Medicinal Flowers

Part 29[1])

Acylated Oleanane-Type Triterpene Bisdesmosides: Perennisaponins G, H, I, J, K, L, and M with Pancreatic Lipase Inhibitory Activity from the Flowers of *Bellis perennis*

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The MeOH extract from the flowers of *Bellis perennis* was found to show pancreatic-lipase inhibitory activity (IC_{50} 455 μg/ml). From the extract, seven new triterpene saponins named perennisaponins G (**1**; IC_{50} 163 μM), H (**2**; 137 μM), I (**3**; 147 μM), J (**4**; 148 μM), K (**5**; 223 μM), L (**6**; 81.4 μM), and M (**7**; 195 μM) were isolated as pancreatic lipase inhibitors. The stereostructures of **1**–**7** were elucidated on the basis of chemical and spectroscopic evidence.

Introduction. – 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][3]. This herbal medicine is also well-known as an ornamental plant, and its flowers and young leaves are edible as a salad. During the course of our studies on bioactive constituents from medicinal flowers [1–20], we found that the MeOH extract from the flowers of *B. perennis* was found to inhibit the increase of plasma triglyceride (TG) levels in olive-oil-loaded mice [2]. From the MeOH extract, 13 acylated saponin constituents, perennisosides I–VII [2] and perennisaponins A–F [3], were isolated, together with eight saponins and two glycosides [2][3].

Our continuing search now led to the isolation and characterization of seven new acylated oleanane-type triterpene oligoglycosides named perennisaponins G–M (**1**–**7**), which were obtained from the flowers of *B. perennis*. Furthermore, we examined the inhibitory effects against pancreatic lipase of perennisaponins **1**–**7**.

Results and Discussion. – The flowers of *B. perennis* cultivated in Albania were treated with MeOH to give an extract (25.8% from the dried flowers), as described previously [2]. The MeOH extract, which was found to inhibit pancreatic lipase activity (IC_{50} 455 mg/ml), was partitioned between AcOEt and H_2O (1:1) to furnish an

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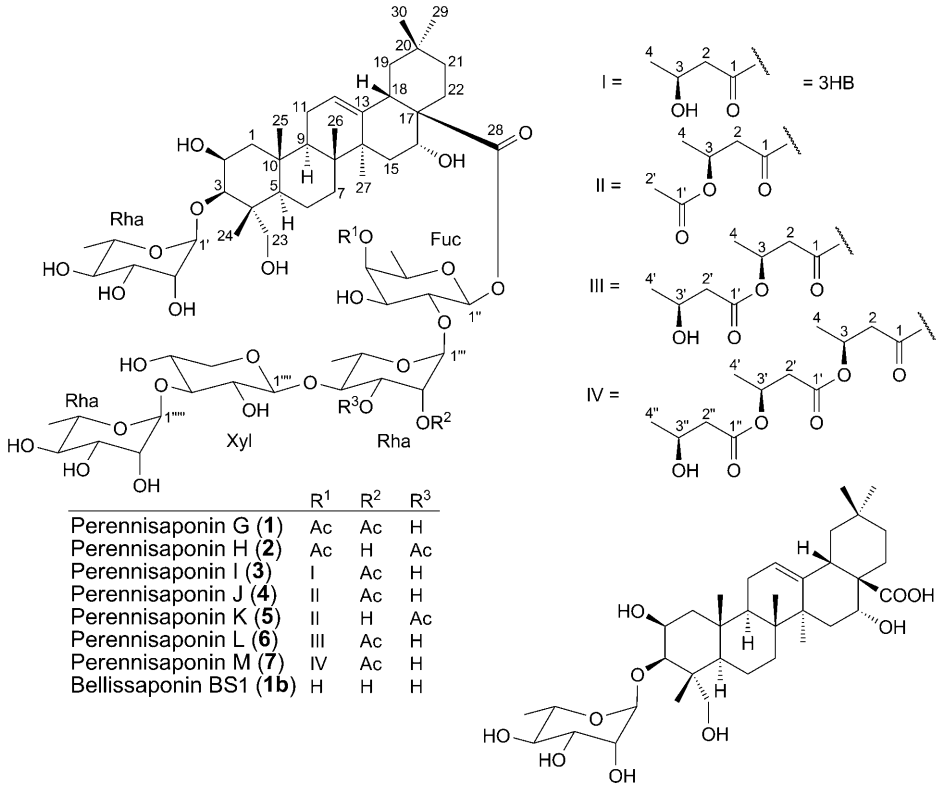
AcOEt-soluble fraction (6.7%) and an aqueous phase. The aqueous phase was subjected to column chromatography (*Diaion HP-20*, $H_2O \rightarrow$ MeOH) to give H_2O- and MeOH-eluted fractions (12.5 and 6.4%), respectively [2]. The MeOH-eluted fraction was subjected to normal- and reversed-phase column chromatographies and finally HPLC to give **1** (0.124%), **2** (0.139%), **3** (0.0038%), **4** (0.060%), **5** (0.027%), **6** (0.0028%), and **7** (0.065%) (*Fig. 1*).



	R^1	R^2	R^3
Perennisaponin G (**1**)	Ac	Ac	H
Perennisaponin H (**2**)	Ac	H	Ac
Perennisaponin I (**3**)	I	Ac	H
Perennisaponin J (**4**)	II	Ac	H
Perennisaponin K (**5**)	II	H	Ac
Perennisaponin L (**6**)	III	Ac	H
Perennisaponin M (**7**)	IV	Ac	H
Bellissaponin BS1 (**1b**)	H	H	H

Polygalacic acid 3-(α-L-rhamnopyranoside) (**1a**)

Fig. 1. *Perennisaponins G–M* (**1**–**7**) *isolated from* Bellis perennis

Compound **1** was obtained as an amorphous powder with a negative optical rotation. The IR spectrum of **1** showed absorption bands at 1736 and 1638 cm^{-1} ascribable to ester C=O and olefin moieties, and broad bands at 3450 and 1049 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion-mode FAB-MS of **1**, quasi-molecular-ion peaks were observed at *m/z* 1327 ($[M+Na]^+$) and 1303 ($[M-H]^-$), respectively, and a positive-mode HR-FAB-MS analysis revealed the molecular formula of **1** to be $C_{63}H_{100}O_{28}$. On alkaline hydrolysis of **1** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1, polygalacic acid 3-(α-L-rhamnopyranoside) (**1a**) [21] was obtained together with AcOH, which was identified by HPLC analysis of the

corresponding 4-nitrobenzyl derivative [3–7][18]. Treatment of **1** with 0.5% MeONa/MeOH provided a deacyl derivative, bellissaponin BS1 (=(2β,3β,4α,16α)-3-[(6-deoxy-α-L-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic acid *O*-6-deoxy-α-L-mannopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-6-deoxy-β-D-galactopyranosyl ester; **1b**) [3]. The ^{1}H- and ^{13}C-NMR data of **1** (*Tables 1* and *2*), which were assigned by various NMR experiments (including DEPT, DQF, HMQC, HMBC, and TOCSY) showed signals assignable to six Me *s* at δ(H) 0.93 (Me(29)), 1.03 (Me(30)), 1.17 (Me(26)), 1.23 (Me(24)), 1.58 (Me(25)), and 1.75 (Me(27)), an oxygenated CH_2 group at δ(H) 3.72 (2 br. *s*, CH_2(23)), three oxygenated CH groups at δ(H) 4.39 (br. *s*, H–C(3)), 4.72–4.76 (*m*, H–C(2)), and 5.16 (br. *s*, H–C(16)), an olefinic H-atom at δ(H) 5.64 (*t*-like, $J \approx 3$ Hz, H–C(12)), a fucopyranosyl moiety at δ(H) 1.24 (*d*, $J = 6.4$ Hz, Me(6″)) and 6.02 (*d*, $J = 8.3$ Hz, H–C(1″)), three rhamnopyranosyl moieties at δ(H) 1.60 (*d*, $J = 6.0$ Hz, Me(6′)), 1.63 (*d*, $J = 6.0$ Hz, Me(6″″)), 1.75 (*d*, $J = 6.2$ Hz, Me(6‴)), 5.76 (br. *s*, H–C(1′)), 6.07 (br. *s*, H–C(1‴)), and 6.21 (br. *s*, H–C(1″″)), and a xylopyranosyl moiety at δ(H) 5.14 (*d*, $J = 7.6$ Hz, H–C(1″′)), together with two AcO groups at δ(H) 1.98 and 1.99 (2 *s*, 2 Me). Comparison of the ^{13}C-NMR data of **1** with those of the deacetyl derivative **1b** revealed acetylation shifts around the 4″-position of the β-D-fucopyranosyl moiety (**1**: δ(C) 73.9 (C(3″)), 74.4 (C(4″)), and 70.4 (C(5″)); **1b**: δ(C) 76.5 (C(3″)), 73.1 (C(4″)), and 72.5 (C(5″))) and around the 2‴-position of the inner α-L-rhamnopyranosyl moiety of the 28-*O*-sugars (**1**: δ(C) 98.8 (C(1‴)), 73.3 (C(2‴)), and 70.0 (C(3‴)); **1b**: δ(C) 101.4 (C(1‴)), 71.9 (C(2‴)), and 72.6 (C(3‴))). The positions of the two AcO groups in **1** were clarified on the basis of an HMBC experiment, which showed ^{1}H → ^{13}C long-range correlations between the following pairs as shown in *Fig. 2:* δ(H) 1.98 (Me of Ac) and 5.51 (br. *s*, H–C(4″) of Fuc)/δ(C) 171.1 (C=O of Ac), and δ(H) 1.99 (Me of Ac) and 5.99–6.01 (*m*, H–C(2‴) of the inner Rha)/δ(C) 170.4 (C=O of Ac). On the basis

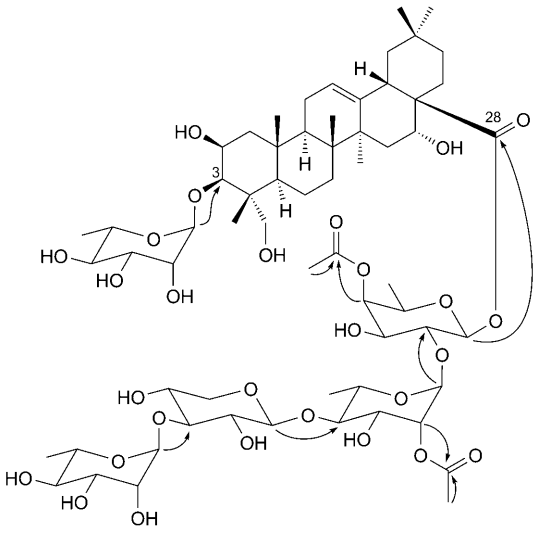


Fig. 2. *Selected HMBCs* (H → C) *of* **1**

of the above evidence, the structure of perennisaponin G (**1**) was determined as 3-*O*-*α*-L-rhamnopyranosylpolygalacic acid 28-[*O*-*α*-L-rhamnopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-2-*O*-acetyl-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-acetyl-*β*-D-fucopyranosyl] ester.

Table 1. *Selected ^{1}H-NMR Data* (600 MHz, 40°, (D_5)pyridine) *of* **1**–**3**. δ in ppm, J in Hz.

	1	**2**	**3**
H–C(2)	4.72–4.76 (*m*)	4.70–4.74 (*m*)	4.72–4.76 (*m*)
H–C(3)	4.39 (br. *s*)	4.38 (*d*, *J* = 2.8)	4.40 (*d*, *J* = 3.1)
H–C(12)	5.64 (*t*-like, *J* ≈ 3)	5.63 (*t*-like, *J* ≈ 3)	5.65 (*t*-like, *J* ≈ 3)
H–C(16)	5.16 (br. *s*)	5.16 (br. *s*)	5.16 (br. *s*)
H–C(18)	3.39 (*dd*-like)	3.41 (*dd*, *J* = 3.4, 14.4)	3.40 (*dd*-like)
CH_2(23)	3.72 (br. *s*)	3.70 (br. *s*)	3.71 (br. *s*)
Me(24)	1.23 (*s*)	1.26 (*s*)	1.24 (*s*)
Me(25)	1.58 (*s*)	1.55 (*s*)	1.59 (*s*)
Me(26)	1.17 (*s*)	1.14 (*s*)	1.19 (*s*)
Me(27)	1.75 (*s*)	1.75 (*s*)	1.76 (*s*)
Me(29)	0.93 (*s*)	0.92 (*s*)	0.93 (*s*)
Me(30)	1.03 (*s*)	1.03 (*s*)	1.03 (*s*)
3-O-*Rha:*			
H–C(1′)	5.76 (br. *s*)	5.76 (br. *s*)	5.78 (br. *s*)
Me(6′)	1.60 (*d*, *J* = 6.0)	1.60 (*d*, *J* = 6.2)	1.62 (*d*, *J* = 6.1)
28-O-*Sugars:*			
Fuc:			
H–C(1″)	6.02 (*d*, *J* = 8.3)	6.06 (*d*, *J* = 6.9)	6.03 (*d*, *J* = 8.3)
H–C(4″)	5.51 (br. *s*)	5.47 (br. *s*)	5.59 (br. *d*, *J* ≈ 4)
Me(6″)	1.24 (*d*, *J* = 6.4)	1.24 (*d*, *J* = 6.2)	1.32 (*d*, *J* = 6.4)
AcO–C(4″)	1.98 (*s*)	1.99 (*s*)	
3HBO–C(4′):			
CH_2(2)			2.68 (*dd*, *J* = 5.2, 14.4), 2.78 (*dd*, *J* = 7.6, 14.4)
H–C(3)			4.46–4.48 (*m*)
Me(4)			1.39 (*d*, *J* = 6.4)
Inner Rha:			
H–C(1‴)	6.07 (br. *s*)	6.07 (br. *s*)	6.09 (*d*, *J* = 1.5)
H–C(2‴)	5.99–6.01 (*m*)	5.00–5.02 (*m*)	5.99 (*dd*, *J* = 1.5, 3.4)
H–C(3‴)	4.79 (*dd*, *J* = 3.4, 9.8)	5.87 (*dd*, *J* = 2.7, 9.6)	4.78 (*dd*, *J* = 3.6, 9.8)
Me(6‴)	1.75 (*d*, *J* = 6.2)	1.75 (*d*, *J* = 6.2)	1.75 (*d*, *J* = 6.1)
AcO–C(2‴)	1.99 (*s*)		1.96 (*s*)
AcO–C(3‴)		2.04 (*s*)	
Xyl:			
H–C(1⁗)	5.14 (*d*, *J* = 7.6)	4.98 (*d*, *J* = 7.6)	5.15 (*d*, *J* = 7.7)
Terminal Rha:			
H–C(1⁗)	6.21 (br. *s*)	6.21 (br. *s*)	6.17 (br. *s*)
Me(6⁗)	1.63 (*d*, *J* = 6.0)	1.66 (*d*, *J* = 6.2)	1.64 (*d*, *J* = 6.1)

Compound **2** was also obtained as an amorphous powder with a negative optical rotation. The IR spectrum of **2** showed absorption bands at 3450, 1734, 1638, and 1049 cm^{-1}, ascribable to OH, ester C=O, olefin, and ether functions. The molecular formula of **2**, $C_{63}H_{100}O_{28}$, was determined from the positive- and negative-ion-mode

Table 2. *^{13}C-NMR Data of* **1**–**3**. At 150 MHz, 40°, in (D_5)pyridine; δ in ppm.

	1	**2**	**3**		**1**	**2**	**3**
C(1)	44.8	44.8	44.8	*28*-O-*Sugars:*			
C(2)	71.1	71.1	71.1	*Fuc:*			
C(3)	81.4	81.4	81.5	C(1″)	94.3	94.2	94.3
C(4)	43.0	43.0	43.0	C(2″)	75.1	76.8	75.2
C(5)	47.6	47.7	47.6	C(3″)	73.9	74.1	73.5
C(6)	18.3	18.3	18.3	C(4″)	74.4	74.4	74.4
C(7)	33.2	33.2	33.3	C(5″)	70.4	70.3	70.5
C(8)	40.2	40.2	40.3	C(6″)	16.4	16.4	16.5
C(9)	47.5	47.5	47.5	*Ac*O–C(4″)	171.1, 20.7	171.1, 20.7	
C(10)	37.2	37.1	37.2	*3HBO–C(4″):*			
C(11)	24.1	24.1	24.1	C(1)			172.3
C(12)	122.8	122.8	122.8	C(2)			45.0
C(13)	144.2	144.2	144.2	C(3)			64.8
C(14)	42.3	42.2	42.3	C(4)			24.0
C(15)	36.2	36.1	36.2	*Inner Rha:*			
C(16)	74.1	74.1	74.1	C(1‴)	98.8	102.7	98.7
C(17)	49.7	49.7	49.5	C(2‴)	73.3	69.4	73.2
C(18)	41.6	41.6	41.6	C(3‴)	70.0	75.5	70.1
C(19)	47.3	47.3	47.3	C(4‴)	83.8	77.9	83.8
C(20)	30.7	30.8	30.8	C(5‴)	68.5	68.9	68.5
C(21)	35.9	35.9	36.0	C(6‴)	18.6	19.2	18.5
C(22)	32.0	31.9	32.0	*Ac*O–C(2‴)	170.4, 20.9		170.4, 20.9
C(23)	65.3	65.2	65.3	*Ac*O–C(3‴)		170.9, 21.4	
C(24)	14.9	15.0	15.0	*Xyl:*			
C(25)	17.7	17.7	17.8	C(1⁗)	106.8	105.8	106.8
C(26)	17.7	17.6	17.7	C(2⁗)	76.2	75.0	76.2
C(27)	27.1	27.1	27.1	C(3⁗)	83.3	83.3	83.4
C(28)	176.1	176.2	176.2	C(4⁗)	69.2	69.8	69.3
C(29)	33.1	33.1	33.1	C(5⁗)	67.3	67.1	67.3
C(30)	24.5	24.6	24.6	*Terminal Rha:*			
3-O-*Rha:*				C(1⁗)	102.6	102.6	102.6
C(1′)	104.2	104.1	104.2	C(2⁗)	72.5	72.5	72.5
C(2′)	72.4	72.4	72.5	C(3⁗)	72.6	72.7	72.6
C(3′)	72.7	72.7	72.8	C(4⁗)	73.9	73.9	74.0
C(4′)	73.2	73.5	74.0	C(5⁗)	69.9	69.8	70.0
C(5′)	70.3	70.3	70.3	C(6⁗)	18.5	18.6	18.6
C(6′)	18.6	18.6	18.6				

FAB-MS (*m*/*z* 1327 ($[M+Na]^+$) and 1303 ($[M-H]^-$), resp.) and by positive-ion-mode HR-FAB-MS, which was the same as that of **1**. Alkaline hydrolysis of **2** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 liberated **1a** together with AcOH. Treatment of **2** with 0.5% MeONa/MeOH yielded **1b**. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*) indicated the presence of the following functions: a polygalacic acid part (six Me *s* at δ(H) 0.92 (Me(29)), 1.03 (Me(30)), 1.14 (Me(26)), 1.26 (Me(24)), 1.55 (Me(25)), and 1.75 (Me(27)), an oxygenated CH_2 group at δ(H) 3.70 (br. *s*, CH_2(23)), three oxygenated CH groups at δ(H) 4.38 (*d*, $J = 2.8$ Hz, H–C(3)), 4.70–4.74 (*m*, H–C(2)), and 5.16 (br. *s*, H–C(16)), and an olefinic H-atom at δ(H) 5.63 (*t*-like, $J \approx 3$ Hz,

H–C(12))), a fucopyranosyl moiety at δ(H) 1.24 (*d*, *J* = 6.2 Hz, Me(6″)) and 6.06 (*d*, *J* = 6.9 Hz, H–C(1″)), three rhamnopyranosyl moieties at δ(H) 1.60 (*d*, *J* = 6.2 Hz, Me(6′)), 1.66 (*d*, *J* = 6.2 Hz, Me(6‴″)), 1.75 (*d*, *J* = 6.2 Hz, Me(6‴)), 5.76 (br. *s*, H–C(1′)), 6.07 (br. *s*, H–C(1‴)), and 6.21 (br. *s*, H–C(1‴″)), and a xylopyranosyl moiety at δ(H) 4.98 (*d*, *J* = 7.6 Hz, H–C(1‴′)), together with two AcO groups at δ(H) 1.99 and 2.04 (2*s*, 2 Me). The ^{1}H- and ^{13}C-NMR data of **2** were superimposable on those of **1**, except for the signals due to the part around the inner Rha unit of the 28-*O*-sugars. The positions of the two AcO groups in **2** were determined by an HMBC experiment, which showed the following ^{1}H → ^{13}C long-range correlations: δ(H) 1.99 (*s*, Me of Ac) and 5.47 (br. *s*, H–C(4″) of Fuc)/δ(C) 171.1 (C=O of Ac), and δ(H) 2.04 (*s*, Me of Ac) and 5.87 (*dd*, *J* = 2.7, 9.6 Hz, H–C(3‴) of the inner Rha)/δ(C) 170.9 (C=O of Ac). Consequently, the structure of **2** was elucidated as 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-3-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-acetyl-β-D-fucopyranosyl] ester.

Compound **3** was also obtained as an optically active amorphous powder. The positive- and negative-ion-mode FAB-MS of **3** showed quasi-molecular-ion peaks at *m*/*z* 1371 ([*M* + Na]$^+$) and 1347 ([*M* – H]$^-$), respectively. The positive-ion-mode HR-FAB-MS of **3** revealed the molecular formula to be $C_{65}H_{104}O_{29}$. Alkaline hydrolysis of **3** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 liberated **1a** and two organic acids, AcOH and 3-hydroxybutanoic acid (3HBOH), which were identified by HPLC analysis of the corresponding 4-nitrobenzyl derivatives. In addition, treatment of **3** with 0.5% MeONa/MeOH gave **1b**, together with methyl (+)-(3*S*)-3-hydroxybutanoate [22], which was identified by HPLC and an optical-rotation detector [23–27]. The H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*, resp.) were superimposable on those of **1**, except for the signals due to the acyl groups: an AcO group at δ(H) 1.96 (*s*, Me of Ac) and a (3*S*)-3-hydroxybutanoyl (3HB) group at δ(H) 1.39 (*d*, *J* = 6.4 Hz, Me(4)), 2.68 (*dd*, *J* = 5.2, 14.4 Hz, 1 H–C(2)), 2.78 (*dd*, *J* = 7.6, 14.4 Hz, 1 H–C(2)), and 4.46–4.48 (*m*, H–C(3)). In the HMBC experiment with **3**, the following ^{1}H → ^{13}C long-range correlations were observed: CH_2(2) of 3HB, H–C(3) of 3HB, and δ(H) 5.59 (br. *d*, *J* ≈ 4 Hz, H–C(4″) of Fuc)/δ(C) 172.3 (C=O of 3HB); and δ(H) 1.96 (*s*, Me of Ac) and 5.99 (*dd*, *J* = 1.5, 3.4 Hz, H–C(2‴) of the inner Rha)/δ(C) 170.4 (C=O of Ac). Thus, the connectivities of the acyloxy groups in **3** were elucidated. On the basis of above-mentioned evidence, the structure of **3** was determined as 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(3*S*)-3-hydroxybutanoyl-β-D-fucopyranosyl] ester.

Compounds **4** and **5** were obtained as amorphous powders with negative optical rotations. The molecular formula of both **4** and **5**, $C_{67}H_{106}O_{30}$, was determined from the positive- and negative-ion-mode FAB-MS at *m*/*z* 1413 ([*M* + Na]$^+$) and 1389 ([*M* – H]$^-$) and by positive-ion-mode HR-FAB-MS measurement. Alkaline hydrolysis of **4** and **5** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 provided **1a** and two organic acids, AcOH and 3HBOH, respectively. Treatment of each **4** and **5** with 0.5% MeONa/MeOH gave **1b**, together with methyl (+)-(3*S*)-3-hydroxybutanoate, respectively. The H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **4** (*Tables 3* and *4*) indicated the presence of an aglycon part, containing six Me *s* at δ(H) 0.95 (Me(29)), 1.06 (Me(30)), 1.18 (Me(26)), 1.22 (Me(24)), 1.58 (Me(25)), and 1.75 (Me(27)), a CH_2 and

three CH groups bearing an O-atom function at δ(H) 3.69 (br. *s*, CH_2(23)), 4.35 (*d*, *J* = 3.5 Hz, H−C(3)), 4.68–4.72 (*m*, H−C(2)), and 5.12 (br. *s*, H−C(16)), an olefinic H-atom at δ(H) 5.65 (*t*-like, $J \approx 3$ Hz, H−C(12)), and a fucopyranosyl moiety at δ(H) 1.25 (*d*, *J* = 6.3 Hz, Me(6″)) and 5.99 (*d*, *J* = 8.0 Hz, H−C(1″)), three rhamnopyranosyl moieties at δ(H) 1.60 (*d*, *J* = 6.1 Hz, Me(6′)), 1.67 (*d*, *J* = 6.3 Hz, Me(6‴″)), 1.75 (*d*, *J* = 6.4 Hz, Me(6‴)), 5.71 (br. *s*, H−C(1′)), 6.04 (*d*, *J* = 1.8 Hz, H−C(1‴)), and 6.08 (br. *s*, H−C(1‴″)), and a xylopyranosyl moiety at δ(H) 5.10 (*d*, *J* = 7.7 Hz, H−C(1‴)), together with two AcO groups at δ(H) 1.98 (2*s*, 2 Me) and a 3 HBO group at δ(H) 1.30 (*d*, *J* = 6.3 Hz, Me(4)), 2.68 (*dd*, *J* = 5.5, 15.8 Hz, 1 H−C(2)), 2.78 (*dd*, *J* = 7.5, 15.8 Hz, 1 H−C(2)), and 5.49–5.51 (*m*, H−C(3)). The H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **4** resembled those of **3**, except for the signals due to the additional AcO group. Comparison of the ^{13}C-NMR spectra of **4** with those of **3** revealed an acetylation shift around the 3-position of the *3HBO*−C(4″) group (**4**: δ(C) 40.8 (C(2)), 67.5 (C(3)), and 19.9 (C(4)); **3**: δ(C) 45.0 (C(2)), 64.8 (C(3)), and 24.0 (C(4))). The connectivity of the above-mentioned AcO group in **4** was clarified by an HMBC experiment, which showed ^{1}H → ^{13}C long-range correlations between δ(H) 1.98 (*s*, Me of Ac) and H−C(3) of 3HBO−C(4″) and δ(C) 170.0 (C=O of Ac). In turn, the H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **5** (*Tables 3* and *4*) resembled those of **4**, except for the signals due to the inner Rha of the 28-*O*-sugars. The position of an AcO group at the inner Rha of **5** was established by an HMBC experiment, which showed ^{1}H → ^{13}C long-range correlations between δ(H) 2.05 (*s*, Me of Ac) and 5.84 (*dd*, *J* = 2.5, 8.9 Hz, H−C(3‴) of the inner Rha) and δ(C) 170.9 (C=O of Ac). On the basis of the above-mentioned evidence, the structures of **4** and **5** were elucidated to be 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(3*S*)-3-(acetyloxy)butanoyl]-β-D-fucopyranosyl} ester and 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-3-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(3*S*)-3-(acetyloxy)butanonyl]-β-D-fucopyranosyl} ester, respectively.

The molecular formulas of compound **6**, $C_{69}H_{110}O_{31}$, and compound **7**, $C_{73}H_{116}O_{33}$, were determined from their respective positive- and negative-ion-mode FAB-MS and by positive-ion-mode HR-FAB-MS data. Alkaline hydrolysis of **6** and **7** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 provided **1a** and two organic acids, AcOH and 3HBOH, respectively. Treatment of **6** and **7** with 0.5% MeONa/MeOH gave **1b**, together with (+)-(3*S*)-3-hydroxybutanoic acid. The H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **6** (*Tables 3* and *4*) showed signals assignable to a bellissaponin BS1 (**1b**) moiety, an AcO group at δ(H) 1.99 (*s*, Me) and two 3HBO groups at δ(H) 1.35 (*d*, *J* = 6.2 Hz, Me(4)), 1.38 (*d*, *J* = 6.9 Hz, Me(4′)), 2.61–2.65 and 2.71–2.75 (2 *m*, CH_2(2′)), 2.68–2.72 and 2.82–2.86 (2 *m*, CH_2(2)), 4.53–4.57 (*m*, H−C(3′)), and 5.59–5.61 (*m*, H−C(3)). The connectivities of the acyl moieties in **6** were determined through an HMBC experiment, which exhibited ^{1}H → ^{13}C long-range correlations between the following pairs: δ(H) 1.99 (*s*, Me of Ac) and 6.01–6.03 (*m*, H−C(2‴) of the inner Rha)/δ(C) 170.5 (C=O of Ac), CH_2(2) of 3HBO−C(4″), H−C(3) of 3HBO−C(4″), and δ(H) 5.53 (br. *d*, $J \approx 3$ Hz, H−C(4″) of Fuc)/δ(C) 170.8 (C=O of 3HBO−C(4″)), and CH_2(2′) of 3HBO−C(3), H−C(3′) of 3HBO−C(3), and H−C(3) of 3HBO−C(4″)/δ(C) 171.3 (C=O of 3HBO−C(3)). By comparison of

Table 3. *Selected ^{1}H-NMR Data* (600 MHz, 40°, (D_5)pyridine) *of* **4**–**7**. δ in ppm, J in Hz.

	4	**5**	**6**	**7**
H–C(2)	4.68–4.72 (*m*)	4.71 (br. *d*, $J\approx3$)	4.74–4.78 (*m*)	4.70 (br. *s*)
H–C(3)	4.35 (*d*, $J=3.5$)	4.39 (*d*, $J=2.9$)	4.41 (br. *s*)	4.36 (*d*, $J=3.2$)
H–C(12)	5.65 (*t*-like, $J\approx3$)	5.65 (*t*-like, $J\approx3$)	5.67 (*t*-like, $J\approx3$)	5.63 (*t*-like, $J\approx3$)
H–C(16)	5.12 (br. *s*)	5.14 (br. *s*)	5.16 (br. *s*)	5.12 (br. *s*)
H–C(18)	3.39 (*dd*, $J=4.3, 14.1$)	3.40 (*dd*, $J=3.4, 11.9$)	3.41 (*dd*, $J=4.2, 13.7$)	3.40 (*dd*-like)
CH_2(23)	3.69 (br. *s*)	3.70 (br. *s*)	3.72 (br. *s*)	3.70 (br. *s*)
Me(24)	1.22 (*s*)	1.24 (*s*)	1.25 (*s*)	1.25 (*s*)
Me(25)	1.58 (*s*)	1.57 (*s*)	1.60 (*s*)	1.59 (*s*)
Me(26)	1.18 (*s*)	1.17 (*s*)	1.19 (*s*)	1.18 (*s*)
Me(27)	1.75 (*s*)	1.75 (*s*)	1.76 (*s*)	1.75 (*s*)
Me(29)	0.95 (*s*)	0.94 (*s*)	0.94 (*s*)	0.94 (*s*)
Me(30)	1.06 (*s*)	1.07 (*s*)	1.04 (*s*)	1.06 (*s*)
3-O-*Rha:*				
H–C(1′)	5.71 (br. *s*)	5.72 (br. *s*)	5.79 (br. *s*)	5.72 (br. *s*)
Me(6′)	1.60 (*d*, $J=6.1$)	1.60 (*d*, $J=6.0$)	1.62 (*d*, $J=6.2$)	1.60 (*d*, $J=6.0$)
28-O-*Sugars:*				
Fuc:				
H–C(1″)	5.99 (*d*, $J=8.0$)	6.03 (*d*, $J=7.6$)	6.02 (*d*, $J=8.2$)	6.00 (*d*, $J=8.0$)
H–C(4″)	5.50 (br. *d*, $J\approx4$)	5.48 (br. *d*, $J\approx3$)	5.53 (br. *d*, $J\approx3$)	5.50 (br. *s*)
Me(6″)	1.25 (*d*, $J=6.3$)	1.25 (*d*, $J=6.0$)	1.27 (*d*, $J=6.2$)	1.25 (*d*, $J=6.3$)
3HBO–C(4″):				
CH_2(2)	2.68 (*dd*, $J=5.5, 15.8$), 2.78 (*dd*, $J=7.5, 15.8$)	2.68 (*dd*, $J=4.6, 13.1$), 2.78 (*dd*, $J=6.2, 13.1$)	2.68–2.72 (*m*), 2.82–2.86 (*m*)	2.65–2.70 (*m*)[a]), 2.74–2.81 (*m*)[b])
H–C(3)	5.49–5.51 (*m*)	5.50–5.52 (*m*)	5.59–5.61 (*m*)	5.52–5.56 (*m*)[c])
Me(4)	1.30 (*d*, $J=6.3$)	1.30 (*d*, $J=6.3$)	1.35 (*d*, $J=6.2$)	1.34 (*d*, $J=6.3$)[d])
AcO–C(3)	1.98 (*s*)	2.00 (*s*)		
3HBO–C(3):				
CH_2(2′)			2.61–2.65 (*m*), 2.71–2.75 (*m*)	2.65–2.70 (*m*)[a]), 2.74–2.81 (*m*)[b])
H–C(3′)			4.53–4.57 (*m*)	5.52–5.56 (*m*)[c])
Me(4′)			1.38 (*d*, $J=6.9$)	1.31 (*d*, $J=6.3$)[d])
3HBO–C(3′):				
CH_2(2″)				2.60 (*dd*, $J=5.2, 14.9$), 2.74–2.81 (*m*)[b])
H–C(3″)				4.52–4.54 (*m*)
Me(4″)				1.38 (*d*, $J=6.1$)
Inner Rha:				
H–C(1‴)	6.04 (*d*, $J=1.8$)	6.03 (br. *s*)	6.10 (br. *s*)	6.05 (br. *s*)
H–C(2‴)	5.94 (*dd*, $J=1.8, 3.5$)	4.95–4.97 (*m*)	6.01–6.03 (*m*)	5.94 (*dd*, $J=1.5, 3.2$)
H–C(3‴)	4.73 (*dd*, $J=3.5, 9.4$)	5.84 (*dd*, $J=2.5, 8.9$)	4.80–4.84 (*m*)	4.74 (*dd*, $J=3.2, 9.5$)
Me(6‴)	1.75 (*d*, $J=6.4$)	1.74 (*d*, $J=6.4$)	1.78 (*d*, $J=6.8$)	1.75 (*d*, $J=6.4$)
AcO–C(2‴)	1.98 (*s*)		1.99 (*s*)	1.99 (*s*)
AcO–C(3‴)		2.05 (*s*)		
Xyl:				
H–C(1⁗)	5.10 (*d*, $J=7.7$)	4.95 (*d*, $J=7.6$)	5.16 (*d*, $J=6.8$)	5.10 (*d*, $J=7.7$)
Terminal Rha:				
H–C(1⁗′)	6.08 (br. *s*)	6.13 (br. *s*)	6.18 (br. *s*)	6.08 (br. *s*)
Me(6⁗′)	1.67 (*d*, $J=6.3$)	1.65 (*d*, $J=6.2$)	1.65 (*d*, $J=6.2$)	1.62 (*d*, $J=6.3$)

[a])[b])[c]) Overlapped. [d]) May be interchangeable within the same column.

Table 4. *^{13}C-NMR Data of* **4**–**7**. At 150 MHz, 40°, in (D_5)pyridine; δ in ppm.

	4	**5**	**6**	**7**		**4**	**5**	**6**	**7**
C(1)	44.8	44.8	44.9	44.8	*28*-O-*Sugars:*				
C(2)	71.0	71.0	71.1	71.0	*Fuc:*				
C(3)	81.6	81.6	81.4	81.6	C(1″)	94.4	94.2	94.3	94.4
C(4)	43.0	43.0	43.0	43.0	C(2″)	75.0	76.7	74.9	75.0
C(5)	47.7	47.7	47.6	47.7	C(3″)	73.3	73.4	73.2	73.3
C(6)	18.4	18.3	18.3	18.4	C(4″)	74.8	74.7	74.7	74.8
C(7)	33.3	33.2	33.3	33.3	C(5″)	70.3	70.3	70.3	70.4
C(8)	40.3	40.3	40.3	40.3	C(6″)	16.4	16.4	16.4	16.4
C(9)	47.6	47.6	47.6	47.6	*3HBO–C(4″):*				
C(10)	37.2	37.2	37.2	37.2	C(1)	170.7	170.7	170.8	170.7
C(11)	24.1	24.1	24.1	24.1	C(2)	40.8	40.8	40.7	40.7[a])
C(12)	122.8	122.8	122.8	122.8	C(3)	67.5	67.5	67.4	67.9[b])
C(13)	144.3	144.2	144.3	144.3	C(4)	19.9	19.8	19.8	19.8[c])
C(14)	42.4	42.2	42.4	42.4	*Ac*O–C(3)	170.0, 21.1	170.1, 21.1		
C(15)	36.2	36.1	36.2	36.2	*3HBO–C(3):*				
C(16)	74.1	74.2	74.1	74.2	C(1′)			171.3	169.7
C(17)	49.6	49.5	49.5	49.6	C(2′)			45.2	41.2[a])
C(18)	41.7	41.6	41.6	41.7	C(3′)			64.4	67.6[b])
C(19)	47.4	47.4	47.3	47.4	C(4′)			23.9	19.9[c])
C(20)	30.8	30.7	30.8	30.8	*3HBO–C(3′):*				
C(21)	36.0	36.0	36.0	36.0	C(1″)				171.4
C(22)	31.9	31.7	32.0	31.9	C(2″)				45.1
C(23)	65.4	65.3	65.3	65.4	C(3″)				64.4
C(24)	15.0	15.0	15.0	15.0	C(4″)				23.9
C(25)	17.8	17.7	17.8	17.7	*Inner Rha:*				
C(26)	17.7	17.7	17.7	17.7	C(1‴)	98.8	102.5	98.7	98.7
C(27)	27.1	27.1	27.1	27.1	C(2‴)	73.3	69.5	73.2	73.3
C(28)	176.2	176.3	176.2	176.2	C(3‴)	70.1	75.5	70.1	70.0
C(29)	33.1	33.1	33.1	33.1	C(4‴)	84.0	77.9	83.7	83.9
C(30)	24.7	24.7	24.5	24.7	C(5‴)	68.6	68.9	68.6	68.6
3-O-*Rha:*					C(6‴)	18.5	19.1	18.5	18.5
C(1′)	104.1	104.0	104.2	104.1	*Ac*O–C(2‴)	170.4, 20.9		170.5, 20.9	170.4, 20.9
C(2′)	72.5	72.4	72.5	72.5	*Ac*O–C(3‴)		170.9, 21.3		
C(3′)	72.8	72.8	72.8	72.8	*Xyl:*				
C(4′)	74.0	74.1	74.0	74.0	C(1⁗)	106.8	105.7	106.7	106.8
C(5′)	70.3	70.2	70.3	70.4	C(2⁗)	76.1	75.0	76.2	76.1
C(6′)	18.6	18.5	18.6	18.6	C(3⁗)	83.7	83.6	83.3	83.7
					C(4⁗)	69.3	69.8	69.3	69.3
					C(5⁗)	67.3	67.1	67.3	67.3
					Terminal Rha:				
					C(1⁗′)	102.6	102.7	102.6	102.6
					C(2⁗′)	72.4	72.4	72.4	72.4
					C(3⁗′)	72.6	72.7	72.6	72.6
					C(4⁗′)	74.0	74.0	74.0	74.0
					C(5⁗′)	70.0	69.8	70.0	70.0
					C(6⁗′)	18.5	18.5	18.6	18.5

[a])[b])[c]) Interchangeable within the same column.

the ^{1}H- and ^{13}C-NMR spectra of **7** (*Tables 3* and *4*) with those of **6**, **7** had one more 3HBO group than **6** at δ(H) (1.31 (*d*, $J = 6.3$ Hz), 1.34 (*d*, $J = 6.3$ Hz), 1.38 (*d*, $J = 6.1$ Hz), Me(4′), Me(4), and Me(4″)), 2.65–2.70 and 2.74–2.81 (2 *m*, 2 H each, CH_2(2) and CH_2(2′)), 2.60 (*dd*, $J = 5.2$, 14.9 Hz, 1 H–C(2″)), 2.74–2.81 (*m*, 1 H–C(2″)), 4.52–4.54 (*m*, H–C(3″)), and 5.52–5.56 (*m*, H–C(3) and H–C(3′)). In the HMBC experiment of **7**, the following $^1H \rightarrow {}^{13}C$ long-range correlations were observed: CH_2(2″) of 3HBO–C(3′), H–C(3″) of 3HBO–C(3′), and H–C(3′) of 3HBO–C(3)/δ(C) 171.4 (C=O of 3HBO–C(3′)). Consequently, the structures of **6** and **7** were elucidated to be 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-{(3*S*)-3-[(3*S*)-3-(hydroxybutanoyl)oxy]butanoyl}-β-D-fucopyranosyl} ester and 3-*O*-α-L-rhamnopyranosylpolygalacic acid 28-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-{(3*S*)-3-{{(3*S*)-3-{[(3*S*)-3-hydroxybutanoyl]oxy}butanoyl}oxy}butanoyl}-β-D-fucopyranosyl} ester, respectively.

Pancreatic lipase is well known to play an important role in lipid digestion. Recently, inhibitory effects of saponins on pancreatic lipase were reported to be involved in anti-obese effects of saponins (*e.g.*, theasaponins, chikusetsusaponins) [28–31]. In the course of our characterization studies on anti-obese constituents from *B. perennis*, inhibitory effects of the constituents on pancreatic lipase activity were examined. Among the saponin constituents, perennisaponins G (**1**; IC_{50} 163 μM), H (**2**; 137 μM), I (**3**;147 μM), J (**4**; 148 μM), K (**5**; 223 μM), L (**6**; 81.4 μM), and M (**7**; 195 μM) were found to inhibit pancreatic lipase activity (*Table 5*). These saponins **1**–**7** are more

Table 5. *Inhibitory Effects of the MeOH Extract and of* **1**–**7** *from the Flowers of* B. perennis *against Pancreatic Lipase*

	Concentration [μg/ml][a])					IC_{50} [μg/ml]
	0	100	200	400	800	
MeOH Extract	0.0 ± 0.6	8.3 ± 1.8	21.4 ± 2.1[c])	43.8 ± 1.9[c])	71.6 ± 4.5[c])	455
	Concentration [μM][a])					IC_{50} [μM]
	0	50	100	200	400	
Perennisaponin G (**1**)	0.0 ± 2.8	− 18.8 ± 10.4	21.3 ± 5.8	58.9 ± 3.7[c])	85.5 ± 1.7[c])	163
Perennisaponin H (**2**)	0.0 ± 1.5	− 33.5 ± 12.2	14.7 ± 9.3	71.9 ± 3.1[c])	87.4 ± 1.3[c])	137
Perennisaponin I (**3**)	0.0 ± 1.1	− 17.0 ± 18.9	27.5 ± 8.9	60.2 ± 2.4[c])	85.2 ± 4.1[c])	147
Perennisaponin J (**4**)	0.0 ± 3.0	16.6 ± 13.6	38.3 ± 10.0[b])	55.9 ± 5.1[c])	55.2 ± 8.6[c])	148
Perennisaponin K (**5**)	0.0 ± 3.8	35.0 ± 5.0[c])	47.1 ± 5.0[c])	43.1 ± 10.1[c])	55.6 ± 6.5[c])	223
Perennisaponin L (**6**)	0.0 ± 4.2	26.9 ± 7.2[b])	55.3 ± 2.8[c])	52.7 ± 5.1[c])	68.5 ± 6.9[c])	81.4
Perennisaponin M (**7**)	0.0 ± 5.7	22.6 ± 12.0	28.5 ± 11.3	53.9 ± 3.8[c])	67.2 ± 5.6[c])	195
	Concentration [μM][a])					IC_{50} [μM]
	0	100	200	400	800	
Theasaponin E_1	0.0 ± 2.2	24.4 ± 4.3[b])	39.2 ± 7.9[c])	57.4 ± 9.1[c])	88.6 ± 2.6[c])	270

[a]) Values represent the means ± s.e.m ($N = 4$). Significantly different from the control group: [b]) $p < 0.05$. [c]) $p < 0.01$.

efficient than theasaponin E_1 (IC_{50} 270 μM) [30], which was isolated from *Camellia sinensis* [32], although their inhibitory activities were considerably weaker than that of the lipase inhibitor orlistat (IC_{50} 56 nM). On the basis of above *in vitro* results and our previous *in vivo* evidence [2], the saponin constituents from the flowers of *B. perennis* may be useful for the prevention of obesity.

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Experimental Part

General. Column chromatography (CC): normal-phase CC with silica gel *BW-200* (SiO_2; 150–300 mesh; *Fuji Silysia Chemical*, *Ltd.*, Japan), reversed-phase CC with *Chromatorex ODS DM1020T* (100–200 mesh; *Fuji Silysia Chemical*, *Ltd.*, Japan). HPLC: *Shimadzu-RID-6A* refractive-index, *SPD-10A* UV/VIS, and *Shodex-OR-2* optical-rotation detectors; *Shimadzu-LC-6AD* pump; *Shimadzu-CTO-10A* column oven; *Shimadzu-C-R6A* chromatopac; *Cosmosil-5C_{18}-MS-II* and *-HILIC* (*Nacalai Tesque, Inc.*), *Wakopak-Navi-C-30-5* (*Wako Pure Chemical Industries Ltd.*), and *YMC-Pack-ODS-A* and *YMC-Pack-ODS-AQ* (*YMC Co.*, *Ltd.*) columns; t_R in min. Optical rotations: *Horiba-SEPA-300* digital polarimeter ($l = 5$ cm). IR Spectra: *Shimadzu-FTIR-8100* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Jeol-JNM-ECA600* spectrometer; at 600 and 150 MHz, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. FAB- and HR-FAB-MS: *Jeol-JMS-SX-102A* mass spectrometer; in *m*/*z*.

Plant Material. The flowers of *B. perennis* cultivated in Albania were purchased from *Tochimoto Tenkaido Co., Ltd.*, in November 2006, as described previously [2][3].

Extraction and Isolation. Compounds **1**–**7** were isolated from previously reported fractions [2][3], *Fr. 6.11* (4.850 g) and *Fr. 6.12* (50.269 g), originally obtained from the MeOH-eluted fraction (6.4%, 140.0 g) of the MeOH extract from flowers of *B. perennis.* An aliquot of *Fr. 6.11* (1820.0 mg) was purified by HPLC (*Wakopak-Navi-C30-5*, 250 × 20 mm i.d., MeCN/MeOH/1% aq. AcOH 32 : 16 : 52; 9.0 ml/min) to afford the following 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). *Fr. 6.11.5* (263.0 mg) was purified by HPLC (*Cosmosil 5C_{18}-MS-II*, 250 × 20 mm i.d., MeCN/MeOH/1% aq. AcOH 32 : 16 : 52; 9.0 ml/min): perennisaponin I (**3**; 33.1 mg, 0.0038%; t_R 48.9). *Fr. 6.11.7* (137.9 mg) was purified by HPLC (*Cosmosil 5C_{18}-MS-II*, 250 × 20 mm i.d., MeCN/MeOH/1% aq. AcOH 32 : 16 : 52; 9.0 ml/min): perennisaponin G (**1**, 42.0 mg, 0.0048%; t_R 64.1). *Fr. 6.11.8* (88.6 mg) was purified by HPLC (*Cosmosil 5C_{18}-MS-II*, 250 × 20 mm i.d., MeCN/MeOH/1% aq. AcOH 32 : 16 : 52; 9.0 ml/min): perennisaponin L (**6**, 24.3 mg, 0.0028%; t_R 70.0). An aliquot of *Fr. 6.12* (2015.0 mg) was further separated by HPLC (*Wakopak-Navi-C30-5*, 250 × 20 mm i.d., MeCN/1% aq. AcOH 40 : 60; 9.0 ml/min) to give the following 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). *Fr. 6.12.1* (893.3 mg) was separated by HPLC (*Cosmosil 5C_{18}-MS-II*, 250 × 20 mm i.d., MeCN/MeOH/1% aq. AcOH 32 : 16 : 52; 9.0 ml/min) to furnish the following fourteen 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. 6.12.10.9* (34.9 mg), *Fr. 6.12.1.10* (125.8 mg, 0.139%; perennisaponin H (**2**); t_R 58.2), *Fr. 6.12.1.11* (57.9 mg), *Fr. 6.12.1.12* (109.2 mg, 0.119%; perennisaponin G (**1**); t_R 64.0), *Fr. 6.12.1.13* (56.0 mg), and *Fr. 6.12.1.14* (22.2 mg). An aliquot of *Fr. 6.12.2* (180.0 mg) was purified by HPLC (*Cosmosil HILIC*, 250 × 20 mm i.d., MeCN/H_2O 90 : 10; 9.9 ml/min): perennisaponins J (**4**, 12.7 mg, 0.026%; t_R 49.9) and M (**7**, 31.7 mg, 0.065%; t_R 52.7) together with perennisaponins C (9.3 mg, 0.019%; t_R 65.2), D (8.1 mg, 0.017%; t_R 57.0), E (10.4 mg, 0.021%; t_R 59.2), and F (28.5 mg, 0.059%; t_R 55.7).

Fr. 6.12.3 (131.3 mg) was purified by HPLC (*Cosmosil HILIC*, 250 × 20 mm i.d., MeCN/H_2O 90:10; 9.9 ml/min): perennisaponins J (**4**, 32.6 mg, 0.034%; t_R 54.2) and K (**5**, 25.6 mg, 0.027%; t_R 57.1).

Perennisaponin G (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-4*-O-*acetyl-6-deoxy-β-*D*-galactopyranosyl Ester*; **1**): Amorphous powder. $[\alpha]_D^{25} = -19.5$ ($c = 4.48$, MeOH). IR (KBr): 3450, 1736, 1638, 1049. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 1327 ($[M+Na]^+$). FAB-MS (neg.): 1303 ($[M-H]^-$), 1157 ($[M-C_6H_{11}O_4]^-$). HR-FAB-MS (pos.): 1327.6296 ($[M+Na]^+$, $C_{63}H_{100}NaO_{28}^+$; calc. 1327.6299).

Perennisaponin H (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-4*-O-*acetyl-6-deoxy-β-*D*-galactopyranosyl Ester*; **2**): Amorphous powder. $[\alpha]_D^{25} = -30.9$ ($c = 4.43$, MeOH). IR (KBr): 3450, 1734, 1638, 1049. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 1327 ($[M+Na]^+$). FAB-MS (neg.): 1303 ($[M-H]^-$), 1157 ($[M-C_6H_{11}O_4]^-$), 649 ($[M-C_{27}H_{43}O_{18}]^-$). HR-FAB-MS (pos.): 1327.6305 ($[M+Na]^+$, $C_{63}H_{100}NaO_{28}^+$; calc. 1327.6299).

Perennisaponin I (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-6-deoxy-4*-O-*[(3*S*)-3-hydroxy-1-oxobutyl]-β-*D*-galactopyranosyl Ester*; **3**): Amorphous powder. $[\alpha]_D^{25} = -22.0$ ($c = 2.28$, MeOH). IR (KBr): 3450, 1738, 1638, 1049. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 1371 ($[M+Na]^+$). FAB-MS (neg.): 1347 ($[M-H]^-$), 1115 ($[M-C_{10}H_{17}O_6]^-$), 649 ($[M-C_{29}H_{47}O_{19}]^-$). HR-FAB-MS (pos.): 1371.6558 ($[M+Na]^+$, $C_{65}H_{104}NaO_{29}^+$; calc. 1371.6561).

Perennisaponin J (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-4*-O-*[(3*S*)-3-(acetyloxy)-1-oxobutyl]-6-deoxy-β-*D*-galactopyranosyl Ester*; **4**): Amorphous powder. $[\alpha]_D^{25} = -48.5$ ($c = 1.20$, MeOH). IR (KBr): 3445, 1734, 1638, 1049. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 1413 ($[M+Na]^+$). FAB-MS (neg.): 1389 ($[M-H]^-$), 649 ($[M-C_{31}H_{49}O_{20}]^-$). HR-FAB-MS (pos.): 1413.6664 ($[M+Na]^+$, $C_{67}H_{106}NaO_{30}^+$; calc. 1413.6667).

Perennisaponin K (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*3*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-4*-O-*[(3*S*)-3-(acetyloxy)-1-oxobutyl]-6-deoxy-β-*D*-galactopyranosyl Ester*; **5**): Amorphous powder. $[\alpha]_D^{25} = -47.1$ ($c = 1.00$, MeOH). IR (KBr): 3445, 1734, 1638, 1049. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 1413 ($[M+Na]^+$). FAB-MS (neg.): 1389 ($[M-H]^-$), 649 ($[M-C_{31}H_{49}O_{20}]^-$). HR-FAB-MS (pos.): 1413.6674 ($[M+Na]^+$, $C_{67}H_{106}NaO_{30}^+$; calc. 1413.6667).

Perennisaponin L (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-6-deoxy-4*-O-*{(3*S*)-3-{[(3*S*)-3-hydroxy-1-oxobutyl]oxy}-1-oxobutyl}-β-*D*-galactopyranosyl Ester*; **6**): Amorphous powder. $[\alpha]_D^{26} = -22.4$ ($c = 1.64$, MeOH). IR (KBr): 3450, 1736, 1655, 1049. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 1457 ($[M+Na]^+$). FAB-MS (neg.): 1433 ($[M-H]^-$), 1115 ($[M-C_{14}H_{23}O_8]^-$), 649 ($[M-C_{33}H_{53}O_{21}]^-$), 503 ($[M-C_{39}H_{62}O_{25}]^-$). HR-FAB-MS (pos.): 1457.6937 ($[M+Na]^+$, $C_{69}H_{110}NaO_{31}^+$; calc. 1457.6929).

Perennisaponin M (=*(2β,3β,4α,16α)-3-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic Acid* O-*6-Deoxy-α-*L*-mannopyranosyl-(1→3)*-O-*β-*D*-*xylopyranosyl-(1→4)*-O-*2*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-6-deoxy-4*-O-*{(3*S*)-3-{[(3*S*)-3-{[(3*S*)-3-hydroxy-1-oxybutyl]-oxy}-1-oxobutyl}oxy}-1-oxobutyl]-β-*D*-galactopyranosyl Ester*; **7**): Amorphous powder. $[\alpha]_D^{26} = -24.2$ ($c = 1.42$, MeOH). IR (KBr): 3445, 1736, 1655, 1049. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 1543 ($[M+Na]^+$). FAB-MS (neg.): 1519 ($[M-H]^-$), 649 ($[M-C_{37}H_{59}O_{23}]^-$). HR-FAB-MS (pos.): 1543.7290 ($[M+Na]^+$, $C_{73}H_{116}NaO_{33}^+$; calc. 1543.7297).

Alkaline Hydrolysis of **1**–**7**. A soln. of each perennisaponins **1**–**7** (6 mg) in H_2O/1,4-dioxane 1:1 (1 ml) was treated with 10% aq. KOH soln. (1 ml), and the mixture was stirred at 40° for 12 h. The mixture was neutralized over *Dowex-HCR-W2* resin (H^+ form), which was then removed by filtration.

The filtrate was concentrated, and the resulting product subjected to reversed-phase CC (2 g of *ODS*; $H_2O \rightarrow$ MeOH) to afford H_2O- and MeOH-eluted fractions, resp. The H_2O-eluted fraction was dissolved in $ClCH_2CH_2Cl$ (2 ml). This soln. was treated with *N,N′*-diisopropyl *O*-(4-nitrobenzyl)-isourea (=(4-nitrophenyl)methyl *N,N′*-bis(1-methylethyl)carbamimidate; 10 mg) and stirred at 80° for 1 h. The mixture was then subjected to HPLC (*YMC-Pack ODS-A*, 250 × 4.6 mm i.d., MeOH/H_2O 65 : 35; 0.8 ml/min, UV detection at 254 nm): 4-nitrobenzyl esters of 3-hydroxybutanoic acid (**a**; t_R 7.0) from **3** – **7** and of AcOH (**b**; t_R 8.3) from **1** – **7**. The MeOH-eluted fraction was subjected to normal-phase CC (2 g of SiO_2, $CHCl_3$/MeOH/H_2O 10 : 3 : 1, lower layer) to give (2*β*,3*β*,4*α*,16*α*)-3-[(6-deoxy-*α*-L-mannopyranosyl)oxy]-2,16,23-trihydroxyolean-12-en-28-oic acid (**1a**; 2 mg each from **1** – **7**).

Deacylation of **1** – **7**. A soln. of each perennisaponin **1** – **7** (2 mg) in 0.5% MeONa/MeOH (1 ml) was stirred at r.t. for 3 h. An aliquot of the mixture was subjected to HPLC (*YMC-Pack ODS-AQ*, 250 × 4.6 mm i.d.; MeOH/H_2O 20 : 80; 0.7 ml/min, optical-rotation detector): methyl (+)-(3*S*)-3-hydroxybutanoate (t_R 9.2, pos. optical rotation) from **3** – **7**. The rest of each mixture was neutralized over *Dowex-HCR-W2* resin (H^+ form), which was then removed by filtration. The filtrate was concentrated and the resulting product purified by HPLC (*Cosmosil 5C_{18}-MS-II*, 250 × 20 mm i.d., MeCN/MeOH/H_2O 32 : 16 : 52; 9.0 ml/min): bellissaponin BS1 (**1b**; 1.5 mg each from **1** – **7**).

Effect on Pancreatic Lipase Activity. A suspension of triolein (80 mg), phosphatidylcholine (10 mg), and sodium taurocholate (5 mg) in 9 ml of 0.1M *Tris*·HCl buffer (pH 7.0) containing 0.1M NaCl was homogeneously emulsified with a homogenizer (strait *Teflon* pestle with strait glass tube, volume 20 ml). The substrate suspension (0.1 ml) in a test tube was preincubated with 5 µl of test sample in DMSO and 95 µl of *Tris*·HCl buffer for 3 min at 37°. An aliquot of porcine pancreatic lipase (250 µg/ml, type II, *Sigma – Aldrich, Inc.*; 50 µl) or *Tris*·HCl buffer (50 µl) as a blank test was then added to start the reaction. After 30 min of incubation, the test tube was immediately immersed in boiling water for 2 min to stop the reaction and then cooled with water. Free fatty acid concentration was determined by a commercial kit (*Wako NEFA C* test, *Wako Pure Chemical Industries, Ltd.*). Theasaponin E_1 was isolated from *Camellia sinensis* [32] and used as a reference compound [28]. IC_{50} was determined graphically ($N = 4$).

Statistics. Values were expressed as means ± s.e.m. For statistical analysis, one-way analysis of variance followed by *Dunnett*'s test was used. Probability (p) values less than 0.05 were considered significant.

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