Medicinal Flowers. XXI.[1] Structures of Perennisaponins A, B, C, D, E, and F, Acylated Oleanane-Type Triterpene Oligoglycosides, from the Flowers of *Bellis perennis*

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Six new acylated oleanane-type triterpene oligoglycosides, perennisaponins A (1), B (2), C (3), D (4), E (5), and F (6), were isolated from the flowers of *Bellis perennis* (Daisy flower) together with 14 saponins, nine flavonoids, and two glycosides. The structures of 1—6 were elucidated on the basis of chemical and physicochemical evidence.

Key words *Bellis perennis*; Asteraceae; triterpene oligoglycoside; perennisaponin; 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] The chemical constituents of this plant, several triterpene saponins,[3—11] anthocyanins,[12] flavonoids,[13] and polyacetylenes[14,15] have been isolated from the roots and flowers of *B. perennis*. The flowers and young leaves of this herbal medicine are used as a vegetable. In the course of our characterization studies on medicinal flowers,[1,16—34] we have reported the isolation and structure elucidation of perennisosides I—VII (**7**—**13**) from the flowers of *B. perennis* (Daisy flower) as well as their anti-hyperlipidemic activities.[1] Our continuing search led us to the additional isolation of six new acylated oleanane-type triterpene oligoglycosides, perennisaponins A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**), from the flowers of *B. perennis* together with 14 saponins (**7**—**21**), nine flavonoids (**22**—**30**), and two glycosides (**31**, **32**). This paper deals with the structure elucidation of six new saponins (**1**—**6**).

The flowers of *B. perennis*, which were cultivated in Albania, were extracted with methanol. The methanolic extract (25.8% from the dried flowers) 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%, 6.4%, respectively), which was described previously.[1] The MeOH-eluted fraction was subjected to normal- and reversed-phase column chromatographies, and finally HPLC to give perennisaponins A (**1**, 0.0004%), B (**2**, 0.0048%), C (**3**, 0.019%), D (**4**, 0.017%), E (**5**, 0.021%), and F (**6**, 0.059%), bellisaponin BS1[10] (**14**, 0.0035%), bellisosides D[4] (**15**, 0.060%), E[4] (**16**, 0.19%), and F[4] (**17**, 0.026%), bellidioside A[35] (**18**, 0.058%), asterbatanoside D[36] (**19**, 0.0063%), bernardioside B_2[37] (**20**, 0.013%), bellissaponin BS6[8] (**21**, 0.023%), apigenin[38—41] (**22**, 0.0035%), apigenin 7-*O*-*β*-D-glucopyranoside[38—41] (**23**, 0.10%), apigenin 7-*O*-*β*-D-glucuronopyranoside[42] (**24**, 0.0009%), apigenin 7-*O*-*β*-D-glucuronopyranoside methyl ester[43] (**25**, 0.0008%), rutin[38—41] (**26**, 0.016%), isorhanmnetin 3-*O*-*β*-D-glucopyranoside[44] (**27**, 0.029%), isorhanmnetin 3-*O*-*β*-D-glucuronopyranoside[45] (**28**, 0.022%), isorhanmnetin 3-*O*-rutinoside[46] (**29**, 0.0092%), isorhanmnetin 3-*O*-robinobioside[47] (**30**, 0.0094%), methyl syringate 4-*O*-*β*-D-glucopyranoside[48] (**31**, 0.0025%), and (*Z*)-3-hexenyl *β*-D-glucopyranoside[49] (**32**, 0.0013%).

Structures of Perennisaponins A (1), B (2), C (3), D (4), E (5), and F (6) Perennisaponin A (**1**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{24}$ −7.8° in MeOH). The IR spectrum of **1** showed absorption bands at 1736 and 1655 cm^{-1} ascribable to ester carbonyl and olefin functions, and broad bands at 3445 and 1050 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* 1181 $(M+Na)^+$ and 1157 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{57}H_{90}O_{24}$. On alkaline hydrolysis of **1** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v), polygalacic acid[50] (**1a**) was obtained together with acetic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[25,29] The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra (pyridine-d_5) of **1**, which were assigned by various NMR experiments,[51] showed signals assignable to six methyls [δ 0.93, 1.04. 1.22, 1.41, 1.63, 1.79 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3)], a methylene and three methines bearing an oxygen function [δ 3.76, 4.18 (1H each, both d, J=9.6 Hz, 23-H_2), 4.23 (1H, br s, 3-H), 4.55 (1H, br s, 2-H), 5.18 (1H, br s, 16-H)], an olefin [δ 5.67 (1H, t-like, 12-H)], a fucopyranosyl [δ 1.25 (3H, d, J=6.2 Hz, Fuc-6-H_3), 6.04 (1H, d, J=8.2 Hz, Fuc-1-H)], two rhamnopyranosyl [δ 1.67 (3H, d, J=6.2 Hz, *terminal*-Rha-6-H_3), 1.77 (3H, d, J=6.2 Hz, *inner*-Rha-6-H_3), 6.12 (1H, br s, *inner*-Rha-1-H), 6.17 (1H, br s, *terminal*-Rha-1-H)], and a xylopyranosyl moieties [δ 5.16 (1H, d, J=7.7 Hz, Xyl-1)] together with two acetyl groups [δ 1.98, 2.01 (3H each, both s, Ac-H_3)]. The positions of the acetyl groups in **1** were clarified on the basis of an HMBC experiment, which showed long-range correlations between the 4-proton in the fucopyranosyl moiety [δ 5.53 (1H, br d, J=*ca.* 3.4 Hz)] and the acetyl carbonyl carbon (δ_C 171.1) and between the 2-proton in the *inner*-rhamnopyranosyl moiety [δ 6.01 (1H, m)] and the acetyl carbonyl carbon (δ_C 170.5), as shown in Fig. 1. Furthermore, treatment of **1** with 0.5% sodium methoxide

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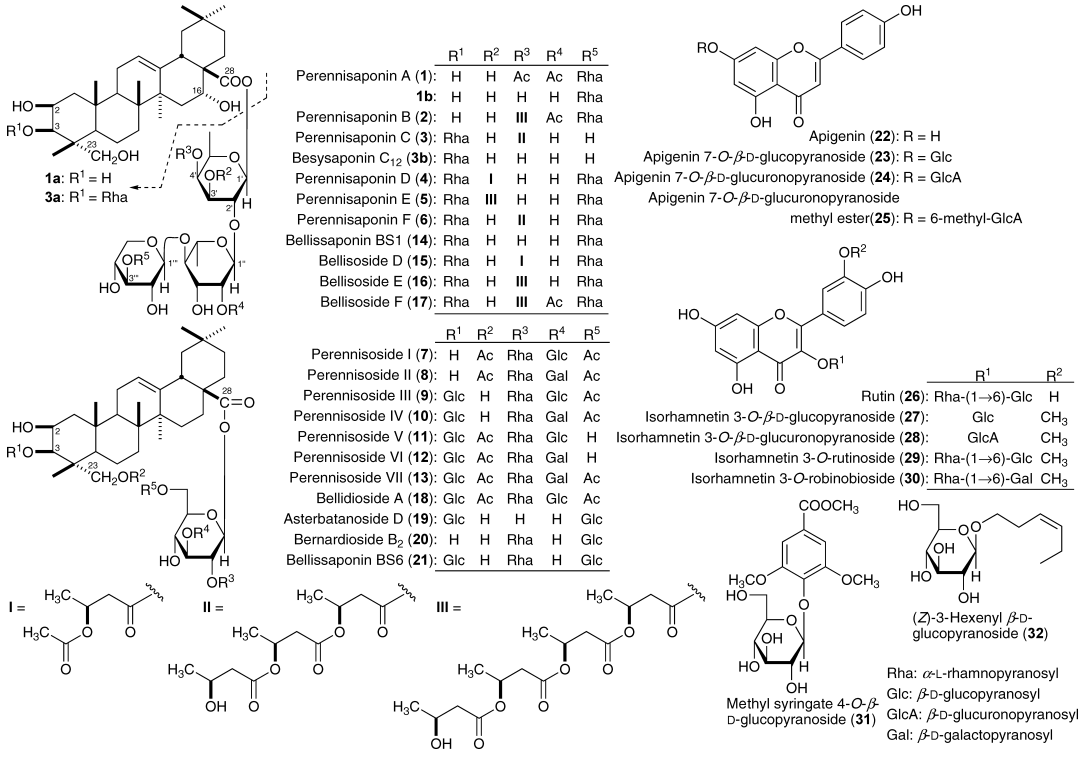

Chart 1

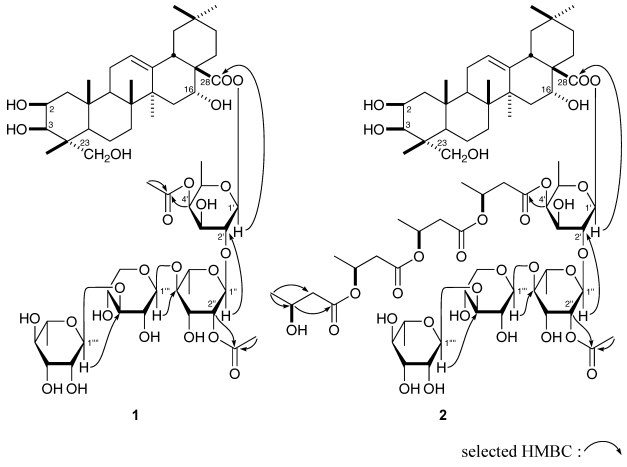

Fig. 1. Selected HMBC Correlations of **1** and **2**

(NaOMe)–MeOH provided polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (**1b**).[52] Comparison of the ^{13}C-NMR data for **1** with those for **1b** revealed acylation shifts around the 4-position in the fucopyranosyl moiety and the 2-position in the *inner*-rhamnopyranosyl moiety [**1**: δ_C 73.2 (*inner*-Rha-2-C), 75.0 (Fuc-4-C), **1b**: δ_C 71.9 (*inner*-Rha-2-C), 73.2 (Fuc-4-C)]. On the basis of the above-mentioned evidence, the structure of perennisaponin A was determined to be polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-2-*O*-acetyl-α-L-rhamnopyranosyl(1→2)-4-*O*-acetyl-β-D-fucopyranoside (**1**).

Perennisaponin B (**2**) was also obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{26}$ −13.4° in MeOH). The IR spectrum of **2** showed absorption bands at 3445, 1736, 1655, and 1080 cm^{-1}, ascribable to hydroxyl, ester carbonyl, olefin, and ether functions. The molecular formula, $C_{71}H_{112}O_{31}$, of **2** was determined from the positive- and negative-ion FAB-MS [*m/z* 1483 $(M+Na)^+$ and 1459 $(M-H)^-$] and by high-resolution positive-ion FAB-MS measurement. The alkaline hydrolysis of **2** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated **1a**[50] and two organic acids, acetic acid and 3-hydroxybutyric acid, which were identified by HPLC analysis of those *p*-nitrobenzyl derivatives.[25,29] In addition, treatment of **2** with 0.5% NaOMe–MeOH gave **1b**[52] together with methyl (*S*)-(+)-3-hydroxybutrate,[53] which was identified by HPLC using an optical rotation detector.[54,55] The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra[51] (pyridine-d_5) of **2** indicated the presence of the following functions: a polygalacic acid part {{six methyls [δ 0.94, 1.05, 1.20, 1.38, 1.61, 1.77 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3)], a methylene and three methines bearing an oxygen function {δ [3.73 (1H, d, J=10.3 Hz), 4.15 (1H, m), 23-H_2], 4.22 (1H, m, 3-H), 4.50 (1H, br s, 2-H), 5.14 (1H, br s, 16-H)}, an olefin [δ 5.67 (1H, t-like, 12-H)]}}, a fucopyranosyl [δ 1.25 (3H, d, J=6.4 Hz, Fuc-6-H_3), 6.00 (1H, d, J=8.3 Hz, Fuc-1-H)], two rhamnopyranosyl [δ 1.63 (3H, d, J=6.2 Hz, *terminal*-Rha-6-H_3), 1.75 (3H, d, J=6.2 Hz, *inner*-Rha-6-H_3), 6.06 (1H, br s, *terminal*-Rha-1-H), 6.08 (1H, br s, *inner*-Rha-1-H)], and a xylopyranosyl moieties [δ 5.10 (1H, d, J=7.7 Hz, Xyl-1)] together with five ester carbonyl carbons (δ_C 169.6, 169.9, 170.4, 170.7, 171.4) suggesting the presence of an acetyl [δ 2.00 (3H, s, Ac-H_3)] and four (*S*)-3-hydroxybutyryl (3HB) moieties [δ 1.29—1.35 (9H, m, 3HB-4, 4′, 4″-H_3), 1.38 (3H, d, J=6.2 Hz, 3HB-4‴-H_3), 2.60—2.65, 2.69—2.84 (4H each, both m, 3HB-2, 2′, 2″, 2‴-H_2), 4.54 (1H, m, 3HB-3‴-H), 5.49—5.55 (3H, m, 3HB-3, 3′, 3″-H)]. The ^{1}H- and ^{13}C-

Table 1. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) of Perennisaponins A (**1**), B (**2**), and C (**3**)

Position	1	2	3
1	1.35 m	1.32 m	1.33 m
	2.40 m	2.39 m	2.31 m
2	4.55 br s	4.50 br s	4.72 m
3	4.22 br s	4.22 br s	4.34 d, 3.4
5	1.73 m	1.67 m	1.90 m
6	1.35 m	1.33 m	1.32 m
	2.13 m	2.20 m	2.21 m
7	1.73 m	1.67 m	1.64 m
	1.90 m	1.88 m	1.85 m
9	1.90 m	1.89 m	1.79 m
11	2.14 m	2.12 m	2.12 m
	2.23 m	2.22 m	2.22 m
12	5.67 t-like	5.67 t-like	5.66 t-like
15	1.90 m	1.88 m	1.99 m
	2.28 m	2.24 m	2.30 m
16	5.18 br s	5.14 br s	5.13 br s
18	3.42 dd, 4.1, 14.1	3.40 dd, 4.3, 14.1	3.41 dd, 4.4, 14.8
19	1.30 m	1.33 m	1.33 m
	2.76 dd, 13.1, 14.1	2.73 m	2.74 m
21	1.30 m	1.32 m	1.32 m
	2.39 m	2.34 m	2.43 m
22	2.23 m	2.20 m	2.22 m
	2.39 m	2.40 m	2.43 m
23	3.76 d, 9.6	3.73 d, 10.3	3.67 m (2H)
	4.18 d, 9.6	4.15 m	
24	1.41 s	1.38 s	1.21 s
25	1.63 s	1.61 s	1.59 s
26	1.22 s	1.20 s	1.18 s
27	1.79 s	1.77 s	1.77 s
29	0.93 s	0.94 s	0.94 s
30	1.04 s	1.05 s	1.04 s
C3-sugar			(Rha)
1			5.72 br s
2			4.67 m
3			4.55 m
4			4.26 dd, 9.2, 9.2
5			4.55 m
6			1.60 d, 6.3

Position	1	2	3
C28-sugars	(Fuc)	(Fuc)	(Fuc)
1	6.04 d, 8.2	6.00 d, 8.3	6.00 d, 8.3
2	4.49 m	4.46 m	4.52 m
3	4.32 m	4.30 dd, 3.6, 9.4	4.35 m
4	5.53 br d, 3.4	5.50 m	5.51 m
5	4.02 m	4.01 m	4.02 m
6	1.25 d, 6.2	1.25 d, 6.4	1.25 d, 6.4
4-*O*-Ac	1.98 s		
4-*O*-*acyl*-2		2.60—2.65*	2.67—2.71*
		2.69—2.84**	2.74—2.81**
3		5.46—5.55***	5.52—5.56***
4		1.29—1.35****	1.33 d, 6.4[a]
2′		2.60—2.65*	2.67—2.71*
		2.69—2.84**	2.74—2.81**
3′		5.49—5.55***	5.52—5.56***
4′		1.29—1.35****	1.33 d, 6.4[a]
2″		2.60—2.65*	2.62 dd, 5.3, 14.6
		2.69—2.84**	2.74—2.81**
3″		5.49—5.55***	4.55 m
4″		1.29—1.35****	1.39 d, 6.2
2‴		2.60—2.65*	
		2.69—2.84**	
3‴		4.54 m	
4‴		1.38 d, 6.2	
	(*i*-Rha)	(*i*-Rha)	(*i*-Rha)
1	6.12 br s	6.08 d, 1.2	6.25 br s
2	6.01 m	5.93 m	4.75 dd-like
3	4.78 m	4.74 dd, 3.3, 9.5	4.66 m
4	4.26 m	4.20 m	4.36 m
5	4.49 m	4.46 m	4.52 m
6	1.77 d, 6.2	1.75 d, 6.2	1.78 d, 6.0
2-*O*-Ac	2.01 s	2.00 s	
	(Xyl)	(Xyl)	(Xyl)
1	5.16 d, 7.7	5.10 d, 7.7	5.10 d, 7.6
2	4.08 m	4.01 m	4.06 m
3	4.26 m	4.15 m	4.06 m
4	4.13 m	4.08 m	4.14 m
5	3.53 dd, 10.9, 11.0	3.51 dd, 10.4, 11.2	3.50 dd, 10.8, 11.4
	4.20 m	4.20 m	4.18 dd, 5.2, 11.4
	(*t*-Rha)	(*t*-Rha)	
1	6.17 br s	6.06 br s	
2	4.78 m	4.72 m	
3	4.60 dd, 3.4, 9.6	4.54 m	
4	4.31 m	4.25 dd, 9.4, 9.5	
5	4.93 m	4.84 m	
6	1.67 d, 6.2	1.63 d, 6.2	

i-Rha: inner Rha; *t*-Rha: terminal Rha. *, **, ***, **** Overlapped signals. *a*) May be interchangeable within the same column.

NMR data of **2** were superimposable on those of bellisoside F (**17**), except for the signals due to the 3-*O*-α-L-rhamnopyranosyl moiety of **17**. In the HMBC experiment on **2**, long-range correlations were observed as shown in Fig. 1, so that the connectivities of the ester carbonyl carbons and the positions of acyl groups were elucidated to be the same as those of **17**. On the basis of above-mentioned evidence, the structure of perennisaponin B (**2**) was determined.

Perennisaponin C (**3**), $[\alpha]_D^{26}$ −13.3° (MeOH), was also obtained as an amorphous powder. The positive- and negative-ion FAB-MS of **3** showed quasimolecular ion peaks at *m*/*z* 1355 $(M+Na)^+$ and *m*/*z* 1331 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **3** revealed the molecular formula to be $C_{65}H_{104}O_{28}$. The alkaline hydrolysis of **3** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated polygalacic acid 3-*O*-α-L-rhamnopyranoside[56] (**3a**) and an organic acid, 3-hydroxybutyric acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[25,29] Treatment of **3** with 0.5% NaOMe–MeOH yielded besysaponin C_{12}[10] (**3b**) together with methyl (*S*)-(+)-3-hydroxybutrate,[53] which was identified by HPLC using an optical rotation detector.[54,55] The proton and carbon signals in the ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra[51] (pyridine-d_5) of **3** indicated the presence of a besysaponon C_{12} part {six methyls [δ 0.94, 1.04, 1.18, 1.21, 1.59, 1.77 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3)], a methylene and three methines bearing an oxygen function [δ 3.67 (2H, m, 23-H_2), 4.37 (1H, d, J=3.4 Hz, 3-H), 4.72 (1H, m, 2-H), 5.13 (1H, br s, 16-H)], and an olefin [δ 5.66 (1H, t-like, 12-H)], a fucopyranosyl [δ 1.25 (3H, d, J=6.4 Hz, Fuc-6-H_3), 6.00 (1H, d, J=8.3 Hz, Fuc-1-H)], two rhamnopyranosyl [δ 1.60 (3H, d, J=6.2 Hz, 3-*O*-Rha-6-H_3), 1.78 (3H, d, J=6.0 Hz, 28-*O*-

Table 2. ^{13}C-NMR Data (150 MHz, Pyridine-d_5) of Perennisaponins A (**1**), B (**2**), and C (**3**), and **1b**, and Besysaponin C_{12} (**3b**)

Position	1	1b	2	3	3b
1	45.0	45.1	45.0	44.8	44.9
2	71.6	71.6	71.5	71.0	71.0
3	73.4	73.5	73.4	81.5	81.5
4	42.4	43.0	42.4	42.9	43.0
5	48.6	48.7	48.8	47.6	47.6
6	18.5	18.3	18.7	18.3	18.3
7	33.3	33.4	33.4	33.2	33.2
8	40.3	40.3	40.3	40.3	40.2
9	47.6	47.6	47.7	47.6	47.6
10	37.3	37.3	37.3	37.1	37.1
11	24.1	24.1	24.1	24.1	24.1
12	122.8	122.6	122.8	122.5	122.4
13	144.3	144.5	144.3	144.5	144.0
14	42.4	42.4	42.3	42.3	42.3
15	36.2	36.3	36.2	36.2	36.2
16	74.1	74.2	74.1	74.0	74.0
17	49.5	49.4	49.6	49.4	49.3
18	41.7	41.7	41.7	41.6	41.6
19	47.4	47.4	47.5	47.4	47.4
20	30.8	30.6	30.8	30.7	30.8
21	36.0	36.0	36.0	36.0	36.0
22	32.1	33.2	32.0	31.8	32.0
23	68.6	68.6	68.8	65.3	65.3
24	14.6	14.6	14.5	14.8	14.9
25	17.6	17.5	17.6	17.6	17.5
26	17.7	17.6	17.7	17.6	17.6
27	27.1	27.1	27.1	27.1	27.1
28	176.2	176.2	176.2	176.1	176.1
29	33.1	33.2	33.1	33.0	33.2
30	24.5	24.5	24.7	24.6	24.4
C3-sugar				(Rha)	(Rha)
1				104.0	104.3
2				72.4	72.6
3				72.7	72.8
4				73.9	74.0
5				70.4	70.4
6				18.6	18.6
C28-sugars	(Fuc)	(Fuc)	(Fuc)	(Fuc)	(Fuc)
1	94.4	94.8	94.4	94.5	94.9
2	74.5	74.4	74.8	74.2	74.8
3	73.6	76.5	73.8	73.9	76.8
4	75.0	73.2	74.8	75.0	73.2
5	70.4	72.5	70.3	70.2	70.3
6	16.4	16.9	16.4	16.4	16.9
4-*O*-Ac-1	171.1				
2	20.8				
4-*O*-acyl-1			170.7	170.6	
2			40.6[a]	40.6[a]	
3			68.0[b]	67.9[b]	
4			19.8[c]	19.7[c]	
1′			169.9[d]	169.7	
2′			41.0[a]	41.2[a]	
3′			67.9[b]	67.6[b]	
4′			19.8[c]	19.9[c]	
1″			169.6[d]	171.4	
2″			41.1[a]	45.0	
3″			67.6[b]	64.4	
4″			19.9[c]	23.8	
1‴			171.4		
2‴			45.1		
3‴			64.5		
4‴			23.9		
	(*i*-Rha)	(*i*-Rha)	(*i*-Rha)	(*i*-Rha)	(*i*-Rha)
1	98.8	101.4	98.7	101.6	99.8
2	73.2	71.9	73.3	71.8	72.0
3	70.1	72.6	70.1	72.4	72.5
4	83.8	84.6	84.0	84.0	84.0
5	68.5	68.4	68.5	68.5	68.5
6	18.6	18.5	18.5	18.5	18.6
2-*O*-Ac-1	170.5		170.4		
2	20.9		20.9		
	(Xyl)	(Xyl)	(Xyl)	(Xyl)	(Xyl)
1	106.9	107.2	106.9	106.9	106.7
2	76.2	76.8	76.1	76.0	76.1
3	83.7	83.7	84.0	78.5	78.7
4	69.3	69.3	69.4	70.9	71.0
5	67.3	67.3	67.3	67.3	67.4
	(*t*-Rha)	(*t*-Rha)	(*t*-Rha)		
1	102.7	102.7	102.7		
2	72.5	72.5	72.4		
3	72.7	72.7	72.6		
4	74.0	74.0	74.0		
5	70.0	70.0	70.0		
6	18.6	18.7	18.5		

i-Rha: inner Rha; *t*-Rha: terminal Rha. *a*—*d*) May be interchangeable within the same column.

Rha-6-H_3), 5.72 (1H, br s, 3-*O*-Rha-1-H), 6.25 (1H, br s, 28-*O*-Rha-1-H)], and a xylopyranosyl moieties [δ 5.10 (1H, d, J=7.6 Hz, Xyl-1)]} together with three ester carbonyl carbons (δ_C 169.7, 170.6, 171.4) suggesting the presence of three (*S*)-3-hydroxybutyryl moieties {δ 1.33 (6H, d, 3HB-4, 4′-H_3), 1.39 (3H, d, J=6.2 Hz, 3HB-4″-H_3), [2.62 (1H, dd, J=5.3, 14.6 Hz), 2.67—2.71 (2H, m), 2.74—2.81 (3H, m), 3HB-2, 2′, 2″-H_2], [4.55 (1H, m), 5.52—5.56 (2H, m), 3HB-3, 3′, 3″-H]}. In the HMBC experiments on **3**, long-range correlations were observed between the 4-proton in the fucopyranosyl moiety [δ 5.51 (1H, m)] and the (*S*)-3-hydroxybutyryl ester carbonyl carbon (δ_C 170.6) as shown in Fig. 2, so that the connectivities of ester carbonyl groups were clarified. Consequently, the structure of perennisaponin C (**3**) was determined to be as shown.

Perennisaponins D (**4**) and E (**5**) were isolated as amorphous powders with negative optical rotation (**4**: $[\alpha]_D^{26}$ −14.9°, **5**: $[\alpha]_D^{26}$ −13.7° both in MeOH, respectively). In the positive- and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at *m/z* 1371 $(M+Na)^+$ and *m/z* 1347 $(M-H)^-$, respectively, and high-resolution positive-ion FAB-MS revealed the molecular formula to be $C_{65}H_{104}O_{29}$. The molecular formula, $C_{75}H_{120}O_{34}$, of **5** was also determined from the positive- and negative-ion FAB-MS [*m/z* 1587 $(M+Na)^+$ and *m/z* 1563 $(M-H)^-$] and by high-resolution positive-ion FAB-MS measurement. Alkaline hydrolysis of **4** and **5** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v) provided (**3a**)[56] together with acetic acid (from **4**) and 3-hydroxybutyric acid (from **4**, **5**), which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[25,29] Treatment of **4** and **5** with 0.5% NaOMe–MeOH yielded bellissaponin BS1[10] (**14**) together

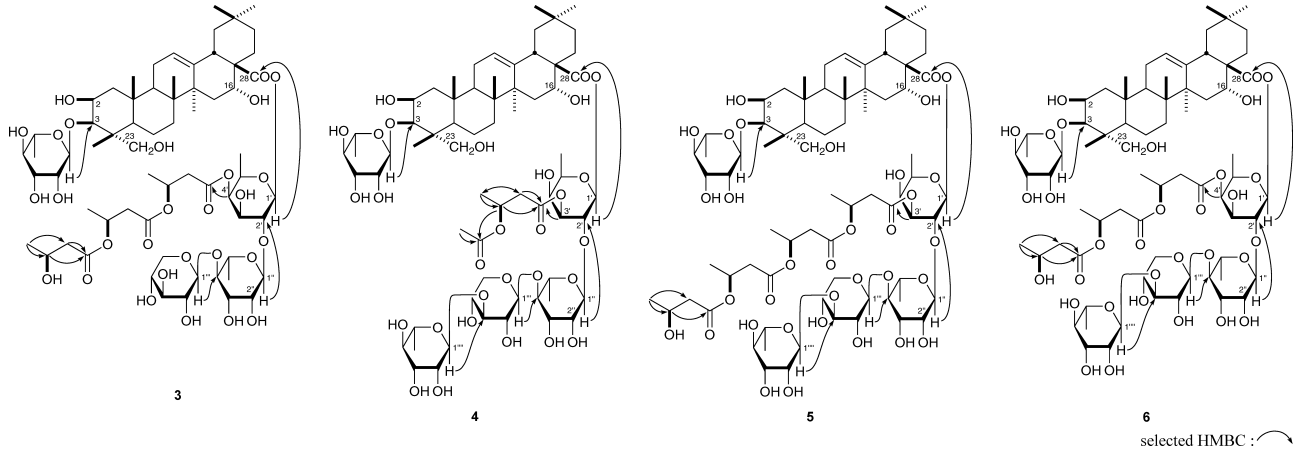

Fig. 2. Selected HMBC Correlations of **3**—**6**

Table 3. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) of Perennisaponins D (**4**) and E (**5**)

Position	**4**	**5**	Position	**4**	**5**
1	1.33 m	1.32 m	C28-sugars	(Fuc)	(Fuc)
	2.30 m	2.30 m	1	6.05 d, 7.9	6.05 d, 8.1
2	4.72 m	4.71 m	2	4.71 m	4.71 m
3	4.36 d, 3.6	4.36 d, 3.6	3	5.34 dd, 3.2, 9.6	5.35 dd, 3.3, 9.8
5	1.89 m	1.88 m	4	4.25 m	4.26 m
6	1.31 m	1.33 m	5	3.93 m	3.94 q-like
	2.20 m	2.20 m	6	1.39 d, 6.4	1.40 d, 6.4
7	1.65 m	1.63 m	3-*O*-*acyl*-2	2.66 dd, 5.0, 15.8	2.60—2.69*
	1.87 m	1.80 m		2.79 dd, 8.1, 15.8	2.75—2.83**
9	1.80 m	1.80 m	3	5.48 m	5.45—5.55***
11	2.20 m	2.11 m	4	1.19 d, 6.4	1.20 d, 6.5[a)]
	2.28 m	2.28 m	2′	2.00 s	2.60—2.69*
12	5.63 t-like	5.63 t-like			2.75—2.83**
15	1.87 m	1.63 m	3′		5.45—5.55***
	2.28 m	2.25 m	4′		1.27 d, 6.4[a)]
16	5.11 br s	5.11 br s	2″		2.60—2.69*
18	3.39 dd-like	3.39 dd, 4.3, 14.4			2.75—2.83**
19	1.33 m	1.32 m	3″		5.45—5.55***
	2.73 br d, *ca.* 13	2.73 m	4″		1.33 d, 6.3
21	1.30 m	1.30 m	2‴		2.60—2.69*
	2.36 m	2.37 m			2.75—2.83**
22	2.23 m	2.23 m	3‴		4.54 m
	2.39 m	2.39 m	4‴		1.39 d, 6.2
23	3.69 m (2H)	3.68 m (2H)		(*i*-Rha)	(*i*-Rha)
24	1.21 s	1.21 s	1	5.68 d, 1.6	5.68 d, 1.4
25	1.58 s	1.58 s	2	4.53 m	4.53 m
26	1.18 s	1.18 s	3	4.46 dd, 3.1, 9.3	4.45 dd, 3.1, 9.3
27	1.74 s	1.74 s	4	4.25 m	4.25 m
29	0.95 s	0.95 s	5	4.32 m	4.32 m
30	1.01 s	1.02 s	6	1.63 d, 6.1	1.63 d, 6.0
C3-sugar	(Rha)	(Rha)		(Xyl)	(Xyl)
1	5.72 br s	5.72 br s	1	4.98 d, 7.7	4.97 d, 7.7
2	4.67 dd, 1.4, 3.2	4.67 dd, 1.3, 3.3	2	4.00 m	4.00 dd, 7.7, 8.6
3	4.55 m	4.55 m	3	4.15 m	4.14 m
4	4.26 m	4.26 m	4	4.06 m	4.05 m
5	4.55 m	4.56 m	5	3.44 dd, 10.5, 11.4	3.45 dd, 10.8, 11.0
6	1.60 d, 6.2	1.60 d, 6.4		4.16 m	4.14 m
				(*t*-Rha)	(*t*-Rha)
			1	6.11 br s	6.10 d, 1.1
			2	4.71 m	4.70 m
			3	4.51 dd, 3.4, 9.3	4.51 dd, 3.4, 9.3
			4	4.26 m	4.26 m
			5	4.86 m	4.85 dq, 9.6, 6.2
			6	1.63 d, 6.1	1.63 d, 6.2

i-Rha: inner Rha; *t*-Rha: terminal Rha. *, **, *** Overlapped signals. *a*) May be interchangeable within the same column.

Table 4. ^{13}C-NMR Data (150 MHz, Pyridine-d_5) of Perennisaponins D (**4**) and E (**5**), and Bellissaponin BS1 (**14**)

Position	**4**	**5**	**14**	Position	**4**	**5**	**14**
1	44.8	44.8	44.8	C28-sugars	(Fuc)	(Fuc)	(Fuc)
2	71.0	71.1	71.1	1	94.6	94.6	94.8
3	81.6	81.6	81.6	2	69.7	69.7	74.4
4	43.1	43.1	43.0	3	78.3	78.4	76.5
5	47.7	47.7	47.6	4	72.1	72.1	73.1
6	18.4	18.4	18.3	5	72.4	72.4	72.5
7	33.3	33.3	33.3	6	16.6	16.6	16.9
8	40.4	40.4	40.3	3-*O*-acyl-1	170.4	170.0	
9	47.6	47.6	47.6	2	41.0	40.9[a]	
10	37.2	37.2	37.2	3	67.4	68.1[b]	
11	24.1	24.1	24.1	4	19.8	19.7[c]	
12	122.9	122.9	122.6	1′	170.1	169.9[d]	
13	144.3	144.3	144.4	2′	21.1	41.0[a]	
14	42.4	42.4	42.3	3′		67.8[b]	
15	36.3	36.3	36.3	4′		19.8[c]	
16	74.1	74.0	74.4	1″		169.9[d]	
17	49.5	49.5	49.4	2″		41.1[a]	
18	41.7	41.8	41.7	3″		67.6[b]	
19	47.4	47.4	47.6	4″		20.0[c]	
20	30.8	30.8	30.8	1‴		171.4	
21	36.0	36.1	36.0	2‴		45.2	
22	31.9	31.9	33.1	3‴		64.5	
23	65.4	65.4	65.5	4‴		23.9	
24	15.0	15.0	15.0		(*i*-Rha)	(*i*-Rha)	(*i*-Rha)
25	17.7	17.7	17.6	1	101.9	101.8	101.4
26	17.7	17.7	17.6	2	71.6	71.6	71.9
27	27.2	27.2	27.1	3	72.5	72.5	72.6
28	175.9	175.9	176.2	4	84.3	84.3	84.7
29	33.2	33.2	33.2	5	69.0	68.9	68.4
30	24.6	24.6	24.5	6	18.5	18.5	18.5
C3-sugar	(Rha)	(Rha)	(Rha)		(Xyl)	(Xyl)	(Xyl)
1	104.1	104.1	104.2	1	107.0	107.0	107.1
2	72.6	72.6	72.5	2	76.1	76.1	76.6
3	72.9	72.9	72.8	3	83.8	83.9	83.4
4	74.0	74.0	74.0	4	69.3	69.3	69.3
5	70.4	70.4	70.4	5	67.3	67.3	67.4
6	18.5	18.6	18.6		(*t*-Rha)	(*t*-Rha)	(*t*-Rha)
				1	102.7	102.7	102.6
				2	72.5	72.5	72.5
				3	72.6	72.5	72.7
				4	74.0	74.0	74.2
				5	70.0	70.0	69.9
				6	18.5	18.6	18.6

i-Rha: inner Rha; *t*-Rha: terminal Rha. *a*—*d*) May be interchangeable within the same column.

with methyl (*S*)-(+)-3-hydroxybutrate,[53] which was identified by HPLC using an optical rotation detector.[54,55] The ^{1}H- (Table 3) and ^{13}C-NMR (Table 4) spectra[51] (pyridine-d_5) of **4** showed signals assignable to six methyls [δ 0.95, 1.01, 1.18, 1.21, 1.58, 1.74 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3)], a methylene and three methines bearing an oxygen function [δ 3.69 (2H m, 23-H_2), 4.36 (1H, d, J=3.6 Hz, 3-H), 4.72 (1H, m, 2-H), 5.11 (1H, br s, 16-H)], an olefin [δ 5.63 (1H, t-like, 12-H)], a fucopyranosyl [δ 1.39 (3H, d, J=6.4 Hz, Fuc-6-H_3), 6.05 (1H, d, J=7.9 Hz, Fuc-1-H)], three rhamnopyranosyl [δ 1.60 (3H, d, J=6.2 Hz, 3-*O*-Rha-6-H_3), 1.63 (6H, d, J=6.1 Hz, 28-*O*-*inner*-Rha-6, 28-*O*-*terminal*-Rha-6-H_3), 5.72 (1H, br s, 3-*O*-Rha-1-H), 5.68 (1H, br s, 28-*O*-*inner*-Rha-1-H), 6.11 (1H, br s, 28-*O*-*terminal*-Rha-1-H)], and a xylopyranosyl moieties [δ 4.98 (1H, d, J=7.7 Hz, Xyl-1-H)] together with an acetyl [δ 2.00 (3H, s, Ac-H_3)] and a (*S*)-3-hydroxybutyryl groups {δ 1.19 (3H, d, J=6.4 Hz, 3HB-4-H_3), [2.66 (1H, dd, J=5.0, 15.8 Hz), 2.79 (1H, dd, J=8.1, 15.8 Hz), 3HB-2-H_2], 5.48 (1H, m, 3HB-3-H)}. The proton and carbon signals in the ^{1}H- (Table 3) and ^{13}C-NMR (Table 4) spectra[51] (pyridine-d_5) of **5** were found to be similar to those of **4**, except for the signals due to the acyl group: [δ 1.20 (3H, d, J=6.5 Hz, 3HB-4-H_3), 1.27 (3H, d, J=6.4 Hz, 3HB-4′-H_3), 1.33 (3H, d, J=6.3 Hz, 3HB-4″-H_3), 1.39 (3H, d, J=6.2 Hz, 3HB-4‴-H_3), 2.60—2.69, 2.75—2.83 (4H each, both m, 3HB-2, 2′, 2″, 2‴-H_2), 4.54 (1H, m, 3HB-3‴-H), 5.45—5.55 (3H, m, 3HB-3, 3′, 3″-H)]. In the HBMC experiment of **4**, long-range correlations were observed between the Fuc-3-proton [δ 5.34 (1H, dd, J=3.2, 9.6 Hz)] and the (*S*)-3-hydroxybutyryl ester carbonyl carbon (δ_C 170.4) and between the 3HB-3-proton and the acetyl carbonyl carbon (δ_C 170.1), while the long-range correlation in the HMBC experiment of **5** was observed between the Fuc-3-proton [δ 5.35 (1H, dd, J=3.3, 9.8 Hz)] and the (*S*)-3-hydroxybutyryl ester carbonyl carbon (δ_C 170.0) (Fig. 2). Thus, the structures of perennisaponins D (**4**) and E (**5**) were determined to be as shown.

Perennisaponin F (**6**) was also obtained as an amorphous

Table 5. ^{1}H-NMR Data (600 MHz, Pyridine-d_5) of Perennisaponin F (**6**)

Position	**6**	Position	**6**
1	1.33 m	C28-sugars	(Fuc)
	2.30 m	1	6.05 d, 8.3
2	4.74 br d, 4.0	2	4.52 m
3	4.40, br s	3	4.33 m
5	1.89 m	4	5.50 m
6	1.30 m	5	4.02 m
	1.96 m	6	1.25 d, 6.5
7	1.73 m	4-*O-acyl*-2	2.60—2.70*
	1.82 m		2.74—2.81**
9	1.82 m	3	5.53—5.56***
11	2.10 m	4	1.30 d, 7.0[a]
	2.23 m	2′	2.60—2.70*
12	5.67 t-like		2.74—2.81**
15	1.98 m	3′	5.53—5.56***
	2.28 m	4′	1.32 d, 6.4[a]
16	5.22 br s	2″	2.62 m
18	3.41 dd-like		2.78 m
19	1.33 m	3″	4.58 m
	2.72 m	4″	1.38 d, 6.1
21	1.32 m		(*i*-Rha)
	2.40 m	1	6.25 br s
22	2.22 m	2	4.77 dd-like
	2.42 m	3	4.64 dd, 3.1, 9.5
23	3.71 m (2H)	4	4.33 m
24	1.22 s	5	4.52 m
25	1.61 s	6	1.76 d, 6.0
26	1.19 s		(Xyl)
27	1.76 s	1	5.02 d, 7.6
29	0.93 s	2	4.07 m
30	1.03 s	3	4.22 m
C3-sugar	(Rha)	4	4.08 m
1	5.77 br s	5	3.45 dd, 10.4, 11.0
2	4.71 m		4.15 dd, 5.2, 11.0
3	4.59 m		(*t*-Rha)
4	4.31 m	1	6.22 br s
5	4.59 m	2	4.79 m
6	1.61 d, 6.2	3	4.58 m
		4	4.31 m
		5	4.94 m
		6	1.66 d, 6.1

i-Rha: inner Rha; *t*-Rha: terminal Rha. *, **Overlapped signals. *a*) May be interchangeable within the same column.

Table 6. ^{13}C-NMR Data (150 MHz, Pyridine-d_5) of Perennisaponin F (**6**)

Position	**6**	Position	**6**
1	44.8	C28-sugars	(Fuc)
2	71.1	1	94.4
3	81.4	2	74.6
4	43.0	3	73.8
5	47.6	4	74.9
6	18.3	5	70.3
7	33.2	6	16.5
8	40.3	4-*O*-acyl-1	170.7
9	47.6	2	40.5[a]
10	37.2	3	67.8[b]
11	24.1	4	19.7[c]
12	122.8	1′	169.8
13	144.4	2′	41.2[a]
14	42.3	3′	67.6[b]
15	36.3	4′	20.0[c]
16	74.1	1″	171.5
17	49.4	2″	45.1
18	41.7	3″	64.4
19	47.4	4″	24.0
20	30.7		(*i*-Rha)
21	36.0	1	101.9
22	31.8	2	71.8
23	65.3	3	72.5
24	15.0	4	84.7
25	17.7	5	68.6
26	17.8	6	18.6
27	27.1		(Xyl)
28	176.2	1	107.2
29	33.1	2	76.4
30	24.6	3	83.5
C3-sugar	(Rha)	4	62.9
1	104.2	5	67.3
2	72.5		(*t*-Rha)
3	72.8	1	102.6
4	74.0	2	72.5
5	70.4	3	72.6
6	18.7	4	74.0
		5	69.9
		6	18.7

i-Rha: inner Rha; *t*-Rha: terminal Rha. *a*—*c*) May be interchangeable within the same column.

powder with negative optical rotation ($[\alpha]_D^{24}$ −21.2° in MeOH). The positive- and negative-ion FAB-MS of **6** showed quasimolecular ion peaks at *m*/*z* 1501 $(M+Na)^+$ and *m*/*z* 1477 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **6** revealed the molecular formula to be $C_{71}H_{114}O_{32}$. The IR spectrum of **6** showed absorption bands at 3445, 1736, 1655, and 1049 cm^{-1}, ascribable to hydroxyl, ester carbonyl, olefin, and ether functions. The alkaline hydrolysis of **6** liberated **3a** and an organic acid, 3-hydroxybutyric acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[25,29] In addition, the alkaline treatment of **6** gave **14**[52] together with methyl (*S*)-(+)-3-hydroxybutrate,[53] which was identified by HPLC using an optical rotation detector.[54,55] The proton and carbon signals of the aglycon part in the ^{1}H- (Table 5) and ^{13}C-NMR (Table 6) spectra[51] (pyridine-d_5) of **6** were similar to those of **3**, except for the signals due to an additional α-L-rhamnopyranosyl moiety [δ 1.66 (3H, d, J=6.1 Hz, 28-*O-terminal*-Rha-6-H$_3$), 6.22 (1H, br s, 28-*O-terminal*-Rha-1-H)]. The HMBC experiment on **6**, long-range correlations were observed as shown in Fig. 2, so that the connectivities of the ester carbonyl carbons and position of acyl group were elucidated to be the same as those of **3**. Consequently, the structure of perennisaponin F (**6**) was determined to be as shown.

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., 150—350 mesh); reverse-phase silica gel column chromatography, Diaion HP-20 (Nippon Rensui) and Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 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_2SO_4 followed by heating.

Plant Material The flowers of *B. perennis* were cultivated in Albania and purchased *via* Tochimoto Tenkaido Co., Ltd., Osaka, Japan at November 2006. A voucher specimen (Lot. No. 20860501K) of this plant is on file in

our laboratory.[1)]

Extraction and Isolation The dried flowers of *B. perennis* (3.0 kg, cultivated in Albania) 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% from the dried flowers). The methanolic extract (720.0 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture, and removal of the solvent *in vacuo* furnished 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_2O→MeOH, twice) to give H_2O-eluted fraction (350.0 g, 12.5%) and 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_2O (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)] as reported previously.[1)] Fraction 2 (5.67 g) was subjected to reversed-phase silica gel column chromatography [200 g, MeOH–H_2O (40 : 60→50 : 50→70 : 30→80 : 20, v/v)→MeOH] to give apigenin (**22**, 76.2 mg, 0.0035%). Fraction 3 (2.41 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (40 : 60→50 : 50→70 : 30→80 : 20, v/v)→MeOH] to give apigenin 7-*O*-*β*-D-glucuronopyranoside (**24**, 20.0 mg, 0.0009%) and methyl syringate 4-*O*-*β*-D-glucopyranoside (**31**, 54.6 mg, 0.0025%). Fraction 4 (1.24 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH–H_2O (40 : 60→50 : 50→70 : 30→80 : 20, v/v)→MeOH] to afford 12 fractions [Fr. 4-1 (25.0 mg), Fr. 4-2 (157.0 mg), Fr. 4-3 (119.5 mg), Fr. 4-4 (151.9 mg), Fr. 4-5 (179.6 mg), Fr. 4-6 (32.6 mg), Fr. 4-7 (34.9 mg), Fr. 4-8 (7.6 mg), Fr. 4-9 (150.5 mg), Fr. 4-10 (149.1 mg), Fr. 4-11 (9.1 mg), and Fr. 4-12 (20.5 mg)]. Fraction 4-4 (151.9 mg) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–H_2O, (10 : 16 : 74, v/v/v)] to furnish (*Z*)-3-hexenyl *β*-D-glucopyranoside (**32**, 28.9 mg, 0.0013%). Fraction 4-5 (179.6 mg) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–H_2O, (20 : 16 : 64, v/v/v)] to furnish apigenin 7-*O*-*β*-D-glucuronopyranoside methyl ester (**25**, 16.3 mg, 0.0008%). Fraction 5 (7.73 g) was subjected to reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (40 : 60→50 : 50→70 : 30→80 : 20, v/v)→MeOH] to afford 12 fractions {Fr. 5-1 (868.5 mg), Fr. 5-2 (296.5 mg), Fr. 5-3 (449.3 mg), Fr. 5-4 [=apigenin 7-*O*-*β*-D-glucopyranoside (**23**, 2217.0 mg, 0.10%)], Fr. 5-5 (1190.8 mg), Fr. 5-6 (206.2 mg), Fr. 5-7 (182.7 mg), Fr. 5-8 (762.8 mg), Fr. 5-9 (529.6 mg), Fr. 5-10 (104.0 mg), Fr. 5-11 (56.6 mg), and Fr. 5-12 (242.5 mg)}. Fraction 5-5 (750.0 mg) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–1% aqueous AcOH, (26 : 16 : 58, v/v/v)] to furnish isorhamnetin 3-*O*-*β*-D-glucopyranoside (**27**, 360.0 mg, 0.026%). Fraction 5-7 (182.7 mg) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–1% aqueous AcOH, (35 : 16 : 49, v/v/v)] to furnish perennisaponin A (**1**, 9.1 mg, 0.0004%). Fraction 5-8 (182.7 mg) was purified by HPLC [Cosmosil HILIC, CH_3CN–H_2O, (90 : 10, v/v)] to furnish perennisaponin B (**2**, 36.6 mg, 0.0048%). Fraction 6 (96.05 g) was subjected to reversed-phase silica gel column chromatography [1.5 kg, MeOH–H_2O (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 (50.269 g), Fr. 6-13 (13.375 g), Fr. 6-14 (2.208 g), and Fr. 6-15 (1.888 g)] as reported previously.[1)] Fraction 6-6 (205.8 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–H_2O (20 : 80, v/v)] to furnish rutin (**26**, 24.8 mg, 0.016%). Fraction 6-7 (345.0 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–H_2O (19 : 81, v/v)] to furnish isorhamnetin 3-*O*-*β*-D-glucuronopyranoside (**28**, 105.7 mg, 0.022%). Fraction 6-8 (343.9 mg) was separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–H_2O (18 : 82, v/v)] to furnish isorhamnetin 3-*O*-*β*-D-glucopyranoside (**27**, 8.6 mg, 0.0025%), isorhamnetin 3-*O*-rutinoside (**29**, 31.8 mg, 0.0092%), and isorhamnetin 3-*O*-robinobioside (**30**, 32.5 mg, 0.0094%). Fraction 6-11 (1820.0 mg) was purified by HPLC [Wakopak Navi C30-5, CH_3CN–MeOH–1% aqueous AcOH (32 : 16 : 52, v/v/v)] 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)] as reported previously.[1)] 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, v/v/v)] to furnish bernardioside B_2 (**20**, 112.2 mg, 0.013%) together with perennisoside III (**9**, 10.5 mg, 0.0012%).[1)] Fraction 6-12 (2015.0 mg) was further separated by HPLC [Wakopak Navi C30-5, CH_3CN–1% aqueous AcOH (40 : 60, v/v)] 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)] as reported previously.[1)] 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, v/v/v)] 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, v/v/v)] to furnish bellidioside A (**18**, 35.3 mg, 0.039%). Fraction 6-12-2 (180.0 mg) was further purified by HPLC [Cosmosil HILIC, CH_3CN–H_2O (90 : 10, v/v)] to give perennisaponins C (**3**, 9.3 mg, 0.019%), D (**4**, 8.1 mg, 0.017%), E (**5**, 10.4 mg, 0.021%), and F (**6**, 28.5 mg, 0.059%). Fraction 6-12-4 (350.4 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to furnish four fractions {Fr. 6-12-4-1 (46.1 mg), Fr. 6-12-4-2 (127.7 mg), Fr. 6-12-4-3 (69.0 mg), and Fr. 6-12-4-4 [=bellisoside E (**16**, 68.7 mg, 0.079%)]}. Fraction 6-12-4-2 (127.7 mg) was further purified by HPLC [Cosmosil HILIC, CH_3CN–H_2O (90 : 10, v/v)] to give bellisoside F (**17**, 25.0 mg, 0.026%). Fraction 6-13 (782.5 mg) was separated by HPLC [Wakopak Navi C30-5, CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to furnish 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, v/v)] to furnish bellidioside A (**18**, 26.0 mg, 0.019%) together with perennisoside VII (**13**, 12.2 mg, 0.0089%).[1)] Fraction 6-13-3 (75.7 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to furnish bellisoside D (**15**, 35.8 mg, 0.0060%). Fraction 6-13-5 (395.9 mg) was further purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to furnish bellisoside E (**16**, 153.7 mg, 0.11%). 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, 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.[1)] Fraction 7-4 (713.4 mg) separated by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–MeOH–H_2O (22 : 16 : 62, v/v/v)] 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 (**19**, 174.5 mg, 0.0063%)], and Fr. 7-4-6 (80.0 mg)}. 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 bellissaponin BS1 (**14**, 11.9 mg, 0.0035%) together with perennisosides V (**11**, 14.2 mg, 0.0042%) and VI (**12**, 20.4 mg, 0.0060%).[1)] 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.[1)] 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 (**21**, 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, v/v)] to give bellissaponin BS6 (**21**, 102.4 mg, 0.019%).

Perennisaponin A (**1**): An amorphous powder, $[\alpha]_D^{24}$ −7.8° (c=0.76, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{90}O_{24}Na$ $(M+Na)^+$: 1181.5720. Found: 1181.5724. IR (KBr): 3445, 1736, 1655, 1260, 1050 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 1181 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1157 $(M-H)^-$, 1115 $(M-C_2H_3O)^-$, 1011 $(M-C_6H_{11}O_4)^-$, 649 $(M-C_{21}H_{33}O_{14})^-$, 503 $(M-C_{27}H_{43}O_{18})^-$.

Perennisaponin B (**2**): An amorphous powder, $[\alpha]_D^{26}$ −13.4° (c=1.68, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{71}H_{112}O_{31}Na$ $(M+Na)^+$: 1483.7085. Found: 1483.7076. IR (KBr): 3445, 1736, 1655, 1260, 1080 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 1483 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1459 $(M-H)^-$, 1115 $(M-C_{16}H_{25}O_8)^-$, 649 $(M-C_{35}H_{55}O_{21})^-$, 503 $(M-C_{41}H_{65}O_{25})^-$.

Perennisaponin C (**3**): An amorphous powder, $[\alpha]_D^{26}$ −13.3° (c=0.83, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{65}H_{104}O_{28}Na$ $(M+Na)^+$: 1355.6612. Found: 1355.6617. IR (KBr): 3445, 1735, 1655,

1261, 1051 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 1355 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1331 $(M-H)^-$, 1119 $(M-C_5H_9O_4)^-$, 1073 $(M-C_{12}H_{19}O_6)^-$, 649 $(M-C_{29}H_{47}O_{18})^-$, 503 $(M-C_{35}H_{57}O_{22})^-$.

Perennisaponin D (**4**): An amorphous powder, $[\alpha]_D^{26}$ −14.9° (c=0.68, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{65}H_{104}O_{29}Na$ $(M+Na)^+$: 1371.6561. Found: 1371.6558. IR (KBr): 3445, 1736, 1656, 1261, 1049 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 3. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 4. Positive-ion FAB-MS *m/z*: 1371 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1347 $(M-H)^-$, 1219 $(M-C_6H_9O_3)^-$, 1073 $(M-C_{12}H_{19}O_7)^-$, 941 $(M-C_{17}H_{27}O_{11})^-$, 649 $(M-C_{29}H_{47}O_{19})^-$.

Perennisaponin E (**5**): An amorphous powder, $[\alpha]_D^{26}$ −13.7° (c=0.83, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{75}H_{120}O_{34}Na$ $(M+Na)^+$: 1587.7559. Found: 1587.7556. IR (KBr): 3445, 1736, 1656, 1250, 1051 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 3. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 4. Positive-ion FAB-MS *m/z*: 1587 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1563 $(M-H)^-$, 1417 $(M-C_6H_{11}O_4)^-$, 1287 $(M-C_{11}H_{17}O_8)^-$, 1219 $(M-C_{16}H_{26}O_8)^-$, 1141 $(M-C_{17}H_{27}O_{12})^-$, 649 $(M-C_{39}H_{63}O_{24})^-$.

Perennisaponin F (**6**): An amorphous powder, $[\alpha]_D^{24}$ −21.2° (c=1.97, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{71}H_{114}O_{32}Na$ $(M+Na)^+$: 1501.7191. Found: 1501.7188. IR (KBr): 3445, 1736, 1655, 1260, 1049 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 5. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 6. Positive-ion FAB-MS *m/z*: 1501 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1477 $(M-H)^-$, 1219 $(M-C_{12}H_{19}O_6)^-$, 795 $(M-C_{29}H_{47}O_{18})^-$, 649 $(M-C_{35}H_{77}O_{22})^-$.

Alkaline Hydrolysis of Perennisaponins A (1), B (2), C (3), D (4), E (5), and F (6) A solution of each perennisaponins (**1**—**6**: 6 mg each) in 50% aqueous 1,4-dioxane (1.0 ml) was treated with 10% aqueous KOH (1.0 ml) and the whole was stirred at 40 °C for 12 h. The each 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 yielded a product, which was subjected to reversed-phase silica gel column chromatography [2.0 g, H_2O→MeOH] to afford H_2O-eluted and MeOH-eluted fractions, respectively. The H_2O-eluted fraction was dissolved in $(CH_2)_2Cl_2$ (2.0 ml) and the solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopyopylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction mixture was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeOH–H_2O (65 : 35, v/v); detection: UV (254 nm); flow rate: 0.8 ml/min] to identify the *p*-nitrobenzyl esters of 3-hydroxybutyric acid (**a**, t_R 7.0 min) from **2**—**6**, and acetic acid (**b**, t_R 8.3 min) from **1**, **2**, and **4**. The MeOH-eluted fraction was subjected to ordinary-phase silica gel column chromatography [2.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, v/v/v, lower layer)] to give polygalacic acid (**1a**, 2 mg each from **1** and **2**) and polygalacic acid 3-*O*-α-L-rhamnopyranoside (**3a**, 3 mg each from **3**—**6**).

Deacylation of Perennisaponins A (1), B (2), C (3), D (4), E (5), and F (6) A solution of perennisaponin A (**1**, 4.5 mg) in 0.5% sodium methoxide (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. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–MeOH–H_2O (32 : 16 : 52, v/v/v)] to furnish **1b** (3.8 mg, 91.1%). A solution of perennisaponins B (**2**, 2.0 mg), C (**3**, 4.0 mg), D (**4**, 2.0 mg), E (**5**, 2.5 mg), and F (**6**, 2.0 mg) in 0.5% NaOMe–MeOH (1.0 ml) was stirred at room temperature for 3 h, respectively. A part of the reaction mixture was subjected to HPLC analysis [column: YMC-Pack ODS-AQ, 250×4.6 mm i.d.; mobile phase: MeOH–H_2O (20 : 80, v/v); detection: optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; flow rate: 0.7 ml/min] to identify methyl (*S*)-(+)-3-hydroxybutrate [**i**, t_R 9.2 min (positive)] (from **2**—**6**, respectively), which was identified by commercially obtained sample {$[\alpha]_D^{20}$ +41.3° (c=0.32, $CHCl_3$)}.[53] The rest of 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, CH_3CN–MeOH–H_2O (32 : 16 : 52, v/v/v)] to furnish **1b** (1.3 mg, 88.4% from **2**), besysaponin C_{12} (**3b**, 3.0 mg, 88.5% from **3**), and bellissaponin BS1 (**14**, 1.6 mg, 88.4% from **4**; 1.8 mg, 92.3% from **5**; 1.5 mg, 88.8% from **6**).

1b: An amorphous powder, $[\alpha]_D^{25}$ −2.4° (c=0.26, MeOH). IR (KBr): 3440, 1736, 1655, 1049 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.93, 1.00, 1.22, 1.39, 1.62, 1.79 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3), 1.48 (3H, d, J=6.4 Hz, Fuc-1-H), 1.64 (3H, d, J=6.2 Hz, *inner*-Rha-1-H), 1.67 (3H, d, J=6.4 Hz, *terminal*-Rha-H), 3.42 (1H, dd-like, 18-H), 3.76, 4.16 (1H each, both d, J=10.0 Hz, 23-H_2), 4.22 (1H, br s, 3-H), 4.53 (1H, br s, 2-H), 5.03 (1H, d, J=7.7 Hz, Xyl-1-H), 5.18 (1H, br s, 16-H), 5.65 (1H, t-like, 12-H), 6.03 (1H, d, J=8.1 Hz, Fuc-1-H), 6.22 (1H, br s, *terminal*-Rha-1-H), 6.43 (1H, br s, *inner*-Rha-1-H). ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2.

Besysaponin C_{12} (**3b**): An amorphous powder, $[\alpha]_D^{27}$ −20.6° (c=0.27, MeOH). IR (KBr): 3445, 1735, 1655, 1051 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.94, 0.97, 1.21, 1.22, 1.60, 1.76 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3), 1.48 (3H, d, J=6.4 Hz, Fuc-1-H), 1.62 (3H, d, J=5.5 Hz, 3-*O*-Rha-1-H), 1.64 (3H, d, J=6.2 Hz, 28-*O*-Rha-1-H), 3.41 (1H, dd, J=4.5, 14.6 Hz, 18-H), 3.69 (2H, m, 23-H_2), 4.32 (1H, br s, 3-H), 4.73 (1H, br s, 2-H), 5.04 (1H, d, J=7.6 Hz, Xyl-1-H), 5.18 (1H, br s, 16-H), 5.65 (1H, t-like, 12-H), 5.79 (1H, br s, 3-*O*-Rha-1-H), 6.01 (1H, d, J=8.3 Hz, Fuc-1-H), 6.46 (1H, br s, 28-*O*-Rha-1-H). ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2.

Bellissaponin BS1 (**14**): $[\alpha]_D^{25}$ −24.3° (c=0.89, MeOH). IR (KBr): 3440, 1736, 1655, 1049 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.93, 0.99, 1.20, 1.22, 1.58, 1.74 (3H each, all s, 29, 30, 26, 24, 25, 27-H_3), 1.48 (3H, d, J=6.4 Hz, Fuc-1-H), 1.62 (3H, d, J=6.2 Hz, 3-*O*-Rha-1-H), 1.62 (3H, d, J=6.2 Hz, 28-*O*-*inner*-Rha-1-H), 1.63 (3H, d, J=6.2 Hz, 28-*O*-*terminal*-Rha-H), 3.40 (1H, dd-like, 18-H), 3.70 (2H, m, 23-H_2), 4.40 (1H, d, J=3.7 Hz, 3-H), 4.71 (1H, br s, 2-H), 5.03 (1H, d, J=7.7 Hz, Xyl-1-H), 5.18 (1H, br s, 16-H), 5.64 (1H, t-like, 12-H), 5.76 (1H, br s, 3-*O*-Rha-1-H), 6.01 (1H, d, J=8.1 Hz, Fuc-1-H), 6.23 (1H, br s, 28-*O*-*terminal*-Rha-1-H), 6.38 (1H, br s, 28-*O*-*inner*-Rha-1-H). ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 4.

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References and Notes

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