Perennisosides I−VII, Acylated Triterpene Saponins with Antihyperlipidemic Activities from the Flowers of *Bellis perennis*

Toshio Morikawa,[†,‡] Xuezheng Li,[†] Eriko Nishida,[‡] Yuki Ito,[†] Hisashi Matsuda,[†] Seikou Nakamura,[†] Osamu Muraoka,[‡] and Masayuki Yoshikawa*[,†]

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan, and Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

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The methanolic extract and its saponin fraction (methanol-eluted fraction) of the flowers of *Bellis perennis* were found to suppress serum triglyceride elevation in olive oil-treated mice. From the saponin fraction, seven new triterpene saponins, perennisosides I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**), and VII (**7**), were isolated together with four known saponins, bellidioside A (**8**), asterbatanoside D (**9**), bernardioside B_2 (**10**), and bellissaponin BS6 (**11**). The stereostructures of **1**−**7** were elucidated on the basis of chemical and spectroscopic evidence. Among these saponins, perennisosides I (**1**) and II (**2**) showed inhibitory effects on serum triglyceride elevation at doses of 25−50 mg/kg, po.

The Asteraceae plant *Bellis perennis* is distributed widely in Europe and North Africa, and the whole flowering plant has been used for bruises, bleeding, muscular pain, purulent skin diseases, and rheumatism in European folk medicine.[2] This herbal medicine is also well-known as an ornamental plant, and its flowers and young leaves are edible as a salad. The chemical constituents of the roots and flowers of *B. perennis*, several triterpene saponins,[3–11] anthocyanins,[12] flavonoids,[13] and polyacetylenes,[14,15] have been reported. During the course of characterization studies on medicinal flowers,[1] we found that a methanol extract and its saponin fraction of the flowers of *B. perennis* showed an inhibitory effect on serum triglyceride (TG) elevation in olive oil-treated mice. From this saponin fraction, seven new acylated triterpene saponins named perennisosides I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**), and VII (**7**) were isolated together with four known saponins, bellidioside A (**8**),[16] asterbatanoside D (**9**),[17] bernardioside B_2 (**10**),[18] and bellissaponin BS6 (**11**).[8] This paper deals with the structure elucidation of these seven new saponins (**1**−**7**) as well as the antihyperlipidemic activities of **1** and **2**.

Results and Discussion

The flowers of *B. perennis* cultivated in Albania were extracted with methanol to give a methanolic extract (25.8% from the dried flowers). As shown in Table S1 (Supporting Information), the methanolic extract significantly suppressed serum TG elevation 2 h after administration of olive oil at a dose of 500 mg/kg, po. 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. The MeOH-eluted fraction (saponin fraction) significantly suppressed serum TG elevation 2 h after administration of olive oil at a dose of 200 mg/kg, po, and its activity was more potent than that of a hypolipidemic drug, clofibrate. This fraction was subjected to normal- and reversed-phase column chromatographies and finally HPLC to give perennisosides I (**1**, 0.012%), II (**2**, 0.011%), III (**3**, 0.0026%), IV (**4**, 0.0012%), V (**5**, 0.0042%), VI (**6**, 0.0060%), and VII (**7**, 0.0089%), bellidioside A (**8**, 0.058%), asterbatanoside D (**9**, 0.0063%), bernardioside B_2 (**10**, 0.013%), and bellissaponin BS6 (**11**, 0.023%).

Perennisoside I (**1**) was obtained as an amorphous powder with a positive optical rotation ($[\alpha]^{26}_D$ +16.1 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 3442 and 1075 cm^{-1} suggestive of an oligoglycoside structure. In the positive- and negative-ion FABMS of **1**, quasimolecular ion peaks were observed at *m/z* 1065 $[M + Na]^+$ and 1041 $[M - H]^-$, and HRFABMS analysis revealed the molecular formula to be $C_{52}H_{82}O_{21}$. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra (pyridine-d_5) of **1**, which were assigned by various NMR experiments,[19] showed signals assignable to six methyls [δ 0.89, 0.93, 1.15, 1.23, 1.26, 1.56 (3H each, all s, H_3-29, 30, 26, 24, 27, 25)], a methylene and two methines bearing an oxygen function [δ 3.88 (1H, m, H-3), 4.02 (1H, brs, H-2), 4.12, 4.38 (1H each, both d, J = 11.6 Hz, H_2-23)], an olefin [δ 5.47 (1H, t-like, J = ca. 3 Hz, H-12)], and two glucopyranosyl units and a rhamnopyranosyl moiety [δ 5.06 (1H, d, J = 7.6 Hz, *terminal*-Glc-H-1), 6.09 (1H, d, J = 6.9 Hz, *inner*-Glc-H-1), 6.25 (1H, brs, Rha-H-1)], together with two acetyl groups [δ 1.96, 2.01 (3H each, both s, *inner*-Glc-6-*O*-H_3-Ac and 23-*O*-H_3-Ac)]. Treatment of **1** with 0.5% sodium methoxide (NaOMe)−MeOH provided a new saponin, desacyl-perennisoside I (**1a**). Acid hydrolysis of **1a** with 5% aqueous sulfuric acid (H_2SO_4)−1,4-dioxane (1:1) liberated bayogenin (**1b**)[20] together with L-rhamnose and D-glucose, which were identified by HPLC analysis.[21] The 28-*O*-oligoglycoside structure of **1a** was characterized by the heteronuclear multiple-bond correlations (HMBC) experiment, in which long-range correlations were observed between the following proton and carbon pairs: *inner*-Glc-H-1 [δ 6.22 (1H, d, J = 7.6 Hz)] and C-28 (δ_C 176.3), Rha-H-1 [δ6.49 (1H, brs)] and *inner*-Glc-C-2 (δ_C 75.5), and *terminal*-Glc-H-1 [δ 5.17 (1H, d, J = 8.2 Hz)] and *inner*-Glc-C-3 (δ_C 88.1). Consequently, the structure of desacyl-perennisoside I was elucidated as bayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside (**1a**). Furthermore, comparison of the ^{13}C NMR spectra of **1** with those of **1a** revealed two acetylation shifts around the C-23 position of the aglycon moiety [**1**: δ_C 41.6 (C-4), 67.4 (C-23), 14.1 (C-24); **1a**: δ_C 42.5 (C-4), 67.9 (C-23), 14.6 (C-24)] and the C-6 position of the *inner*-β-D-glucopyranosyl part [**1**: δ_C 75.0 (*inner*-Glc-C-5), 64.0 (*inner*-Glc-C-6); **1a**: δ_C 78.4 (*inner*-Glc-C-5), 62.0 (*inner*-Glc-C-6)]. In the HMBC experiment of **1**, long-range correlations were observed between H_2-23 and the acetyl carbonyl carbon (δ_C 170.7) and between *inner*-Glc-H_2-6 [δ4.58 (1H, dd, J = 4.2, 11.0 Hz), 4.62 (1H, m)] and the acetyl carbonyl carbon (δ_C 170.5), as shown in Figure 1. On the basis of the above-mentioned evidence, the

* To whom correspondence should be addressed. Tel: +81-75-595-4633. Fax: +81-75-595-4768. E-mail: myoshika@mb.kyoto-phu.ac.jp.
[†] Kyoto Pharmaceutical University.
[‡] Kinki University.

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Table 1. ^{1}H NMR Data (600 MHz, pyridine-d_5) of Perennisosides I (**1**), II (**2**), and III (**3**) and Desacyl Perennisosides I (**1a**) and II (**2a**)

position	**1**	**1a**	**2**	**2a**	**3**
1	1.26 m	1.27 m	1.26 m	1.29 m	1.17 m
	2.31 brd,ca. 14	2.37 brd,ca. 12	2.32 brd,ca.14	2.38 dd,2.4,14.1	2.29 brd,ca.12
2	4.02 brs	4.52 brs	4.41 m	4.53 brs	4.83 brd,ca.3
3	3.88 m	4.26 m	3.88 d 3.7	4.26 m	4.33 m
5	1.45 m	1.72 m	1.46 m	1.72 m	1.76 m
6	1.23 m	1.32 m	1.24 m	1.31 m	1.23 m
	1.74 m	1.85 m	1.76 m	1.80 m	1.73 m
7	1.53 m	1.53 m	1.48 m	1.52 m	1.55 m
	1.68 m	1.72 m	1.67 m	1.72 m	1.67 m
9	1.70 m	1.77 m	1.70 m	1.79 m	1.66 m
11	1.98 m	2.06 m	1.99 m	2.06 m	2.01 m
	2.15 m	2.23 m	2.15 m	2.23 m	2.14 m
12	5.47 t-like ca.3	5.51 t-like ca.3	5.47 t-like ca.3	5.50 t-like ca.3	5.47 t-like ca.3
15	1.46 m	1.53 m	1.46 m	1.53 m	1.48 m
	2.05 m	2.10 m	2.03 m	2.07 m	2.04 m
16	1.68 m	2.08 m	1.60 m	2.05 m	2.01 m
	2.17 m	2.15 m	2.15 m	2.22 m	2.16 m
18	3.11 brd,ca.14	3.11 dd,4.1,13.7	3.11 dd,3.1,13.7	3.11 dd,4.3,14.1	3.13 dd,4.3,13.5
19	1.26 m	1.23 m		1.23 m	1.20 m
	1.77 m	1.74 m	1.77 m	1.70 m	1.74 m
21	1.13 m	1.08 m	1.16 m	1.10 m	1.15 m
	1.35 m	1.35 m	1.35 m	1.33 m	1.34 m
22	1.74 m	1.76 m	1.77 m	1.76 m	1.74 m
	2.03 m	1.89 m	2.00 m	1.89 m	2.00 m
23	4.12 d,11.6	3.65 d,10.3	4.13 d,11.0	3.65 d,10.1	3.63 d,11.6
	4.38 d,11.6	4.13 d,10.3	4.39 d,11.0	4.14 d,10.1	4.35 d,11.6
24	1.23 s	1.35 s	1.24 s	1.35 s	1.34 s
25	1.56 s	1.66 s	1.57 s	1.67 s	1.62 s
26	1.15 s	1.23 s	1.15 s	1.24 s	1.17 s
27	1.26 s	1.23 s	1.26 s	1.23 s	1.23 s
29	0.89 s	0.85 s	0.88 s	0.85 s	0.87 s
30	0.93 s	0.79 s	0.92 s	0.78 s	0.91 s
23-*O*-Ac	2.01 s		2.00 s		
C-3-sugar					(Glc)
1					5.18 d,7.7
2					4.04 m
3					4.18 m
4					4.20 m
5					3.91 m
6					4.33 dd-like
					4.48 m
C-28-sugars	(*i*-Glc)[a]	(*i*-Glc)[a]	(Glc)	(Glc)	(*i*-Glc)[a]
1	6.09 d,6.9	6.22 d,7.6	6.05 d,7.7	6.18 d,7.6	6.15 d,7.4
2	4.38 m	4.51 m	4.30 m	4.45 m	4.47 m
3	4.19 m	4.34 m	4.13 m	4.32 m	4.28 m
4	3.99 m	4.40 m	3.96 m	4.30 m	4.07 m
5	4.00 m	4.00 m	4.04 dd,2.2,9.2	3.97 m	4.08 m
6	4.58 dd,4.2,11.0	4.28 m	4.61 m (2H)	4.28 m	4.68 m (2H)
	4.62 m	4.38 m		4.38 m	
6-*O*-Ac	1.96 s		1.97 s		1.95 s
	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)
1	6.25 brs	6.49 brs	6.25 brs	6.45 brs	6.38 brs
2	4.68 m	4.79 m	4.69 m	4.80 m	4.77 m
3	4.46 m	4.50 m	4.46 m	4.50 m	4.48 m
4	4.19 m	4.34 m	4.19 m	4.34 m	4.29 m
5	4.37 m	4.49 m	4.39 m	4.47 m	4.43 m
6	1.67 d,5.5	1.76 d,6.2	1.66 d,6.1	1.75 d,6.1	1.74 d,6.1
	(*t*-Glc)[b]	(*t*-Glc)[b]	(Gal)	(Gal)	(*t*-Glc)[b]
1	5.06 d,7.6	5.17 d,8.2	4.96 d,7.8	5.07 d,7.9	5.14 d,7.9
2	4.00 m	4.07 m	4.41 m	4.51 m	4.08 m
3	4.14 m	4.21 m	4.09 m	4.19 m	4.20 m
4	4.39 m	4.13 m	4.41 m	4.47 m	4.12 m
5	4.00 m	4.08 m	3.96 m	4.13 dd,3.1,10.3	4.04 m
6	4.19 m	4.28 m	4.28 dd,5.6,11.3	4.28 m	4.24 dd-like
	4.50 m	4.61 brd,10.3	4.41 m	4.41 brd,10.3	4.59 brd,*ca.*10

[a] *i*-Glc: inner Glc. [b] *t*-Glc: terminal Glc.

structure of perennisoside I was determined as 23-*O*-acetylbayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-6-*O*-acetyl-β-D-glucopyranoside (**1**).

Perennisoside II (**2**) was also obtained as an amorphous powder with a positive optical rotation ($[\alpha]^{26}_{D}$ +19.5 in MeOH). The IR spectrum of **2** showed absorption bands at 3445, 1745, 1655, and 1070 cm^{-1}, ascribable to hydroxyl, ester carbonyl, olefin, and ether functions. The molecular formula, $C_{52}H_{82}O_{21}$, of **2** was determined from the positive- and negative-ion FABMS (*m/z* 1065 $[M + Na]^+$ and 1041 $[M - H]^-$, respectively) and by HRFABMS, which was the same as that of **1**. Treatment of **2** with 0.5% NaOMe−MeOH yielded a new saponin, desacyl perennisoside II (**2a**). Acid

Table 2. ^{13}C NMR Data (150 MHz, pyridine-d_5) of Perennisosides I (**1**), II (**2**) III (**3**), IV (**4**), V (**5**), VI (**6**), and VII (**7**) and Desacyl-perennisosides I (**1a**), II (**2a**), and IV (**4a**)

position	1	1a	2	2a	3	4	4a	5	6	7
1	44.9	45.1	44.9	45.1	44.3	44.3	44.2	43.9	43.9	44.1
2	71.4	71.6	71.4	71.6	70.6	70.6	70.7	70.2	70.2	70.2
3	71.9	73.2	71.9	73.2	83.0	83.0	83.0	83.1	83.1	83.1
4	41.6	42.5	41.6	42.5	42.8	42.8	42.8	41.6	41.6	41.7
5	48.4	48.3	48.4	48.3	47.8	47.8	47.8	48.5	48.5	48.4
6	18.5	18.4	18.5	18.4	18.1	18.1	18.1	18.2	18.2	18.2
7	33.0	33.1	33.0	33.1	33.1	33.1	33.1	33.0	33.0	33.0
8	40.1	40.1	40.1	40.1	40.1	40.1	40.1	40.1	40.1	40.1
9	48.8	48.8	48.8	48.6	48.6	48.6	48.6	48.8	48.6	48.7
10	37.2	37.3	37.2	37.3	37.0	37.0	37.0	37.0	37.0	37.0
11	24.0	24.1	24.0	24.1	24.0	24.1	24.1	24.0	24.0	24.0
12	122.8	122.8	122.8	122.8	123.0	122.9	122.9	122.7	122.7	122.9
13	144.1	144.3	144.1	144.3	144.1	144.1	144.2	144.2	144.2	144.0
14	42.4	42.4	42.4	42.4	42.4	42.5	42.5	42.4	42.5	42.4
15	28.4	28.6	28.4	28.6	28.5	28.5	28.6	28.5	28.5	28.4
16	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3	23.3
17	47.2	47.1	47.2	47.1	47.2	47.2	47.1	47.2	47.2	47.2
18	42.1	42.1	42.1	42.1	42.0	42.0	42.1	42.2	42.3	42.2
19	46.5	46.4	46.5	46.4	46.3	46.3	46.4	46.4	46.4	46.4
20	30.7	30.7	30.7	30.7	30.7	30.7	30.7	30.7	30.7	30.7
21	34.1	34.1	34.1	34.1	34.1	34.1	34.0	34.1	34.1	34.1
22	32.3	32.2	32.3	32.2	32.3	32.3	32.2	32.2	32.2	32.3
23	67.4	67.9	67.4	67.9	65.5	65.5	65.5	66.7	66.7	66.6
24	14.1	14.6	14.1	14.6	15.1	15.1	15.1	14.7	14.7	14.7
25	17.3	17.4	17.3	17.4	17.4	17.4	17.4	17.2	17.2	17.2
26	17.5	17.6	17.5	17.6	17.5	17.5	17.6	17.5	17.5	17.5
27	25.7	26.0	25.7	26.0	26.0	26.0	26.0	25.7	25.7	25.7
28	176.3	176.3	176.3	176.3	176.4	176.3	176.4	176.3	176.3	176.4
29	33.1	33.1	33.1	33.1	33.1	33.1	33.1	33.1	33.1	33.1
30	23.7	23.7	23.7	23.7	23.7	23.6	23.7	23.6	23.6	23.6
23-*O*-Ac-1	170.7		170.6					170.8	170.8	170.8
2	20.7		20.7					20.9	20.9	20.9
C-3-sugar					(Glc)	(Glc)	(Glc)	(Glc)	(Glc)	(Glc)
1					105.8	105.8	105.8	106.1	106.1	106.1
2					75.4	75.5	75.4	75.1	75.3	75.3
3					78.3	78.3	78.3	78.5	78.5	78.5
4					71.6	71.6	71.6	71.6	71.6	71.6
5					78.6	78.6	78.6	78.6	78.6	78.6
6					62.6	62.6	62.7	62.7	62.7	62.7
C-28-sugars	(*i*-Glc)[a]	(*i*-Glc)[a]	(Glc)	(Glc)	(*i*-Glc)[a]	(Glc)	(Glc)	(*i*-Glc)[a]	(Glc)	(Glc)
1	94.2	94.5	94.2	94.4	94.2	94.2	94.4	94.5	94.5	94.3
2	75.4	75.2	75.5	75.4	75.5	75.5	75.5	75.3	75.3	75.4
3	88.2	88.9	88.2	88.9	88.2	88.2	88.9	88.9	89.0	88.3
4	69.4	69.1	69.4	69.2	69.4	69.4	69.2	69.1	69.2	69.3
5	75.0	78.4	75.1	78.5	74.9	75.0	78.5	78.4	75.2	75.0
6	64.0	62.0	63.9	62.0	64.1	64.1	61.9	61.9	62.1	63.9
6-*O*-Ac-1	170.5		170.5		170.6	170.6				170.6
2	20.6		20.7		20.7	20.7				20.7
	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)
1	101.2	101.2	101.3	101.3	101.4	101.4	101.3	101.2	101.2	101.3
2	72.1	72.4	72.2	72.4	72.3	72.3	72.4	72.3	72.4	72.3
3	72.4	72.6	72.5	72.6	72.5	72.5	72.6	72.5	72.6	72.5
4	73.7	73.8	73.7	73.8	73.7	73.7	73.7	73.8	73.8	73.8
5	70.1	70.1	70.0	70.1	70.2	70.2	70.1	70.1	70.1	70.1
6	18.7	18.8	18.7	18.8	18.8	18.8	18.8	18.8	18.8	18.9
	(*t*-Glc)[b]	(*t*-Glc)[b]	(Gal)	(Gal)	(*t*-Glc)[b]	(Gal)	(Gal)	(*t*-Glc)[b]	(Gal)	(Gal)
1	104.1	104.1	104.7	104.7	104.1	104.7	104.7	104.8	104.8	104.8
2	74.8	75.1	72.4	72.6	75.1	72.5	72.6	75.1	72.6	72.5
3	78.4	78.5	77.4	77.5	78.5	77.5	77.5	78.5	77.5	77.6
4	71.5	71.6	70.1	70.1	71.6	70.0	70.1	71.6	70.0	70.2
5	78.5	78.7	74.9	75.2	78.7	75.1	75.2	78.7	75.3	75.2
6	62.4	62.4	62.1	62.1	62.4	62.1	62.1	62.4	62.0	62.1

[a] *i*-Glc: inner Glc. [b] *t*-Glc: terminal Glc.

hydrolysis of **2a** with 5% aqueous H_2SO_4−1,4-dioxane (1:1) liberated bayogenin (**1b**)[20] together with L-rhamnose, D-glucose, and D-galactose, which were identified by HPLC analysis.[21] The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **2a** showed signals assignable to six methyls [δ 0.78, 0.85, 1.23, 1.24, 1.35, 1.67 (3H each, all s, H_3-30, 29, 27, 26, 24, 25)], a methylene and two methines bearing an oxygen function [δ 3.65, 4.14 (1H each, both d, J = 10.1 Hz, H_2-23), 4.26 (1H, m, H-3), 4.53 (1H, brs, H-2)], an olefin [δ 5.50 (1H, t-like, J = ca. 3 Hz, H-12)], a β-D-galactopyranosyl [δ 5.07 (1H, d, J = 7.9 Hz, Gal-H-1)], a β-D-glucopyranosyl [δ 6.18 (1H, d, J = 7.6 Hz, Glc-H-1)], and an α-L-rhamnopyranosyl moiety [δ 6.45 (1H, brs, Rha-H-1)], which were superimposable on those of **1a**, except for the signals due to the β-D-galactopyranosyl unit. Comparison of the ^{13}C NMR data for **2** with those for **2a** revealed acetylation shifts around the C-23 position of the aglycon moiety [**1**: δ_C 41.6 (C-4), 67.4 (C-23), 14.1

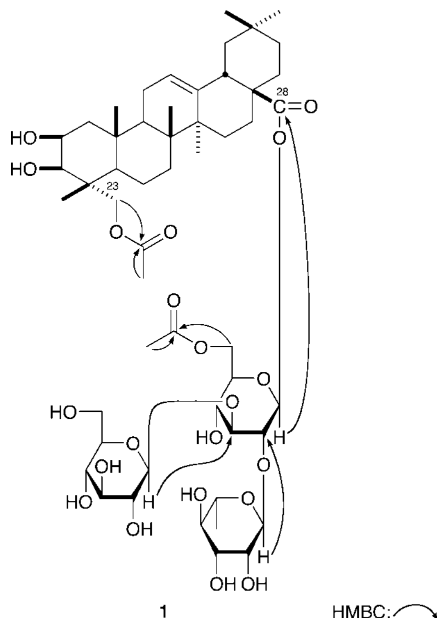


Figure 1. Selected HMBC correlations of **1**.

(C-24); **1a**: δ_C 42.5 (C-4), 67.9 (C-23), 14.6 (C-24)] and the C-6 position of the *inner-β*-D-glucopyranosyl part [**2**: δ_C 75.1 (*inner*-Glc-C-5), 63.9 (*inner*-Glc-C-6); **2a**: δ_C 78.5 (*inner*-Glc-C-5), 62.0 (*inner*-Glc-C-6)]. Furthermore, the positions of two acetyl groups and the oligoglycoside linkage in **2** were characterized in an HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: H_2-23 and the acetyl carbonyl carbon (δ_C 170.6), Glc-H_2-6 and the acetyl carbonyl carbon (δ_C 170.5), Glc-H-1 and C-28 (δ_C 176.3), Rha-H-1 and Glc-C-2 (δ_C 75.5), and Gal-H-1 and Glc-C-3 (δ_C 88.2) (Figure S1). Consequently, the structure of perennisoside II was elucidated as 23-*O*-acetylbayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[*β*-D-galactopyranosyl(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside (**2**).

Perennisosides III (**3**) and IV (**4**) were obtained as amorphous powders with positive optical rotations (**3**: $[\alpha]^{27}_D$ +1.7, **4**: $[\alpha]^{27}_D$ +4.6 both in MeOH). The molecular formula, $C_{56}H_{90}O_{25}$, of both **3** and **4** was determined from the positive- and negative-ion FABMS (*m/z* 1185 [M + Na]$^+$ and 1161 [M − H]$^-$) and by HRFABMS. By treatment of **3** and **4** with 0.5% NaOMe−MeOH, besysaponin U_{D2} (**3a**)[22] and desacyl-perennisoside IV (**4a**), respectively were obtained. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **3** indicated the presence of an aglycon part, constructed by six methyls [δ 0.87, 0.91, 1.17, 1.23, 1.34, 1.62 (3H each, all s, H_3-29, 30, 26, 27, 24, 25)], a methylene and two methines bearing an oxygen function [δ 4.33 (1H, m, H-3), 4.83 (1H, br d, J = ca. 3 Hz, H-2), 3.63 (1H, d, J = 11.6 Hz), 4.35 (1H, d, J = 11.6 Hz), H_2-23], an olefin [δ 5.47 (1H, t-like, J = ca. 3 Hz, H-12)], three *β*-D-glucopyranosyl units [δ 5.14 (1H, d, J = 7.9 Hz, 28-*O*-*terminal*-Glc-H-1), 5.18 (1H, d, J = 7.7 Hz, 3-*O*-Glc-H-1), 6.15 (1H, d, J = 7.4 Hz, 28-*O*-*inner*-Glc-H-1)], an α-L-rhamnopyranosyl moiety [δ 6.38 (1H, brs, Rha-H-1)], together with an acetyl group [δ 1.95 (3H, s, *inner*-Glc-6-*O*-H_3-Ac)]. The connectivity of the acetyl group in **3** was clarified by an HMBC experiment, which showed long-range correlations between the C-6 protons of the 28-*O*-*inner*-Glc [δ4.68 (2H, m)] and the acetyl carbonyl carbon (δ_C 170.6). Consequently, the structure of perennisoside III was established as 3-*O*-*β*-D-glucopyranoside of bayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside (**3**). In turn, acid hydrolysis of **4a** with 5% aqueous H_2SO_4−1,4-dioxane (1:1) liberated bayogenin (**1b**)[20] together with L-rhamnose, D-glucose, and D-galactose, which were identified by HPLC analysis.[21] The proton and carbon signals in the ^{1}H (Table 3) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **4** resembled those of **3**, except for the signals due to the *β*-D-galactopyranosyl part [δ 5.04 (1H, d, J = 7.4 Hz, Gal-H-1)]. The HMBC experiment on **4** showed long-range correlations between the following proton and carbon pairs: 3-*O*-Glc-H-1 [δ 5.18 (1H, d, J = 7.7 Hz)] and C-3 (δ_C 83.0), 28-*O*-*inner*-Glc-H_2-6 [δ 4.66 (2H, m)] and the acetyl carbonyl carbon (δ_C 170.6), 28-*O*-*inner*-Glc-H-1 [δ 6.11 (1H, d, J = 7.6 Hz)] and C-28 (δ_C 176.3), Rha-H-1 [δ 6.36 (1H, brs)] and 28-*O*-*inner*-Glc-C-2 (δ_C 75.5), and 28-*O*-*terminal*-Glc-H-1 [δ 5.04 (1H, d, J = 7.4 Hz)] and 28-*O*-*inner*-Glc-C-3 (δ_C 88.2). On the basis of the above-mentioned evidence, the structure of perennisoside IV was elucidated as 3-*O*-*β*-D-glucopyranoside of bayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[*β*-D-galactopyranosyl(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside (**4**).

The molecular formulas of perennisoside V (**5**) and VI (**6**), both $C_{56}H_{90}O_{25}$, were determined from their respective positive- and negative-ion FABMS (*m/z* 1185 [M + Na]$^+$ and 1161 [M − H]$^-$) and by HRFABMS data. By treatment of **5** and **6** with 0.5% NaOMe−MeOH, **3a** and **4a** were obtained as their respective desacyl derivative. The proton and carbon signals in the ^{1}H (Table 3) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **5** showed signals assignable to a besysaponin U_{D2} moiety and an acetyl group [δ 2.05 (3H, s, 23-*O*-H_3-Ac)]. The connectivity of the acetyl moiety in **5** was characterized by an HMBC experiment, which exhibited a long-range correlation between the C-23 protons [δ 4.22 (1H, m), 4.70 (1H, d, J = 11.0 Hz)] and the acetyl carbonyl carbon (δ_C 170.8). Thus, the structure of perennisoside V was assigned as 3-*O*-*β*-D-glucopyranoside of 23-*O*-acetylbayogenin 28-*O*α-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranoside (**5**). The signals in the ^{1}H (Table 3) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **6** were very similar to those of **4** {six methyls [δ 0.78, 0.86, 1.20, 1.28, 1.29, 1.55 (3H each, all s, H_3-29, 30, 26, 24, 27, 25)], a methylene and two methines bearing an oxygen function [δ 4.02 (1H, brs, H-3), 4.22, 4.70 (1H each, both d, J = 11.3 Hz, H_2-23), 4.78 (1H, m, H-2)], an olefin [δ 5.49 (1H, t-like, J = ca. 3 Hz, H-12)], two *β*-D-glucopyranosyls [δ 5.00 (1H, d, J = 8.0 Hz, 3-*O*-Glc-H-1), 6.18 (1H, d, J = 7.6 Hz, 28-*O*-Glc-H-1)], a *β*-D-galactopyranosyl [δ 5.06 (1H, d, J = 7.7 Hz, Gal-H-1)], and an α-L-rhamnopyranosyl moiety [δ 6.45 (1H, brs, Rha-H-1)], and an acetyl group [δ 2.05 (3H, s, 23-*O*-H_3-Ac)]}. The connectivity of the acetyl group of **6** was elucidated on the basis of an HMBC experiment, in which long-range correlation was observed between the C-23 protons and the acetyl carbonyl carbon (δ_C 170.8). Consequently, the structure of perennisoside VI was determined as 3-*O*-*β*-D-glucopyranoside of 23-*O*-acetylbayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[*β*-D-galactopyranosyl(1→3)]-*β*-D-glucopyranoside (**6**).

Perennisoside VII (**7**) was obtained as an amorphous powder with positive optical rotation ($[\alpha]^{25}_D$ +14.3, in MeOH). In the positive- and negative-ion FABMS of **7**, quasimolecular ion peaks were observed at *m/z* 1227 [M + Na]$^+$ and 1203 [M − H]$^-$, and HRFABMS analysis revealed the molecular formula to be $C_{58}H_{92}O_{26}$. Treatment of **7** with 0.5% NaOMe−MeOH liberated **4a**. Comparison of the ^{1}H (Table 3) and ^{13}C NMR (Table 2) spectra (pyridine-d_5)[19] of **7** with those of **6** showed the presence of an additional acetyl group observed at the C-6 position in the 28-*O*-*β*-D-glucopyranosyl part [δ 1.97 (3H, s, 28-*O*-Glc-6-*O*-H_3−Ac), 4.63 (2H, m, 28-*O*-Glc-H_2-6)]. In the HMBC experiment of **7**, long-range correlations were observed between the C-23 protons [δ 4.25 (1H, m), 4.71 (1H, d, J = 11.3 Hz)] and the acetyl carbonyl carbon (δ_C 170.8) and between the C-6 protons in the 28-*O*-*β*-D-glucopyranosyl part and the acetyl carbonyl carbon (δ_C 170.6). Consequently, the structure of perennisoside VII was elucidated as 3-*O*-*β*-D-glucopyranoside of 23-*O*-acetylbayogenin 28-*O*-α-L-rhamnopyranosyl(1→2)[*β*-D-galactopyranosyl(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside (**7**).

The effects of the principal new saponin constituents (**1** and **2**) of *B. perennis* on serum TG elevation in olive oil-treated mice were

Table 3. ^{1}H NMR Data (600 MHz, pyridine-d_5) of Perennisosides IV (**4**), V (**5**), VI (**6**), and VII (**7**) and Desacyl-perennisoside IV (**4a**)

position	4	4a	5	6	7
1	1.20 m	1.22 m	1.18 m	1.16 m	1.24 m
	2.30 brd,ca13	2.39 brd,ca.12	2.28 dd-like	2.26 brd,ca.13	2.30 brd,ca.13
2	4.83 brs	4.83 brs	4.80 brd,ca3	4.78 m	4.82 m
3	4.33 brs	4.34 brs	4.02 brs	4.02 brs	4.03 m
5	1.77 m	1.79 m	1.50 m	1.50 m	1.47 m
6	1.23 m	1.28 m	1.22 m	1.20 m	1.24 m
	1.72 m	1.80 m	1.70 m	1.70 m	1.81 m
7	1.55 m	1.54 m	1.57 m	1.53 m	1.50 m
	1.69 m	1.71 m	1.72 m	1.70 m	1.77 m
9	1.67 m	1.73 m	1.72 m	1.70 m	1.72 m
11	2.01 m	2.04 m	2.04 m	2.04 m	2.03 m
	2.14 m	2.16 m	2.15 m	2.15 m	2.17 m
12	5.46 t-like,ca.3	5.48 t-like,ca.3	5.50 t-like,ca.3	5.49 t-like,ca.3	5.47 t-like,ca.3
15	1.47 m	1.47 m	1.47 m	1.50 m	1.50 m
	2.04 m	2.04 m	2.07 m	2.10 m	2.05 m
16	2.01 m	2.04 m	2.05 m	2.02 m	2.05 m
	2.16 m	2.17 m	2.18 m	2.17 m	2.21 m
18	3.12 dd,4.0,13.4	3.10 dd,4.2,13.1	3.11 dd,3.7,13.5	3.11 dd,4.0,13.7	3.14 dd,4.0,14.3
19	1.17 m	1.23 m	1.28 m	1.25 m	1.29 m
	1.74 m	1.78 m	1.80 brd,*ca*14	1.78 dd-like	1.81 m
21	1.14 m	1.08 m	1.15 m	1.15 m	1.15 m
	1.34 m	1.36 m	1.37 m	1.37 m	1.37 m
22	1.77 m	1.78 m	1.80 m	1.80 m	1.81 m
	2.01 m	1.87 m	2.04 m	2.04 m	2.00 m
23	3.62 d,10.7	3.62 d,11.0	4.22 d,11.0	4.22 d,11.3	4.25 d,11.3
	4.36 d,10.7	4.37 d,11.0	4.70 d,11.0	4.70 d,11.3	4.71 d,11.3
24	1.34 s	1.34 s	1.28 s	1.28 s	1.28 s
25	1.63 s	1.62 s	1.55 s	1.55 s	1.56 s
26	1.18 s	1.22 s	1.20 s	1.20 s	1.17 s
27	1.23 s	1.23 s	1.29 s	1.29 s	1.29 s
29	0.86 s	0.85 s	0.79 s	0.78 s	0.88 s
30	0.90 s	0.78 s	0.86 s	0.86 s	0.91 s
23-*O*-Ac			2.05 s	2.05 s	2.08 s
C-3-sugar	(Glc)	(Glc)	(Glc)	(Glc)	(Glc)
1	5.18 d,7.7	5.20 d,7.6	5.00 d,7.9	5.00 d,8.0	5.01 d,7.6
2	4.06 m	4.05 dd,7.6,8.2	4.00 m	4.00 m	4.01 m
3	4.18 m	4.19 m	4.17 m	4.17 m	4.23 m
4	4.24 m	4.18 m	4.21 m	4.21 m	4.22 m
5	3.91 m	3.92 m	3.94 m	3.94 m	4.23 m
6	4.35 m	4.35 m	4.25 m	4.26 m	4.30 m
	4.49 m	4.48 m	4.50 m	4.43 m	4.53 m
C-28-sugars	(Glc)	(Glc)	(*i*-Glc)[a]	(Glc)	(Glc)
1	6.11 d,7.6	6.16 d,7.6	6.21 d,7.3	6.18 d,7.6	6.12 d,7.7
2	4.41 m	4.45 dd,7.6,7.6	4.45 dd-like	4.45 dd-like	4.41 m
3	4.24 m	4.28 m	4.32 m	4.28 m	4.23 m
4	4.04 m	4.32 m	4.03 m	4.03 m	4.04 m
5	4.04 m	3.97 m	4.03 m	4.03 m	4.00 m
6	4.66 m (2H)	4.24 m	4.29 m	4.24 m	4.63 m (2H)
		4.35	4.38 m	4.35 m	
6-*O*-Ac	1.97 s				1.97 s
	(Rha)	(Rha)	(Rha)	(Rha)	(Rha)
1	6.36 brs	6.45 brs	6.48 brs	6.45 brs	6.26 brs
2	4.77 m	4.80 m	4.78 m	4.78 m	4.79 m
3	4.49 m	4.51 m	4.50 m	4.50 m	4.49 m
4	4.29 dd,9.2,9.2	4.30 m	4.28 m	4.28 m	4.27 m
5	4.46 m	4.48 m	4.48 m	4.48 m	4.47 m
6	1.73 d,5.8	1.74 d,6.2	1.75 d,6.1	1.73 d,6.2	1.72 d,6.1
	(Gal)	(Gal)	(*t*-Glc)[b]	(Gal)	(Gal)
1	5.04 d,7.4	5.07 d,7.6	5.16 d,7.9	5.06 d,7.7	5.03 d,0.0
2	4.51 m	4.53 m	4.54 m	4.54 m	4.51 m
3	4.18 m	4.20 m	4.20 m	4.20 m	4.20 m
4	4.46 m	4.52 m	4.45 m	4.45 m	4.44 m
5	4.10 dd,3.0,9.5	4.12 dd,2.8,8.9	4.09 m	4.09 m	4.11 m
6	4.35 m	4.35 m	4.28 m	4.35 m	4.37 dd,4.6,11.6
	4.53 m	4.53 m	4.60 dd,2.2,11.6	4.50 m	4.51 m

[a] *i*-Glc: inner Glc. [b] *t*-Glc: terminal Glc.

examined. As shown in Table 4, a reference compound, clofibrate, which reduces TG through peroxisome proliferator-activated receptor-α (PPARα)-mediated stimulation of fatty acid oxidation, increased LPL synthesis, and reduced expression of apoCIII as a mechanism of action,[23] showed a weak effect. In contrast, a standard lipase inhibitor, orlistat,[24] showed a potent effect in this assay model (Table 4). Among the principal saponin constituents, **1** and **2** significantly suppressed the increase in serum TG levels 2 h after administration of olive oil at doses of 25−50 mg/kg, po (Table 4).

Experimental Section

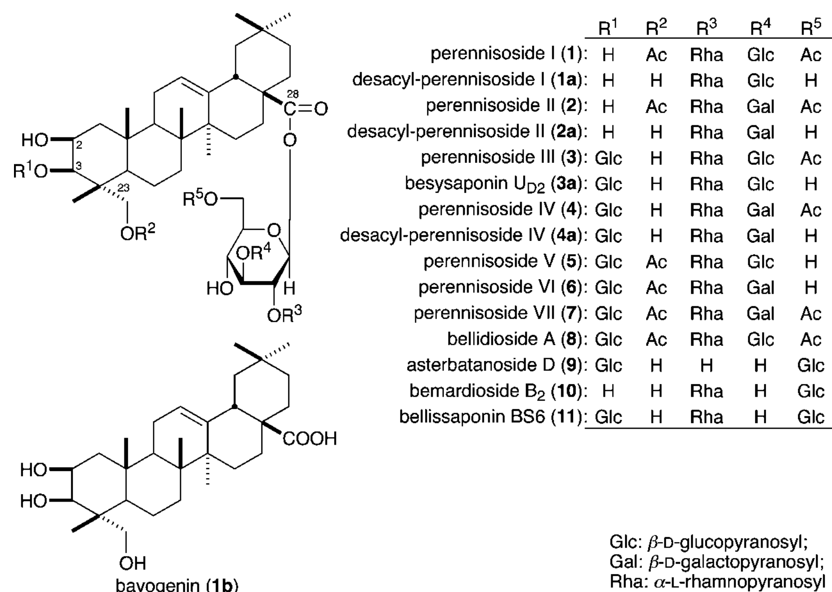
General Experimental Procedures. The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); UV spectra, Shimadzu UV-1600 spectrometer;

Table 4. Inhibitory Effects of Perennisosides I (**1**) and II (**2**) on Serum Triglyceride Elevation in Olive Oil-Treated Mice

treatment	dose (mg/kg, p.o.)	n	serum triglyceride (mg/dL)^a 2.0 h	4.0 h	6.0 h
normal		6	141.4 ± 9.1^c	101.6 ± 10.7	81.7 ± 9.3
control		6	501.5 ± 64.0	239.0 ± 58.6	167.2 ± 26.6
perennisoside I (**1**)	25	6	337.0 ± 47.7	357.7 ± 65.6	222.7 ± 41.3
	50	6	326.9 ± 50.5^b	355.8 ± 67.5	203.7 ± 30.7
	100	6	135.7 ± 33.5^c	278.7 ± 78.2	208.2 ± 31.4
control		6	338.5 ± 61.9	207.0 ± 26.3	142.1 ± 18.4
perennisoside II (**2**)	25	6	204.6 ± 25.5^b	147.0 ± 31.2	87.0 ± 9.5^c
	50	6	232.5 ± 31.8	180.4 ± 33.4	104.6 ± 7.5
	100	6	179.4 ± 15.3^c	155.6 ± 24.5	80.6 ± 7.5^c
normal		10	140.0 ± 7.4^c	124.6 ± 9.3^b	147.6 ± 7.5
control		10	439.4 ± 34.5	267.5 ± 22.4	244.8 ± 22.0
clofibrate[25]	125	10	377.5 ± 22.8	295.0 ± 40.7	333.4 ± 29.4
	250	10	441.2 ± 25.4	340.5 ± 58.6	443.3 ± 59.2^c
	500	10	318.2 ± 18.2^c	239.7 ± 28.7	373.5 ± 28.5^b
normal		10	154.3 ± 9.3^c	138.0 ± 9.8^c	138.1 ± 12.3^c
control		10	387.1 ± 39.2	320.4 ± 61.3	276.5 ± 35.1
orlistat[25]	6.25	10	266.4 ± 31.1^b	179.3 ± 17.2^b	155.6 ± 13.2^c
	12.5	10	187.9 ± 25.5^c	176.0 ± 29.5^c	189.7 ± 28.8^b
	25	10	158.9 ± 28.7^c	132.2 ± 10.5^c	140.1 ± 13.7^c

^{a} Values represent the means ± SEM. ^{b} Significantly different from the control group, $p < 0.05$. ^{c} Significantly different from the control group, $p < 0.01$.

Chart 1



	R1	R2	R3	R4	R5
perennisoside I (**1**):	H	Ac	Rha	Glc	Ac
desacyl-perennisoside I (**1a**):	H	H	Rha	Glc	H
perennisoside II (**2**):	H	Ac	Rha	Gal	Ac
desacyl-perennisoside II (**2a**):	H	H	Rha	Gal	H
perennisoside III (**3**):	Glc	H	Rha	Glc	Ac
besysaponin U_{D2} (**3a**):	Glc	H	Rha	Glc	H
perennisoside IV (**4**):	Glc	H	Rha	Gal	Ac
desacyl-perennisoside IV (**4a**):	Glc	H	Rha	Gal	H
perennisoside V (**5**):	Glc	Ac	Rha	Glc	H
perennisoside VI (**6**):	Glc	Ac	Rha	Gal	H
perennisoside VII (**7**):	Glc	Ac	Rha	Gal	Ac
bellidioside A (**8**):	Glc	Ac	Rha	Glc	Ac
asterbatanoside D (**9**):	Glc	H	H	H	Glc
bemardioside B_2 (**10**):	H	H	Rha	H	Glc
bellissaponin BS6 (**11**):	Glc	H	Rha	H	Glc

IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JEOL ECA-600 (600 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C NMR spectra, JEOL ECA-600 (150 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–vis detectors; HPLC column, Cosmosil 5C$_{18}$-MS-II and HILIC (Nacalai Tesque, Inc.) and Wakopak Navi C-30–5 (Wako Pure Chemical Industries Ltd.) (250 × 4.6 mm i.d. and 250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel 60N (Kanto Chemical Co., Ltd., 63–210 mesh, spherical, neutral); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with silica gel 60F$_{254}$ (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm); detection was carried out by spraying with 1% Ce(SO$_4$)$_2$−10% aqueous H$_2$SO$_4$, followed by heating.

Plant Material. The flowers of *B. perennis* cultivated in Albania were purchased from Tochimoto Tenkaido Co., Ltd., Osaka, Japan, in November 2006. The plant material was identified by one of the authors (M.Y.). A voucher specimen (lot. no. 20860501K) of this plant is on file in our laboratory.

Extraction and Isolation. The dried flowers of *B. perennis* (3.0 kg) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (775.0 g, 25.8%). The methanolic extract (720.0 g) was partitioned between an EtOAc−H$_2$O (1:1) mixture, and removal of the solvents in vacuo yielded an EtOAc-soluble fraction (187.1 g, 6.7%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (4.0 kg, H$_2$O → MeOH, twice) to give a H$_2$O-eluted fraction (350.0 g, 12.5%) and a MeOH-eluted fraction (180.0 g, 6.4%). The MeOH-eluted fraction (140.0 g) 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, 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)]. 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) → 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 (50.269 g), Fr. 6-13 (13.375 g), Fr. 6-14 (2.208 g), and Fr. 6-15 (1.888 g)].

Fraction 6-11 (1820.0 mg) was purified by HPLC [Wakopak Navi C30-5, CH_3CN−MeOH−1% aqueous AcOH (32:16:52)] to afford eight fractions [Fr. 6-11-1 (465.8 mg), Fr. 6-11-2 (272.1 mg), Fr. 6-11-3 (41.7 mg), Fr. 6-11-4 (44.5 mg), Fr. 6-11-5 (263.0 mg), Fr. 6-11-6 (210.0 mg), Fr. 6-11-7 (137.9 mg), and Fr. 6-11-8 (88.6 mg)]. Fraction 6-11-1 (465.8 mg) was further separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−H_2O (25:16:59)] to furnish perennisoside III (**3**, 10.5 mg, 0.0012%) and bernardioside B_2 (**10**, 112.2 mg, 0.013%). Fraction 6-12 (2015.0 mg) was further separated by HPLC [Wakopak Navi C30-5, CH_3CN−1% aqueous AcOH (40:60)] to furnish six fractions [Fr. 6-12-1 (893.3 mg), Fr. 6-12-2 (324.4 mg), Fr. 6-12-3 (131.3 mg), Fr. 6-12-4 (350.4 mg), Fr. 6-12-5 (48.8 mg), and Fr. 6-12-6 (135.0 mg)]. Fraction 6-12-1 (893.3 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−1% aqueous AcOH (32:16:52)] to furnish 14 fractions [Fr. 6-12-1-1 (13.0 mg), Fr. 6-12-1-2 (9.4 mg), Fr. 6-12-1-3 (34.5 mg), Fr. 6-12-1-4 (10.5 mg), Fr. 6-12-1-5 (23.0 mg), Fr. 6-12-1-6 (80.8 mg), Fr. 6-12-1-7 (49.7 mg), Fr. 6-12-1-8 (86.8 mg), Fr. 6-12-1-9 (34.9 mg), Fr. 6-12-1-10 (125.8 mg), Fr. 6-12-1-11 (57.9 mg), Fr. 6-12-1-12 (109.2 mg), Fr. 6-12-1-13 (56.0 mg), and Fr. 6-12-1-14 (22.2 mg)]. Fraction 6-12-1-6 (80.8 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−1% aqueous AcOH (30:16:54)] to afford bellidioside A (**8**, 35.3 mg, 0.039%). Fraction 6-13 (782.5 mg) was separated by HPLC [Wakopak Navi C30-5, CH_3CN−1% aqueous AcOH (40:60)] to yield six fractions [Fr. 6-13-1 (77.1 mg), Fr. 6-13-2 (59.5 mg), Fr. 6-13-3 (75.7 mg), Fr. 6-13-4 (34.9 mg), Fr. 6-13-5 (395.9 mg), and Fr. 6-13-6 (114.5 mg)]. Fraction 6-13-1 (77.1 mg) was further purified by HPLC [Wakopak Navi C30-5, CH_3CN−1% aqueous AcOH (33:67)] to furnish perennisoside VII (**7**, 12.2 mg, 0.0089%) and **8** (26.0 mg, 0.019%). Fraction 6-14 (946.3 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−1% aqueous AcOH (40:60)] to afford six fractions {Fr. 6-14-1 (27.5 mg), Fr. 6-14-2 (91.1 mg), Fr. 6-14-3 (162.7 mg), Fr. 6-14-4 [= perennisoside II (**2**, 110.2 mg, 0.011%)], Fr. 6-14-5 (174.5 mg), and Fr. 6-14-6 (101.5 mg)}. Fraction 6-14-5 (174.5 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−1% aqueous AcOH (40:60)] to furnish perennisoside I (**1**, 122.4 mg, 0.0122%). Fraction 7 (10.11 g) was subjected by reversed-phase silica gel column chromatography [300 g, MeOH−H_2O (20:80 → 30:70 → 40:60 → 50:50 → 70:30) → 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)]. Fraction 7-4 (713.4 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−H_2O (22:16:62)] to afford six fractions {Fr. 7-4-1 (57.7 mg), Fr. 7-4-2 (57.9 mg), Fr. 7-4-3 (36.4 mg), Fr. 7-4-4 (41.4 mg), Fr. 7-4-5 [= astrbatanoside D (**9**, 174.5 mg, 0.0063%)], and Fr. 7-4-6 (80.0 mg)}. Fraction 7-5 (1910.7 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH− H_2O(26:16:58)] to afford 10 fractions [Fr. 7-5-1 (174.1 mg), Fr. 7-5-2 (150.2 mg), 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)]. Fraction 7-5-6 (87.1 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH-H_2O (26:16:58)] to furnish perennisoside IV (**4**, 26.4 mg, 0.0012%). Fraction 7-5-7 (70.7 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−H_2O (26:16:58)] to furnish perennisoside III (**3**, 31.5 mg, 0.0014%). Fraction 7-6 (450.5 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−H_2O (30:16:54)] to furnish perennisosides V (**5**, 14.2 mg, 0.0042%) and VI (**6**, 20.4 mg, 0.0060%). 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) → 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)]. Fraction 8-7 (960.0 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−H_2O (22:16:62)] to afford nine fractions {Fr. 8-7-1 (38.6 mg), Fr. 8-7-2 [= bellissaponin BS6 (**11**, 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 (46.7 mg)}. Fraction 8-8 (506.5 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN−MeOH−1% aqueous AcOH (20:60:64)] to give **11** (102.4 mg, 0.019%).

Perennisoside I (1): amorphous powder; $[\alpha]^{26}_D$ +16.1 (*c* 3.06, MeOH); IR (KBr) ν_{max} 3442, 1744, 1655, 1254, 1075 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1065 $[M + Na]^+$; negative-ion FABMS *m/z* 1041 $[M - H]^-$, 879 $[M - C_6H_{11}O_5]^-$, 529 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m/z* 1065.5253 (calcd for $C_{52}H_{82}O_{21}Na$ $[M + Na]^+$, 1065.5246).

Perennisoside II (2): amorphous powder; $[\alpha]^{26}_D$ +19.5 (*c* 2.76, MeOH); IR (KBr) ν_{max} 3445, 1745, 1655, 1252, 1070 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1065 $[M + Na]^+$; negative-ion FABMS *m/z* 1041 $[M - H]^-$, 879 $[M - C_6H_{11}O_5]^-$, 529 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m/z* 1065.5242 (calcd for $C_{52}H_{82}O_{21}Na$ $[M + Na]^+$, 1065.5246).

Perennisoside III (3): amorphous powder; $[\alpha]^{27}_D$ +1.7 (*c* 2.09, MeOH); IR (KBr) ν_{max} 3445, 1736, 1655, 1256, 1074 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1185 $[M + Na]^+$; negative-ion FABMS *m/z* 1161 $[M - H]^-$, 999 $[M - C_6H_{11}O_5]^-$, 649 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m/z* 1185.5660 (calcd for $C_{56}H_{90}O_{25}Na$ $[M + Na]^+$, 1185.5669).

Perennisoside IV (4): amorphous powder; $[\alpha]^{27}_D$ +4.6 (*c* 1.80, MeOH); IR (KBr) ν_{max} 3440, 1736, 1655, 1256, 1076 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1185 $[M + Na]^+$; negative-ion FABMS *m/z* 1161 $[M - H]^-$, 999 $[M - C_6H_{11}O_5]^-$, 649 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m/z* 1185.5665 (calcd for $C_{56}H_{90}O_{25}Na$ $[M + Na]^+$, 1185.5669).

Perennisoside V (5): amorphous powder; $[\alpha]^{25}_D$ +7.1 (*c* 0.95, MeOH); IR (KBr) ν_{max} 3440, 1736, 1655, 1260, 1074 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1185 $[M + Na]^+$; negative-ion FABMS *m/z* 1161 $[M - H]^-$, 999 $[M - C_6H_{11}O_5]^-$, 691 $[M - C_{18}H_{31}O_{14}]^-$, 529 $[M - C_{24}H_{41}O_{19}]^-$; HRFABMS *m/z* 1185.5663 (calcd for $C_{56}H_{90}O_{25}Na$ $[M + Na]^+$, 1185.5669).

Perennisoside VI (6): amorphous powder; $[\alpha]^{25}_D$ +8.8 (*c* 1.35, MeOH); IR (KBr) ν_{max} 3440, 1736, 1656, 1256, 1075 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1185 $[M + Na]^+$; negative-ion FAB-MS *m/z* 1161 $[M - H]^-$, 999 $[M - C_6H_{11}O_5]^-$, 837 $[M - C_{12}H_{22}O_{10}]^-$; HRFABMS *m/z* 1185.5674 (calcd for $C_{56}H_{90}O_{25}Na$ $[M + Na]^+$, 1185.5669).

Perennisoside VII (7): amorphous powder; $[\alpha]^{25}_D$ +14.3 (*c* 0.71, MeOH); IR (KBr) ν_{max} 3445, 1736, 1655, 1256, 1077 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1227 $[M + Na]^+$; negative-ion FAB-MS *m/z* 1203 $[M - H]^-$, 1041 $[M - C_6H_{11}O_5]^-$, 691 $[M - C_{20}H_{33}O_{15}]^-$, 529 $[M - C_{26}H_{43}O_{20}]^-$; HRFABMS *m/z* 1227.5780 (calcd for $C_{58}H_{92}O_{26}Na$ $[M + Na]^+$, 1227.5775).

Deacylation of Perennisosides I (1), II (2), III (3), IV (4), V (5), VI (6), and VII (7). A solution of perennisoside I (**1**, 12.4 mg) in 0.5% sodium methoxide (NaOMe)−MeOH 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. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by HPLC [Cosmosil $5C_{18}$-MS-II, MeOH−H_2O (70:30)] to furnish desacyl-perennisoside I (**1a**, 11.1 mg, 97.4%). Using the same procedure, desacyl-perennisoside II (**2a**, 10.8 mg, 96.4%), besysaponin U_{D2} (**3a**, 4.8 mg, 92.3% from **3**; 4.4 mg, 91.3% from **5**), and desacyl-perennisoside IV (**4a**, 4.6 mg, 91.8% from **4**; 9.0 mg, 95.3% from **6**; 2.1 mg, 90.3% from **7**) were prepared from perennisosides II (**2**, 12.2 mg), III (**3**, 5.4 mg), IV (**4**, 5.2 mg), V (**5**, 5.0 mg), VI (**6**, 9.7 mg), and VII (**7**, 2.5 mg).

Desacyl-perennisoside I (1a): amorphous powder; $[\alpha]^{28}_D$ +14.7 (*c* 0.81, MeOH); IR (KBr) ν_{max} 3445, 1736, 1655, 1255, 1075 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 981 $[M + Na]^+$; negative-ion FABMS *m/z* 957 $[M - H]^-$, 794 $[M - C_6H_{11}O_5]^-$, 649 $[M - C_{12}H_{21}O_9]^-$, 487 $[M - C_{18}H_{31}O_{14}]^-$; HRFABMS *m/z* 981.5031 (calcd for $C_{48}H_{78}O_{19}Na$ $[M + Na]^+$, 981.5035).

Desacyl-perennisoside II (2a): amorphous powder; $[\alpha]^{28}_D$ +15.8 (*c* 0.59, MeOH); IR (KBr) ν_{max} 3445, 1740, 1655, 1260, 1074 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 981 $[M + Na]^+$; negative-ion FAB-MS *m/z* 957 $[M - H]^-$, 794 $[M - C_6H_{11}O_5]^-$, 649 $[M - C_{12}H_{21}O_9]^-$, 487 $[M - C_{18}H_{31}O_{14}]^-$; HRFABMS *m/z* 981.5030 (calcd for $C_{48}H_{78}O_{19}Na$ $[M + Na]^+$, 981.5035).

Desacyl-perennisoside IV (4a): amorphous powder; $[\alpha]^{25}_D$ +7.2 (*c* 1.00, MeOH); IR (KBr) ν_{max} 3440, 1736, 1655, 1260, 1075 cm^{-1}; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1143 $[M + Na]^+$; negative-ion FAB-MS *m/z* 1119 [M −

H]$^-$, 957 [M − $C_6H_{11}O_5$]$^-$, 649 [M − $C_{18}H_{31}O_{14}$]$^-$, 487 [M − $C_{24}H_{41}O_{19}$]$^-$; HRFABMS *m/z* 1143.5568 (calcd for $C_{54}H_{88}O_{24}$Na [M + Na]$^+$, 1143.5563).

Acid Hydrolysis of 1a, 2a, and 4a. Solutions of **1a** (5.5 mg), **2a** (2.0 mg), and **4a** (2.0 mg) in 5% aqueous H_2SO_4−1,4-dioxane (1:1, 1 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 filtered. On removal of the solvent from the filtrate under reduced pressure, the residue was partitioned in an EtOAc−H_2O (1:1) mixture, and the solvent was removed in vacuo from the EtOAc-soluble fraction and an aqueous phase, respectively. The EtOAc-soluble fraction was purified by HPLC [Cosmosil 5C_{18}-MS-II, MeOH−H_2O (80:20)] to furnish bayogenin (**1b**, 1.2 mg, 67.4% from **1a**; 1.1 mg, 61.8% from **2a**; 0.5 mg, 57.5% from **4a**), respectively. In turn, the aqueous layer was subjected to HPLC analysis under the following conditions, respectively: 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); flow rate 0.5 mL/min]. Identification of L-rhamnose (i) from **1a**, **2a**, and **4a**, and D-glucose (ii) from **1a** and **4a**, and D-galactose (iii) from **2a** and **4a** present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of an authentic samples. t_R: (i) 12.0 min (negative optical rotation), (ii) 20.7 min (positive optical rotation), and (iii) 22.2 min (positive optical rotation).

Bioassay Method. Animals. Male ddY mice weighing about 25–30 g 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 24–26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Inhibitory Effect on Serum Triglyceride (TG) Elevation in Olive Oil-Treated Mice. Each test sample was administered orally to fasted mice and olive oil (5 mL/kg) was administered po 30 min thereafter. Blood was collected from the infraorbital venosus plexus, 2, 4, and 6 h after olive oil treatment. Serum TG was determined by enzymatic method using a triglyceride E test (Wako Pure Chemical Industries Ltd., Osaka, Japan).[25,26]

Statistics. Values were expressed as means ± SEM. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used.

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Supporting Information Available: Inhibitory effects of the MeOH extract and its EtOAc-soluble fraction and MeOH- and H_2O-eluted fractions from the flowers of *Bellis perennis* on serum triglyceride elevation in olive oil-treated mice (Table S1). Selected HMBC correlations of perennisosides II−VII (**2–7**) (Figure S1). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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