluted Fraction of the MeOH Extract from the Flowers of *B. perennis* on Gastric Emptying in Olive Oil-Loaded Mice

Treatment	Dose (mg/kg, *p.o.*)	*n*	Weight of stomach (g)	Gastric emptying (%)	Inhibition (%)
Control	—	6	0.43±0.03	77.0±3.1	—
MeOH-eluted fraction	100	5	0.53±0.03*	65.4±5.9	15.2
	200	6	0.60±0.02**	32.4±5.6**	58.0
Control	—	6	0.42±0.03	81.4±4.3	—
Escin IIa	50	6	0.59±0.07	44.7±8.2**	45.1
	100	5	0.62±0.06*	33.3±2.4**	59.1

Each value represents the mean±S.E.M. Significantly different from the control: $*p<0.05$, $**p<0.01$.

Table 2. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) of Perennisosides VIII (**1**) and IX (**2**) and Their Desacyl Derivatives (**1a** and **2a**)

Position	**1**	**1a**	**2**	**2a**	Position	**1**	**1a**	**2**	**2a**
1	1.29 (m)	1.35 (m)	1.29 (m)	1.35 (m)	3-*O*-Fuc				
	2.34 (br d, *ca.* 13)	2.37 (br d, *ca.* 12)	2.35 (br d, *ca.* 12)	2.36 (br d, *ca.* 12)	1′	4.82 (d, 7.6)	5.02 (d, 7.6)	4.79 (d, 6.9)	5.06 (d, 7.6)
2	4.74 (m)	4.81 (m)	4.71 (m)	4.80 (m)	2′	4.37 (m)	4.42 (m)	4.32 (m)	4.52 (m)
3	3.99 (br s)	4.32 (br s)	3.99 (br s)	4.31 (br s)	3′	4.00 (m)	4.09 (m)	4.00 (m)	4.11 (dd, 3.1, 9.5)
5	1.46 (m)	1.79 (m)	1.47 (m)	1.77 (m)	4′	4.04 (m)	4.04 (m)	4.02 (m)	4.03 (m)
6	1.26 (m)	1.33 (m)	1.27 (m)	1.31 (m)	5′	3.87 (dq-like)	3.80 (dq-like)	3.85 (m)	3.80 (q-like, *ca.* 6)
	1.77 (m)	1.81 (m)	1.78 (m)	1.85 (m)	6′	1.50 (3H, d, 6.2)	1.50 (3H, d, 6.2)	1.50 (3H, d, 5.8)	1.50 (3H, d, 6.4)
7	1.46 (m)	1.50 (m)	1.48 (m)	1.50 (m)	28-*O*-Glc				
	1.70 (m)	1.71 (m)	1.67 (m)	1.74 (m)	1″	6.12 (d, 7.6)	6.21 (d, 7.6)	6.06 (d, 7.6)	6.17 (d, 7.6)
9	1.75 (m)	1.77 (m)	1.75 (m)	1.82 (m)	2″	4.37 (m)	4.51 (m)	4.37 (m)	4.44 (m)
11	2.02 (m)	2.07 (m)	2.01 (m)	2.06 (m)	3″	4.23 (m)	4.33 (m)	4.20 (m)	4.28 (m)
	2.16 (m)	2.22 (m)	2.16 (m)	2.21 (m)	4″	3.96 (m)	4.42 (m)	3.98 (m)	4.29 (m)
12	5.46 (t-like, *ca.* 3)	5.49 (t-like, *ca.* 3)	5.47 (t-like, *ca.* 3)	5.49 (t-like, *ca.* 3)	5″	4.09 (m)	4.00 (m)	3.98 (m)	3.98 (m)
15	1.47 (m)	1.54 (m)	1.54 (m)	1.51 (m)	6″	4.61 (2H, m)	4.29 (m)	4.63 (2H, m)	4.26 (dd, 5.3, 11.9)
	2.07 (m)	2.07 (m)	2.07 (m)	2.06 (m)			4.38 (m)		4.35 (m)
16	2.05 (m)	2.07 (m)	2.07 (m)	2.06 (m)	6″-*O*-Ac	1.94 (3H, s)		1.97 (3H, s)	
	2.20 (m)	2.21 (m)	2.21 (m)	2.17 (m)	2″-*O*-Rha				
18	3.11 (br d, *ca.* 13)	3.11 (dd, 4.3, 13.7)	3.13 (br d, *ca.* 13)	3.10 (dd, 4.3, 13.8)	1‴	6.34 (br s)	6.48 (br s)	6.26 (br s)	6.45 (br s)
19	1.30 (m)	1.21 (m)	1.29 (m)	1.22 (m)	2‴	4.74 (m)	4.78 (m)	4.71 (m)	4.80 (m)
	1.79 (m)	1.73 (m)	1.80 (m)	1.77 (m)	3‴	4.44 (m)	4.51 (m)	4.43 (m)	4.51 (m)
21	1.13 (m)	1.09 (m)	1.16 (m)	1.09 (m)	4‴	4.27 (m)	4.32 (m)	4.22 (m)	4.31 (m)
	1.35 (m)	1.35 (m)	1.38 (m)	1.32 (m)	5‴	4.41 (m)	4.49 (m)	4.41 (m)	4.48 (m)
22	1.77 (m)	1.79 (m)	1.75 (m)	1.78 (m)	6‴	1.70 (3H, d, 6.1)	1.77 (3H, d, 6.2)	1.68 (3H, d, 6.1)	1.75 (3H, d, 6.1)
	2.04 (m)	1.89 (m)	2.05 (m)	1.88 (m)	3″-*O*-Sugar	(Glc)	(Glc)	(Gal)	(Gal)
23	4.23 (m)	3.61 (m)	4.20 (m)	3.60 (m)	1⁗	5.10 (d, 7.6)	5.18 (d, 7.6)	4.99 (d, 7.6)	5.02 (d, 7.7)
	4.64 (m)	4.35 (m)	4.63 (m)	4.36 (m)	2⁗	4.00 (m)	4.07 (m)	4.51 (m)	4.42 (m)
24	1.20 (3H, s)	1.31 (3H, s)	1.23 (3H, s)	1.31 (3H, s)	3⁗	4.17 (m)	4.21 (m)	4.11 (m)	4.19 (m)
25	1.52 (3H, s)	1.63 (3H, s)	1.54 (3H, s)	1.64 (3H, s)	4⁗	4.40 (m)	4.13 (m)	4.34 (m)	4.48 (m)
26	1.12 (3H, s)	1.21 (3H, s)	1.15 (3H, s)	1.22 (3H, s)	5⁗	4.04 (m)	4.05 (m)	3.98 (m)	4.03 (m)
27	1.27 (3H, s)	1.24 (3H, s)	1.29 (3H, s)	1.24 (3H, s)	6⁗	4.28 (dd, 5.6, 11.3)	4.29 (m)	4.29 (dd, 5.4, 11.3)	4.35 (m)
29	0.89 (3H, s)	0.80 (3H, s)	0.89 (3H, s)	0.79 (3H, s)		4.41 (m)	4.60 (dd, 2.0, 11.6)	4.43 (m)	4.50 (m)
30	0.94 (3H, s)	0.86 (3H, s)	0.94 (3H, s)	0.86 (3H, s)					
23-OAc	2.08 (3H, s)		2.08 (3H, s)						

tified by HPLC using an optical rotation detector.[5,6] The ^{1}H- (Table 2) and ^{13}C-NMR (Table 3) spectra (pyridine-d_5) of **1**, which were assigned by various NMR experiments,[15] showed signals assignable to six methyls [δ 0.89, 0.94, 1.12, 1.20, 1.27, 1.52 (3H each, all s, 29, 30, 26, 24, 27, 25-H$_3$)], a methylene and two methines bearing an oxygen function [δ 3.99 (1H, br s, 3-H), 4.23, 4.64 (1H each, m, 23-H$_2$), 4.74 (1H, m, 2-H)], an olefin [δ 5.46 (1H, t-like, J=*ca.* 3 Hz, 12-H)], a fucopyranosyl [δ 1.50 (3H, d, J=6.2 Hz, Fuc-6′-H$_3$), 4.82 (1H, d, J=7.6 Hz, Fuc-1′-H)], two glucopyranosyl [δ 5.10 (1H, d, J=7.6 Hz, *terminal*-Glc-1⁗-H), 6.12 (1H, d, J=7.6 Hz, *inner*-Glc-1″-H)], and a rhamnopyranosyl moieties [δ 1.70 (3H, d, J=6.1 Hz, Rha-6‴-H$_3$), 6.34 (1H, br s, Rha-1‴-H)] together with two acetyl groups [δ 1.94, 2.08 (3H each, both s, Ac-H$_3$)]. The positions of the sugar parts and the acetyl groups in **1** were clarified on the basis of an heteronuclear multiple bond correlation spectroscopy (HMBC) experiment, which showed long-range correlations between the following proton and carbon pairs as shown in Fig. 1: 23-H$_2$ and the acetyl carbonyl carbon (δ_C 170.6), 1′-H and 3-C (δ_C 82.9), 1″-H and 28-C (δ_C 176.3), 1‴-H and 2″-C (δ_C 75.5), 1⁗-H and 3″-C (δ_C 88.1), and 6″-H$_2$ [δ 4.61 (2H, m)] and the acetyl carbonyl carbon (δ_C 170.5). Comparison of the ^{13}C-NMR data for **1** with those for **1a** revealed acetylation shifts around the 23-position in the aglycon part [**1**: δ_C 41.6 (4-C), 48.5 (5-C), 66.7 (23-C), 14.6 (24-C); **1a**: δ_C 42.9 (4-C), 47.9 (5-C), 65.5 (23-C), 15.1 (24-C)] and the 6″-position in the 28-*O*-*inner*-glucopyranosyl moiety [**1**: δ_C 75.0 (5″-C), 64.0 (6″-C); **1a**: δ_C 78.4 (5″-C), 62.0 (6″-C)]. On the basis of the above-mentioned evidence, the structure of

Table 3. ^{13}C-NMR Data (175 MHz, Pyridine-d_5) of Perennisosides VIII (**1**) and IX (**2**) and Their Desacyl Derivatives (**1a** and **2a**)

Position	1	1a	2	2a	Position	1	1a	2	2a
1	44.0	44.5	44.1	44.5	3-*O*-Fuc				
2	70.1	70.7	70.3	70.7	1′	106.2	106.3	106.5	106.3
3	82.9	83.0	82.9	83.0	2′	72.3	72.6	72.3	72.6
4	41.6	42.9	41.6	42.9	3′	75.2	75.2	75.3	75.2
5	48.5	47.9	48.5	47.9	4′	72.5	72.7	72.5	72.7
6	18.2	18.1	18.2	18.1	5′	71.5	71.5	71.5	71.5
7	33.0	33.1	33.0	33.1	6′	17.2	17.4	17.3	17.4
8	40.1	40.1	40.1	40.1	28-*O*-Glc				
9	48.8	48.6	48.8	48.6	1″	94.2	94.5	94.2	94.4
10	36.9	37.1	37.0	37.1	2″	75.5	75.4	75.6	75.4
11	24.0	24.1	24.0	24.1	3″	88.1	88.8	88.2	88.9
12	122.9	122.9	122.9	122.9	4″	69.4	69.1	69.3	69.2
13	144.0	144.2	144.0	144.3	5″	75.0	78.4	75.1	78.5
14	42.4	42.5	42.4	42.5	6″	64.0	62.0	63.9	62.0
15	28.4	28.6	28.4	28.6	6″-*O*-Ac	170.5		170.5	
16	23.3	23.3	23.3	23.3		20.6		20.7	
17	47.2	47.1	47.2	47.1	2″-*O*-Rha				
18	42.1	42.1	42.1	42.1	1‴	101.2	101.2	101.3	101.3
19	46.4	46.3	46.4	46.4	2‴	72.1	72.4	72.3	72.4
20	30.7	30.7	30.7	30.7	3‴	72.4	72.6	72.5	72.6
21	34.1	34.0	34.1	34.0	4‴	73.7	73.8	73.7	73.8
22	32.3	32.2	32.2	32.2	5‴	70.1	70.1	70.0	70.1
23	66.7	65.5	66.6	65.5	6‴	18.7	18.8	18.7	18.8
24	14.6	15.1	14.6	15.1	3″-*O*-Sugar				
25	17.2	17.3	17.2	17.3	1⁗	104.1	104.0	104.8	104.7
26	17.4	17.6	17.5	17.6	2⁗	74.8	75.1	72.5	72.6
27	25.7	26.0	25.7	26.0	3⁗	78.3	78.5	77.5	77.5
28	176.3	176.3	176.4	176.3	4⁗	71.5	71.6	70.1	70.1
29	33.1	33.1	33.1	33.1	5⁗	78.5	78.7	75.0	75.4
30	23.7	23.7	23.6	23.7	6⁗	62.3	62.4	62.1	62.1
23-*O*-Ac	170.6		170.7						
	20.8		20.9						

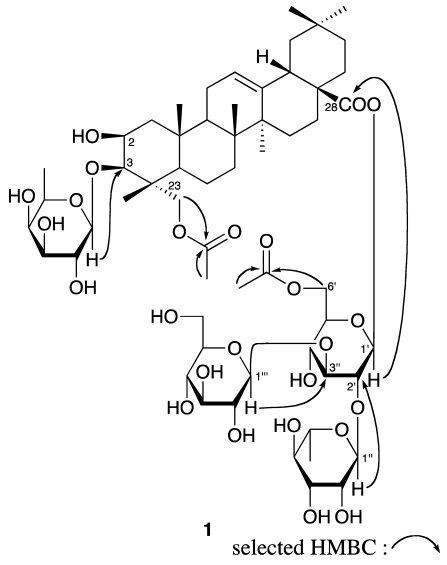

Fig. 1. Selected HMBC Correlations of **1**

perennisoside VIII was determined to be 3-*O*-β-D-fucopyranosyl-23-*O*-acetylbayogenin {28-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-6-*O*-acetyl-β-D-glucopyranosyl} ester (**1**).

Perennisoside IX (**2**) was also obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{27}$ +13.9° in MeOH). The IR spectrum of **2** showed absorption bands at 3440, 1736, 1655, and 1065 cm^{-1}, ascribable to hydroxyl, ester carbonyl, olefin, and ether functions. The molecular formula, $C_{58}H_{92}O_{25}$, of **2** was determined to be the same as that of **1** by high-resolution positive-ion FAB-MS measurement. Treatment of **2** with 0.5% NaOMe–MeOH provided desacyl-perennisoside IX (**2a**). Acid hydrolysis of **2a** with 5% H_2SO_4–1,4-dioxane (1 : 1, v/v) liberated bayogenin together with L-rhamnose, D-fucose, D-glucose, and D-galactose, which were identified by HPLC using an optical rotation detector. The proton and carbon signals in the ^{1}H- (Table 2) and ^{13}C-NMR (Table 3) spectra (pyridine-d_5) of **2** were superimposable on those of **1**, except for the signals due to the β-D-galactopyranosyl part: a bayogenin part {six methyls [δ 0.89, 0.94, 1.15, 1.23, 1.29, 1.54 (3H each, all s, 29, 30, 26, 24, 27, 25-H_3)], a methylene and two methines bearing an oxygen function [δ 3.99 (1H, br s, 3-H), 4.20, 4.63 (1H each, both m, 23-H_2), 4.71 (1H, m, 2-H)], and an olefin [δ 5.47 (1H, t-like, J=*ca.* 3 Hz, 12-H)]}, a fucopyranosyl [δ 1.50 (3H, d, J=5.8 Hz, Fuc-6′-H_3), 4.79 (1H, d, J=6.9 Hz, Fuc-1′-H)], a galactopyranosyl [δ 4.99 (1H, d, J=7.6 Hz, Gal-1⁗-H)], a rhamnopyranosyl moieties [δ 1.68 (3H, d, J=6.1 Hz, Rha-6‴-H_3), 6.26 (1H, br s, Rha-1‴-H)] and a glucopyranosyl [δ 6.06 (1H, d, J=7.6 Hz, Glc-1″-H)], together with two acetyl groups [δ 1.97, 2.08 (3H each, both s, Ac-H_3)]. In the HMBC experiment on **2**, long-range correlations were observed between the 1⁗-proton in the galactopyranosyl part and the 3‴-carbon in the glucopyranosyl part (δ_C 88.2). Consequently, the structure of perennisoside IX was determined to be 3-*O*-β-D-fucopyranosyl-23-*O*-acetylbayogenin {28-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-galactopyranosyl(1→3)]-

Table 4. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) of Perennisosides X (**3**) and XI (**4**) and Their Desacyl Derivatives (**3a** and **4a**)

Position	3	3a	4	4a	Position	3	3a	4	4a
1	1.17 (m)	1.20 (m)	1.16 (m)	1.19 (m)	3-*O*-Glc				
	2.26 (br d, *ca.* 13)	2.26 (br d, *ca.* 13)	2.26 (br d, *ca.* 13)	2.24 (br d, *ca.* 14)	1′	4.98 (d, 8.0)	5.15 (d, 7.6)	4.98 (d, 7.6)	5.12 (d, 7.6)
2	4.78 (m)	4.78 (m)	4.78 (m)	4.78 (m)	2′	4.01 (m)	4.01 (m)	4.02 (m)	4.02 (m)
3	4.02 (br s)	4.35 (br s)	4.02 (br s)	4.32 (br s)	3′	4.18 (m)	4.05 (m)	4.17 (m)	4.04 (m)
5	1.49 (m)	1.79 (m)	1.50 (m)	1.78 (m)	4′	4.05 (m)	4.09 (m)	4.06 (m)	4.09 (m)
6	1.28 (m)	1.30 (m)	1.28 (m)	1.32 (m)	5′	3.94 (m)	3.86 (m)	3.94 (m)	3.81 (m)
	1.78 (m)	1.79 (m)	1.80 (m)	1.79 (m)	6′	4.22 (m)	4.25 (m)	4.26 (m)	4.24 (m)
7	1.61 (m)	1.62 (m)	1.62 (m)	1.62 (m)		4.44 (m)	4.39 (m)	4.43 (m)	4.37 (m)
	1.70 (m)	1.75 (m)	1.70 (m)	1.72 (m)	3′-*O*-Glc				
9	1.70 (m)	1.72 (m)	1.70 (m)	1.72 (m)	1″	5.22 (d, 7.9)	5.23 (d, 7.9)	5.22 (d, 7.8)	5.22 (d, 7.9)
11	2.03 (m)	2.00 (m)	2.04 (m)	2.01 (m)	2″	4.02 (m)	4.05 (m)	4.06 (m)	4.05 (m)
	2.17 (m)	2.13 (m)	2.15 (m)	2.13 (m)	3″	4.20 (m)	4.26 (m)	4.22 (m)	4.26 (m)
12	5.48 (t-like, *ca.* 3)	5.48 (t-like, *ca.* 3)	5.48 (t-like, *ca.* 3)	5.46 (t-like, *ca.* 3)	4″	4.20 (m)	4.20 (m)	4.20 (m)	4.20 (m)
15	1.49 (m)	1.54 (m)	1.50 (m)	1.52 (m)	5″	4.20 (m)	4.01 (m)	4.22 (m)	4.03 (m)
	2.04 (m)	2.15 (m)	2.05 (m)	2.12 (m)	6″	4.22 (m)	4.22 (m)	4.22 (m)	4.24 (m)
16	2.06 (m)	2.03 (m)	2.06 (m)	2.03 (m)		4.60 (m)	4.53 (m)	4.63 (m)	4.54 (m)
	2.22 (m)	2.16 (m)	2.17 (m)	2.14 (m)	28-*O*-Glc				
18	3.14 (dd, 4.3, 13.4)	3.10 (dd, 4.1, 13.7)	3.13 (dd, 4.1, 13.7)	3.07 (br d, *ca.* 13)	1‴	6.15 (d, 7.3)	6.21 (d, 7.6)	6.10 (d, 7.6)	6.14 (d, 7.6)
19	1.28 (m)	1.20 (m)	1.28 (m)	1.19 (m)	2‴	4.46 (m)	4.50 (m)	4.39 (dd, 7.6, 8.9)	4.45 (m)
	1.80 (m)	1.71 (m)	1.80 (dd, 13.7, 13.7)	1.72 (m)	3‴	4.26 (m)	4.35 (m)	4.23 (m)	4.29 (m)
21	1.15 (m)	1.07 (m)	1.15 (m)	1.04 (m)	4‴	4.05 (m)	4.39 (m)	4.03 (m)	4.28 (m)
	1.36 (m)	1.29 (m)	1.37 (m)	1.30 (m)	5‴	4.05 (m)	4.00 (m)	4.03 (m)	3.95 (m)
22	1.80 (m)	1.86 (m)	1.80 (m)	1.85 (m)	6‴	4.65 (2H, m)	4.28 (m)	4.65 (2H, m)	4.25 (m)
	2.03 (m)	2.04 (m)	2.04 (m)	2.04 (m)			4.38 (m)		4.37 (m)
23	4.28 (m)	3.61 (m)	4.30 (m)	3.60 (m)	6‴-*O*-Ac	1.95 (s)		1.98 (3H, s)	
	4.66 (m)	4.32 (m)	4.65 (m)	4.32 (m)	2‴-*O*-Rha				
24	1.28 (3H, s)	1.32 (3H, s)	1.28 (3H, s)	1.30 (3H, s)	1⁗	6.39 (br s)	6.47 (br s)	6.36 (br s)	6.43 (br s)
25	1.55 (3H, s)	1.61 (3H, s)	1.55 (3H, s)	1.60 (3H, s)	2⁗	4.77 (m)	4.78 (m)	4.78 (m)	4.77 (br s)
26	1.15 (3H, s)	1.20 (3H, s)	1.16 (3H, s)	1.19 (3H, s)	3⁗	4.50 (m)	4.51 (m)	4.49 (m)	4.49 (m)
27	1.29 (3H, s)	1.23 (3H, s)	1.29 (3H, s)	1.21 (3H, s)	4⁗	4.28 (m)	4.34 (m)	4.28 (m)	4.28 (m)
29	0.88 (3H, s)	0.79 (3H, s)	0.88 (3H, s)	0.76 (3H, s)	5⁗	4.44 (m)	4.50 (m)	4.48 (m)	4.45 (m)
30	0.92 (3H, s)	0.85 (3H, s)	0.91 (3H, s)	0.82 (3H, s)	6⁗	1.72 (3H, d, 6.1)	1.76 (3H, d, 6.2)	1.71 (3H, d, 6.2)	1.72 (3H, d, 6.2)
23-OAc	2.10 (3H, s)		2.10 (3H, s)		3‴-*O*-Sugar	(Glc)	(Glc)	(Gal)	(Gal)
					1⁗	5.14 (d, 7.6)	5.17 (d, 7.9)	5.03 (d, 7.6)	5.00 (d, 7.6)
					2⁗	4.05 (m)	4.06 (m)	4.51 (m)	4.50 (m)
					3⁗	3.96 (m)	4.21 (m)	4.20 (m)	4.16 (m)
					4⁗	4.20 (m)	4.13 (m)	4.45 (m)	4.48 (m)
					5⁗	4.05 (m)	4.07 (m)	4.09 (m)	4.09 (m)
					6⁗	4.31 (m)	4.26 (m)	4.35 (dd, 4.8, 11.0)	4.35 (m)
						4.50 (m)	4.60 (m)	4.50 (m)	4.54 (m)

6-*O*-acetyl-β-D-glucopyranosyl} ester (**2**).

Perennisosides X (**3**) and XI (**4**) were isolated as amorphous powders with positive optical rotations (**3**: $[\alpha]_D^{25}$ +11.3°, **4**: $[\alpha]_D^{25}$ +31.2° both in MeOH). In the positive- and negative-ion FAB-MS of **3** and **4**, common quasimolecular ion peaks were observed at *m/z* 1389 $(M+Na)^+$ and *m/z* 1365 $(M-H)^-$, and high-resolution positive-ion FAB-MS revealed the molecular formula to be $C_{64}H_{102}O_{31}$. Treatments of **3** and **4** with 0.5% NaOMe–MeOH provided desacylperennisosides X (**3a**) and XI (**4a**), respectively. Acid hydrolysis of **3a** and **4a** with 5% H_2SO_4–1,4-dioxane (1 : 1, v/v) liberated bayogenin together with L-rhamnose, D-fucose, and D-glucose (from **3a** and **4a**), and D-galactose (from **4a**). The proton and carbon signals in the ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectra (pyridine-d_5) of **3** were very similar to those of **1**, except for the signals due to the 3-*O*-glycosyl moiety [δ 4.98 (1H, d, J=8.0 Hz, 3-*O*-*inner*-Glc-1′-H), 5.22 (1H, d, J=7.9 Hz, 3-*O*-*terminal*-Glc-1″-H)]. In the HBMC experiment of **3**, long-range correlations were observed between the 1′-proton in the 3-*O*-*inner*-glucopyranosyl part and the 3-carbon in the aglycon part (δ_C 83.2) and between the 1″-proton in the 3-*O*-*terminal*-glucopyranosyl part and the 3′-carbon in the 3-*O*-*inner*-glucopyranosyl part (δ_C 88.8). On the other hand, the proton and carbon signals in the ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectra (pyridine-d_5) of **4** resembled those of **3** except for the signals due to β-D-galactopyranosyl part [δ 5.03 (1H, d, J=7.6 Hz, Gal-1‴-H)]. Thus, the structures of perennisosides X and XI were determined to be 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-23-*O*-acetylbayogenin {28-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-6-*O*-acetyl-β-D-glucopyranosyl} ester (**3**) and 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-23-*O*-acetylbayogenin {28-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-galactopyranosyl(1→3)]-6-*O*-acetyl-β-D-glucopyranosyl} ester (**4**), respectively.

Perennisoside XII (**5**) was obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{27}$ +0.9° in MeOH). The positive- and negative-ion FAB-MS of **5** showed quasimolecular ion peaks at *m/z* 1143 $(M+Na)^+$ and *m/z* 1119 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **5** revealed the molecular formula to be $C_{54}H_{88}O_{24}$. The IR spectrum of **5** showed absorption bands at 3440, 1736, 1655, and 1075 cm^{-1}, ascribable to hydroxyl, ester carbonyl, olefin, and ether functions. The acid hydroly-

Table 5. ^{13}C-NMR Data (175 MHz, Pyridine-d_5) of Perennisosides X (**3**) and XI (**4**) and Their Desacyl Derivatives (**3a** and **4a**)

Position	3	3a	4	4a	Position	3	3a	4	4a
1	43.9	44.2	44.0	44.4	3-*O*-Glc				
2	70.3	70.8	70.3	70.9	1′	105.7	105.5	105.7	105.6
3	83.2	83.1	83.2	82.8	2′	74.0	74.0	74.0	74.1
4	41.7	42.9	41.7	42.9	3′	88.8	88.8	88.8	88.8
5	48.4	47.7	48.4	47.7	4′	69.7	69.6	69.7	69.7
6	18.2	18.2	18.2	18.2	5′	78.0	77.9	78.0	77.9
7	33.0	33.1	33.0	33.1	6′	62.4	62.5	62.4	62.5
8	40.1	40.1	40.1	40.1	3′-*O*-Glc				
9	48.7	48.3	48.7	48.6	1″	105.9	106.0	105.9	106.1
10	36.9	37.0	37.0	37.0	2″	75.5	75.6	75.5	75.6
11	24.0	24.0	24.0	24.1	3″	78.3	78.3	78.3	78.3
12	122.8	122.8	122.8	122.8	4″	71.5	71.6	71.5	71.6
13	144.1	144.1	144.1	144.1	5″	78.5	78.8	78.6	78.8
14	42.4	42.5	42.4	42.5	6″	62.3	62.4	62.3	62.4
15	28.4	28.6	28.4	28.7	28-*O*-Glc				
16	23.3	23.3	23.3	23.3	1‴	94.3	94.2	94.3	94.4
17	47.2	47.1	47.2	47.1	2‴	75.2	75.2	75.4	75.4
18	42.1	42.1	42.1	42.1	3‴	88.3	88.8	88.3	88.9
19	46.4	46.3	46.4	46.3	4‴	69.4	69.2	69.3	69.2
20	30.7	30.7	30.7	30.7	5‴	74.9	78.4	75.0	78.5
21	34.1	34.1	34.1	34.1	6‴	64.1	61.9	63.9	62.1
22	32.3	32.3	32.3	32.3	6‴-*O*-Ac	170.6		170.6	
23	66.5	64.9	66.5	65.0		20.7		20.7	
24	14.6	15.1	14.6	15.1	2‴-*O*-Rha				
25	17.2	17.4	17.2	17.4	1⁗	101.3	101.2	101.3	101.3
26	17.5	17.6	17.5	17.6	2⁗	72.3	72.4	72.3	72.4
27	25.7	26.0	25.7	26.0	3⁗	72.5	72.5	72.5	72.6
28	176.4	176.3	176.4	176.3	4⁗	73.7	73.8	73.7	73.8
29	33.1	33.1	33.1	33.1	5⁗	70.2	70.1	70.0	70.1
30	23.7	23.7	23.6	23.7	6⁗	18.8	18.8	18.8	18.8
23-*O*-Ac	170.7		170.7		3‴-*O*-Sugar	(Glc)	(Glc)	(Gal)	(Gal)
	20.9		20.9		1⁗	104.2	104.1	104.8	104.7
					2⁗	75.1	75.1	72.5	72.6
					3⁗	78.6	78.5	77.5	77.5
					4⁗	71.5	71.6	70.1	70.1
					5⁗	78.7	78.7	75.1	75.2
					6⁗	62.4	62.2	62.1	62.2

sis of **5** liberated bayogenin together with L-rhamnose, D-glucose, and D-galactose. The ^{1}H- and ^{13}C-NMR (Table 6) spectra (pyridine-d_5) of **5** showed signals assignable to six methyls [δ 0.84, 0.87, 1.22, 1.23, 1.34, 1.66 (3H each, all s, 29, 30, 27, 26, 24, 25-H$_3$)], a methylene and two methines bearing an oxygen function [δ 3.62, 4.11 (1H each, d, J=10.4 Hz, 23-H$_2$), 4.22 (1H, br s, 3-H), 4.53 (1H, m, 2-H)], an olefin [δ 5.47 (1H, t-like, J=*ca.* 3 Hz, 12-H)], two glucopyranosyl [δ 4.95 (1H, d, J=7.7 Hz, *terminal*-Glc-1⁗-H), 6.06 (1H, d, J=7.6 Hz, *inner*-Glc-1′-H)], a galactopyranostyl [δ 5.02 (1H, d, J=8.0 Hz, Gal-1‴-H)], and a rhamnopyranosyl moieties [δ 1.73 (3H, d, J=6.4 Hz, Rha-6″-H$_3$), 6.35 (1H, br s, Rha-1″-H)]. The positions of the sugar parts in **5** were clarified on the basis of an HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: 1′-H and 28-C (δ_C 176.5), 1″-H and 2′-C (δ_C 75.1), 1‴-H and 3′-C (δ_C 88.2), and 1⁗-H and 6′-C (δ_C 69.3). Consequently, the structure of perennisoside XII was determined to be bayogenin {28-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-galactopyranosyl(1→3)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl} ester (**5**).

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL ECA-600 (600 MHz) and JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL ECA-600 (150 MHz) and JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Japan, 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F$_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO$_4$)$_2$–10% aqueous H$_2$SO$_4$ followed by heating.

Plant Material The flowers of *B. perennis*, which were cultivated in Albania, were imported in May 2001, and were purchased *via* Tochimoto Tenkaido Co., Ltd, Osaka, Japan in November 2006 as described previously.[5]

Extraction and Isolation The MeOH-eluted fraction (140.0 g) from the methanolic extract of the dried flowers of *B. perennis* was subjected to normal-phase silica gel column chromatography [3.0 kg, CHCl$_3$–MeOH–H$_2$O (20 : 3 : 1→10 : 3 : 1→7 : 3 : 1, v/v/v, lower layer→6 : 4 : 1)→MeOH] to give eight fractions [Fr. 1 (0.85 g), Fr. 2 (5.67 g), Fr. 3 (2.41 g), Fr. 4 (1.24 g), Fr. 5 (7.73 g), Fr. 6 (96.05 g), Fr. 7 (10.11 g), and Fr. 8 (16.09 g)]. The fraction 6 (96.05 g) was subjected to reversed-phase silica gel column chromatography [1.5 kg, MeOH–H$_2$O (30 : 70→40 : 60→50 : 50→70 : 30, v/v)→MeOH] to afford 15 fractions [Fr. 6-1 (1.398 g), Fr. 6-2 (3.418 g), Fr. 6-3 (1.148 g), Fr. 6-4 (1.290 g), Fr. 6-5 (0.800 g), Fr. 6-6 (3.179 g), Fr. 6-7 (1.680 g), Fr. 6-8 (2.317 g), Fr. 6-9 (1.216 g), Fr. 6-10 (1.682 g), Fr. 6-11 (4.850 g), Fr. 6-12

Table 6. ^{1}H- and ^{13}C-NMR Data (600 and 175 MHz, Pyridine-d_5) of Perennisoside XII (**5**)

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.27 (m)	45.0	28-*O*-Glc		
	2.37 (br d, *ca.* 14)		1′	6.06 (d, 7.6)	94.3
2	4.53 (m)	71.6	2′	4.30 (m)	75.1
3	4.22 (br s)	73.2	3′	4.21 (m)	88.2
4		42.5	4′	4.28 (m)	68.8
5	1.76 (m)	48.3	5′	4.11 (m)	77.2
6	1.28 (m)	18.3	6′	4.17 (m)	69.3
	1.80 (m)			4.55 (br d, *ca.* 11)	
7	1.75 (m)	33.1	2′-*O*-Rha		
	1.98 (m)		1″	6.35 (br s)	101.3
8		40.1	2″	4.75 (br s)	72.3
9	1.66 (m)	48.6	3″	4.47 (m)	72.5
10		37.2	4″	4.28 (m)	73.7
11	2.04 (m)	24.0	5″	4.45 (m)	70.0
	2.19 (m)		6″	1.73 (3H, d, 6.4)	18.8
12	5.47 (t-like, *ca.* 3)	122.8	3′-*O*-Gal		
13		144.2	1‴	5.02 (d, 8.0)	104.6
14		42.4	2‴	4.45 (m)	72.5
15	1.55 (m)	28.7	3‴	4.11 (m)	77.4
	2.03 (m)		4‴	4.45 (m)	70.0
16	2.00 (m)	23.3	5‴	4.08 (dd-like)	75.1
	2.14 (m)		6‴	4.31 (m)	62.0
17		47.2		4.53 (m)	
18	3.12 (dd, 3.4, 13.8)	42.0	6′-*O*-Glc		
19	1.22 (m)	46.4	1⁗	4.95 (d, 7.7)	105.4
	1.74 (m)		2⁗	3.99 (dd-like)	75.2
20		30.7	3⁗	4.21 (m)	78.3
21	1.15 (m)	34.1	4⁗	4.21 (m)	71.4
	1.32 (m)		5⁗	3.85 (m)	78.3
22	1.81 (m)	32.2	6⁗	4.35 (m)	62.5
	1.98 (m)			4.47 (m)	
23	3.62 (d, 10.4)	67.8			
	4.11 (d, 10.4)				
24	1.34 (3H, s)	14.6			
25	1.66 (3H, s)	17.5			
26	1.23 (3H, s)	17.6			
27	1.22 (3H, s)	25.9			
28		176.5			
29	0.84 (3H, s)	33.1			
30	0.87 (3H, s)	23.8			

(50.269 g), Fr. 6-13 (13.375 g), Fr. 6-14 (2.208 g), and Fr. 6-15 (1.888 g)]. The fraction 6-14 (946.3 mg) was separated by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to afford six fractions [Fr. 6-14-1 (=perennisoside II, 27.5 mg, 0.0110%), Fr. 6-14-2 (91.1 mg), Fr. 6-14-3 (162.7 mg), Fr. 6-14-4 (110.2 mg), Fr. 6-14-5 (174.5 mg), and Fr. 6-14-6 (101.5 mg)] as reported previously.[5] The fraction 6-14-2 (91.1 mg) was purified by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–1% aqueous AcOH (37 : 63, v/v)] to give perennnisoside IX (**2**, 75.7 mg, 0.0076%). The fraction 6-14-3 (162.7 mg) was purified by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–1% aqueous AcOH (37 : 63, v/v)] to give perennisoside VIII (**1**, 123.6 mg, 0.0124%). The fraction 7 (10.11 g) was subjected to reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→50 : 50→70 : 30, v/v)→MeOH] to afford nine fractions [Fr. 7-1 (796.8 mg), Fr. 7-2 (2520.6 mg), Fr. 7-3 (641.1 mg), Fr. 7-4 (713.4 mg), Fr. 7-5 (1910.7 mg), Fr. 7-6 (3098.7 mg), Fr. 7-7 (257.8 mg), Fr. 7-8 (286.5 mg), and Fr. 7-9 (361.2 mg)] as reported previously.[5] The fraction 7-5 (1910.7 mg) was separated by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–MeOH–H_2O (26 : 16 : 58, v/v/v)] to afford 10 fractions {Fr. 7-5-1 (174.1 mg), Fr. 7-5-2 [=perennisoside XII (**5**, 150.2 mg, 0.0064%)], Fr. 7-5-3 (272.3 mg), Fr. 7-5-4 (228.7 mg), Fr. 7-5-5 (135.4 mg), Fr. 7-5-6 (87.1 mg), Fr. 7-5-7 (70.7 mg), Fr. 7-5-8 (136.4 mg), Fr. 7-5-9 (68.6 mg), and Fr. 7-5-10 (153.9 mg)}. The fraction 7-6 (450.5 mg) was further purified by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–MeOH–H_2O (30 : 16 : 54, v/v/v)] to furnish perennisosides X (**3**, 42.4 mg, 0.0125%) and XI (**4**, 44.7 mg, 0.0132%) together with perennisosides V (14.2 mg, 0.0042%) and VI (20.4 mg, 0.0060%) and bellissaponin BS1 (11.9 mg, 0.0035%).[5] The fraction 8 (16.09 g) was subjected to reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→50 : 50→70 : 30, v/v)→MeOH] to afford nine fractions [Fr. 8-1 (3977.2 mg), Fr. 8-2 (759.6 mg), Fr. 8-3 (774.2 mg), Fr. 8-4 (5033.2 mg), Fr. 8-5 (427.2 mg), Fr. 8-6 (946.7 mg), Fr. 8-7 (2280.8 mg), Fr. 8-8 (2189.0 mg), and Fr. 8-9 (710.1 mg)] as reported previously.[5] The fraction 8-7 (960.0 mg) was separated by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–MeOH–H_2O (22 : 16 : 62, v/v)] to afford nine fractions {Fr. 8-7-1 (38.6 mg), Fr. 8-7-2 [=bellissaponin BS6 (43.6 mg, 0.0044%)], Fr. 8-7-3 (52.7 mg), Fr. 8-7-4 (39.4 mg), Fr. 8-7-5 (57.6 mg), Fr. 8-7-6 (133.1 mg), Fr. 8-7-7 (126.6 mg), Fr. 8-7-8 (119.9 mg), and Fr. 8-7-9 [=perennisoside XII (**5**, 46.7 mg, 0.0047%)]}.[5]

Perennisoside VIII (**1**): An amorphous powder, $[\alpha]_D^{27}$ +11.9° (c=3.09, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{58}H_{92}O_{25}Na$ $(M+Na)^+$: 1211.5825. Found: 1211.5833. IR (KBr): 3440, 1744, 1655, 1256, 1069 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS m/z: 1211 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1187 $(M-H)^-$, 1041 $(M-C_6H_{11}O_4)^-$, 1025 $(M-C_6H_{11}O_5)^-$, 879 $(M-C_{12}H_{21}O_9)^-$, 675 $(M-C_{20}H_{33}O_{15})^-$, 529 $(M-C_{26}H_{43}O_{19})^-$.

Perennisoside IX (**2**): An amorphous powder, $[\alpha]_D^{27}$ +13.9° (c=3.79, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{58}H_{92}O_{25}Na$ $(M+Na)^+$: 1211.5825. Found: 1211.5819. IR (KBr): 3440, 1736, 1655, 1256, 1065 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS m/z: 1211 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1187 $(M-H)^-$, 1041 $(M-C_6H_{11}O_4)^-$, 1025 $(M-C_6H_{11}O_5)^-$, 879 $(M-C_{12}H_{21}O_9)^-$, 675 $(M-C_{20}H_{33}O_{15})^-$, 529 $(M-C_{26}H_{43}O_{19})^-$.

Perennisoside X (**3**): An amorphous powder, $[\alpha]_D^{25}$ +11.3° (c=1.97, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{64}H_{102}O_{31}Na$ $(M+Na)^+$: 1389.6303. Found: 1389.6296. IR (KBr): 3440, 1736, 1656, 1256, 1077 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 4. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 5. Positive-ion FAB-MS m/z: 1389 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1365 $(M-H)^-$, 1203 $(M-C_6H_{11}O_5)^-$, 1041 $(M-C_{12}H_{21}O_{10})^-$, 733 $(M-C_{24}H_{41}O_{19})^-$, 691 $(M-C_{26}H_{43}O_{20})^-$, 529 $(M-C_{32}H_{53}O_{25})^-$.

Perennisoside XI (**4**): An amorphous powder, $[\alpha]_D^{25}$ +31.2° (c=2.98, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{64}H_{102}O_{31}Na$ $(M+Na)^+$: 1389.6303. Found: 1389.6298. IR (KBr): 3445, 1736, 1656, 1251, 1075 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 4. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 5. Positive-ion FAB-MS m/z: 1389 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1365 $(M-H)^-$, 1203 $(M-C_6H_{11}O_5)^-$, 1041 $(M-C_{12}H_{21}O_{10})^-$, 733 $(M-C_{24}H_{41}O_{19})^-$, 691 $(M-C_{26}H_{43}O_{20})^-$, 529 $(M-C_{32}H_{53}O_{25})^-$.

Perennisoside XII (**5**): An amorphous powder, $[\alpha]_D^{25}$ +0.9° (c=2.53, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{88}O_{24}Na$ $(M+Na)^+$: 1143.5563. Found: 1143.5569. IR (KBr): 3440, 1736, 1655, 1260, 1075 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 6. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 6. Positive-ion FAB-MS m/z: 1143 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1119 $(M-H)^-$, 957 $(M-C_6H_{11}O_5)^-$, 811 $(M-C_{12}H_{21}O_9)^-$, 649 $(M-C_{18}H_{31}O_{14})^-$, 487 $(M-C_{24}H_{41}O_{19})^-$.

Deacylation of Perennisosides VIII (1), IX (2), X (3), and XI (4) A solution of perennisoside VIII (**1**, 12.2 mg) in 0.5% NaOMe–MeOH (1.0 ml) was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure, which was purified by HPLC [Cosmosil 5C_{18}-MS-II, MeOH–H_2O (70 : 30, v/v)] to furnish desacyl-perennisoside VIII (**1a**, 9.6 mg, 84.7%). Using the same procedure, desacyl-perennisosides IX (**2a**, 12.0 mg, 97.8%), X (**3a**, 9.8 mg, 92.4%), and XI (**4a**, 8.6 mg, 84.0%) were prepared from perennisosides IX (**2**, 13.2 mg), X (**3**, 11.5 mg), and XI (**4**, 10.9 mg), respectively.

Desacyl-perennisoside VIII (**1a**): An amorphous powder, $[\alpha]_D^{27}$ +5.5° (c=0.74, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{88}O_{23}Na$ $(M+Na)^+$: 1127.5614. Found: 1127.5609. IR (KBr): 3445, 1736, 1655, 1256, 1067 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS m/z: 1127 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1103 $(M-H)^-$, 957 $(M-C_6H_{11}O_4)^-$, 941 $(M-C_6H_{11}O_5)^-$, 795 $(M-C_{12}H_{23}O_9)^-$, 633 $(M-C_{18}H_{31}O_{14})^-$, 487 $(M-C_{24}H_{41}O_{18})^-$.

Desacyl-perennisoside IX (**2a**): An amorphous powder, $[\alpha]_D^{26}$ +9.0° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{88}O_{23}Na$ $(M+Na)^+$: 1127.5614. Found: 1127.5619. IR (KBr): 3445, 1736, 1655, 1256, 1065 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS m/z: 1127 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1103

$(M-H)^-$, 957 $(M-C_6H_{11}O_4)^-$, 941 $(M-C_6H_{11}O_5)^-$, 795 $(M-C_{12}H_{23}O_9)^-$, 633 $(M-C_{18}H_{31}O_{14})^-$, 487 $(M-C_{24}H_{41}O_{18})^-$.

Desacyl-perennisoside X (**3a**): An amorphous powder, $[\alpha]_D^{26}$ +3.8° (c=0.88, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{98}O_{29}Na$ $(M+Na)^+$: 1305.6091. Found: 1305.6086. IR (KBr): 3440, 1736, 1655, 1230, 1075 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 4. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 5. Positive-ion FAB-MS m/z: 1305 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1281 $(M-H)^-$, 1119 $(M-C_6H_{11}O_5)^-$, 957 $(M-C_{12}H_{21}O_{10})^-$, 811 $(M-C_{18}H_{31}O_{14})^-$, 649 $(M-C_{24}H_{41}O_{19})^-$, 487 $(M-C_{30}H_{51}O_{24})^-$.

Desacyl-perennisoside XI (**4a**): An amorphous powder, $[\alpha]_D^{25}$ +4.0° (c=0.29, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{98}O_{29}Na$ $(M+Na)^+$: 1305.6091. Found: 1305.6085. IR (KBr): 3440, 1736, 1655, 1260, 1051 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 4. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 5. Positive-ion FAB-MS m/z: 1305 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1281 $(M-H)^-$, 1119 $(M-C_6H_{11}O_5)^-$, 957 $(M-C_{12}H_{21}O_{10})^-$, 811 $(M-C_{18}H_{31}O_{14})^-$, 649 $(M-C_{24}H_{41}O_{19})^-$, 487 $(M-C_{30}H_{51}O_{24})^-$.

Acid Hydrolysis of 1a—4a and Perennisoside XII (5) Solutions of **1a** (5.2 mg), **2a** (5.5 mg), **3a** (4.0 mg), **4a** (3.0 mg), and **5** (1.6 mg) in 5% H_2SO_4–1,4-dioxane (1 : 1, v/v, 1.0 ml) were heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. On removal of the solvent from the filtrate under reduced pressure, the residue was partitioned in an EtOAc–H_2O (1 : 1, v/v) mixture, and the solvent was removed *in vacuo* from the EtOAc-soluble fraction and as aqueous phase. The EtOAc-soluble fraction was purified by HPLC [Cosmosil 5C_{18}-MS-II, MeOH–H_2O (80 : 20, v/v)] to furnish bayogenin[5,13,14] (1.2 mg, 52.2% from **1a**, 1.2 mg, 50.0% from **2a**, 1.0 mg, 65.8% from **3a**, 0.7 mg, 61.4%, from **4a**, and 0.5 mg, 71.7% from **5**), respectively. On the other hand, the aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate 0.5 ml/min]. Identification of L-rhamnose (**i**) from **1a**—**4a** and **5**, D-fucose (**ii**) from **1a** and **2a**, D-glucose (**iii**) from **1a**—**4a** and **5**, and D-galactose (**iv**) from **2a**, **4a**, and **5** present in the aqueous layer was carried out by comparison of their retention time and optical rotation with those of authentic samples. t_R: (**i**) 12.0 min (negative optical rotation), (**ii**) 15.5 min (positive optical rotation, (**iii**) 20.7 min (positive optical rotation), and (**iv**) 22.2 min (positive optical rotation).

Animals Male ddY mice were purchased from Kiwa Laboratory Animal Co., Ltd. (Wakayama, Japan). The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 20—24 h prior to the beginning of experiments, but were allowed free access to tap water. All experiments were performed using conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Effect on Gastric Emptying in Olive Oil-Loaded Mice Gastric emptying was determined by a modification of the phenol red method.[16] Briefly, each test sample suspended in 5% (w/v) acacia solution (10 ml/kg) was administrated orally to fasted mice (*ca.* 30 g), and olive oil (0.15 ml/mouse) containing 0.05% phenol red as a marker was given orally 30 min thereafter. Two hours later, the mice were sacrificed by cervical dislocation under ether anesthesia. The abdominal cavity was opened, and the gastroesophageal junction and pylorus were clamped, then the stomach was removed, weighted, and placed in 10 ml of 0.1 M NaOH and homogenized. The suspension was allowed to settle for 1 h at room temperature, 1 ml of the supernatant was added to 0.1 ml of 20% (w/v) trichloroacetic acid, and then the mixture was centrifuged at 3000 rpm for 20 min. The supernatant (0.1 ml) was mixed with 0.1 ml of 0.5 M NaOH, and the amount of phenol red was determined from the optical density (OD) at 560 nm using a microplate reader (SH-1000 Lab., Corona Electric Co., Ltd.). Escin IIa was used as a reference compound.[17—19] Gastric emptying (%) in the 30 min period was calculated according to the following equation:

$$\text{gastric emptying (\%)} = (1 - \text{amount of test sample/amount of standard}) \times 100$$

Statistics Values are expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

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