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MEDICINAL FLOWERS. XXVIII.[1] STRUCTURES OF FIVE NEW GLYCOSIDES, EVERLASTOSIDES A, B, C, D, AND E, FROM THE FLOWERS OF *HELICHRYSUM ARENARIUM*

Li-Bo Wang,[a,b] Toshio Morikawa,[c] Seikou Nakamura,[a] Kiyofumi Ninomiya,[c] Hisashi Matsuda,[a] Osamu Muraoka,[c] Li-Jun Wu,[b] and Masayuki Yoshikawa*,[a]

a) Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan
b) Faculty of Natural Medicines, Shenyang Pharmaceutical University, 103 Wenhua Rd., Shenyang 110016, People's Republic of China
c) Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

Abstract — Five new glycosides, everlastosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), were isolated from the methanolic extract of the flowers of *Helichrysum arenarium*. Their structures were elucidated on the basis of chemical and physicochemical evidence.

During the course of our studies on medicinal flowers,[1–17] we found that the methanolic extract of the flowers of *Helichrysum arenarium* L. MOENCH (Asteraceae, Everlasting in English) was found to inhibit on tumor necrosis factor-α (TNF-α)-induced cytotoxicity in L929 cells.[1] Furthermore, from the methanolic extract, four flavanone and chalcone glycosides, arenariumosides I—IV, were isolated together with 46 known compounds.[1] As a continuing study on the constituents from *H. arenarium*, we additionally isolated five new glycosides called everlastosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**). This paper deals with the isolation and structure elucidation of **1**—**5**.

The methanolic extract from the dried flowers of *H. arenarium* (19.8% from the dried flowers) was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (7.6%) and

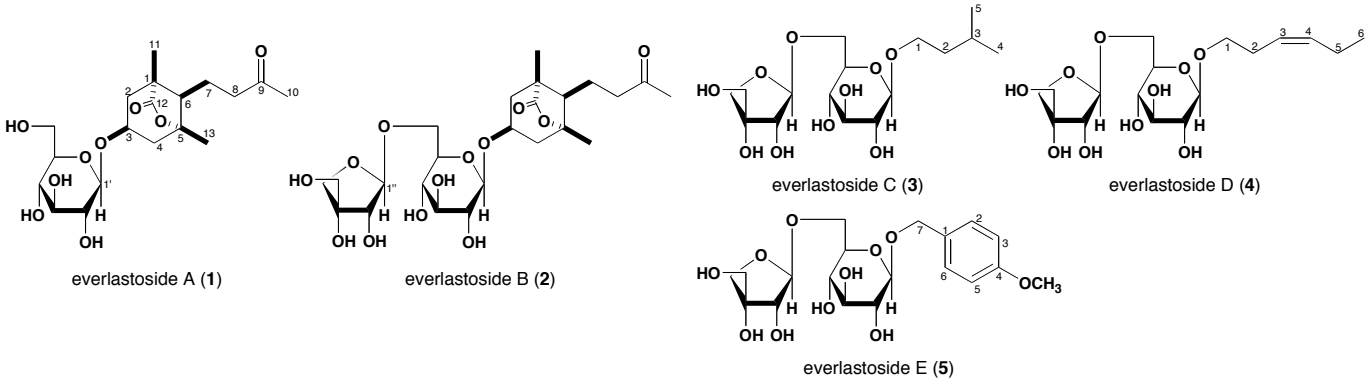

Chart 1

anaqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (H_2O → MeOH) to give H_2O- and MeOH-eluted fractions (8.6% and 3.2%, respectively), which was described previously.[1] From the MeOH-eluted fraction, **1** (0.0005%), **2** (0.0032%), **3** (0.0042%), **4** (0.0015%), and **5** (0.0060%) were purified using normal- and reversed-phase silica gel chromatographies and finally HPLC.

Structures of Everlastosides A (1), B (2), C (3), D (4), and E (5)

Everlastoside A (**1**) was obtained as a white powder and exhibited a negative optical rotation ($[\alpha]_D^{27}$ –14.6° in MeOH). The IR spectrum of **1** showed absorption bands at 1736 and 1718 cm^{-1} assignable to ester carbonyl and carbonyl functions in addition to strong absorption bands at 3450 and 1064 cm^{-1} suggestive of a glycoside moiety. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 425 $(M+Na)^+$, and high-resolution FAB-MS analysis revealed the molecular formula of **1** to be $C_{19}H_{30}O_9$. Acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,3,11,12] The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra of **1**, which were assigned by various NMR experiments,[18] showed signals assignable to three methyls [δ 1.14, 1.33, 2.08 (3H each, all s, 11, 13, 10-H_3)], four methylenes [δ 1.68, 1.72 (1H each, both m, 7-H_2), 1.76 (1H, m, 3β-H), 2.34 (1H, dd, J = 7.1, 13.7 Hz, 3α-H),1.82 (1H, dd, J = 11.0, 13.7 Hz, 2β-H), 2.10 (1H, dd, J = 7.9, 13.7 Hz, 2α-H), 2.46 (2H, m, 8-H_2)], two methines [δ 1.78 (1H, m,

Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Data of **1** and **2**

	1		**2**	
position	**δ_H** ***(J Hz)***	**δ_C**	**δ_H** ***(J Hz)***	**δ_C**
1		45.8		45.9
2α	2.10 (dd, 7.9, 13.7)	35.0	2.26 (dd, 7.0, 13.6)	35.1
2β	1.82 (dd, 11.0, 13.7)		1.88 (dd, 11.0, 13.6)	
3	4.32 (m)	72.5	4.27 (m)	73.2
4α	2.34 (dd, 7.1, 13.7)	35.5	2.24 (m)	35.6
4β	1.76 (m)		1.73 (m)	
5		85.3		85.3
6	1.78 (m)	53.5	1.75 (m)	53.5
7	1.68 (m)	18.8	1.68 (m)	18.8
	1.72 (m)		1.73 (m)	
8	2.46 (2H, m)	42.0	2.45 (2H, m)	41.9
9		206.8		206.8
10	2.08 (s)	29.7	2.06 (s)	29.7
11	1.14 (s)	19.9	1.22 (s)	19.9
12		179.1		179.3
13	1.33 (s)	24.0	1.31 (s)	24.0
	(3-*O*-*Glc*)		(3-*O*-*Glc*^{6}—^{1}Api)	
1'	4.84 (d, 7.7)	103.5	4.75 (d, 7.7)	103.9
2'	3.96 (dd, 7.7, 8.9)	75.2	3.92 (dd, 7.7, 8.9)	75.1
3'	4.19 (dd, 8.9, 9.1)	78.5	4.13 (m)	78.5
4'	4.26 (dd, 9.1, 9.2)	71.5	3.99 (dd, 8.9, 9.3)	71.8
5'	3.82 (m)	78.3	3.96 (m)	77.0
6'	4.34 (dd, 5.0, 12.0)	62.5	4.12 (dd, 6.4, 11.3)	68.8
	4.38 (dd, 2.6, 12.0)		4.59 (dd, 1.9, 11.3)	
			(3-*O*-Glc^6—^{1}*Api*)	
1"			5.69 (d, 2.2)	111.1
2"			4.66 (d, 2.2)	77.9
3"				80.4
4"			4.31 (d, 9.3)	75.1
			4.52 (d, 9.3)	
5"			4.15 (2H, m)	65.9

Measured in pyridine-d_5

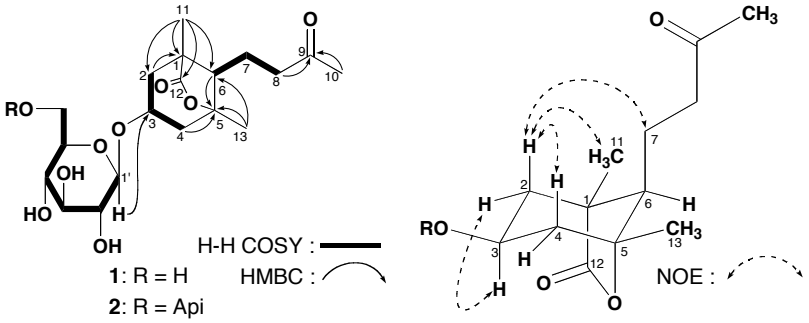

Figure 1

6-H), 4.32 (1H, m, 3-H)], and quaternary carbons (1, 5, 9, 12-C) together with a β-D-glucopyranosyl moiety [δ 4.84 (1H, d, J = 7.7 Hz, Glc-1-H)]. As shown in Figure 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of partial structures written in bold line, and in the heteronuclear multiple-bond correlations (HMBC) experiment, long-range correlations were observed between the following protons and carbons (2-H_2 and 1-C; 4-H_2 and 5-C; 6-H and 5-C; 8-H_2 and 9-C; 10-H_3 and 9-C; 11-H_3 and 1, 2, 6, 12-C; 13-H_3 and 5, 6-C). The stereostructure of **1** was characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs (2α-H and3-H; 2β-H and 4β-H, 7-H_2, 11-H_3). On the basis of this evidence, the structure of **1**, having a γ-lactone linkage between the C-5 and C-12,[19,20] was determined to be as shown.

Everlastoside B (**2**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{26}$ –33.6° in MeOH). The molecular formula, $C_{24}H_{38}O_{13}$, of **2** was determined by the quasimolecular ion peak [*m/z* 557 $(M+Na)^+$] in positive-ion FAB-MS and high-resolution FAB-MS measurements. Acid hydrolysis of **2** with 1.0 M HCl liberated D-apiose[21,22] and D-glucose, which were identified by HPLC analysis. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra[18] of **2** were very similar to those of **1**, except for an apiofuranosyl part [δ 4.98 (1H, d, J = 2.5 Hz, Api-1-H)]. The position of the apiofuranosyl part in **2** was confirmed by the HMBC experiment, which showed a long-range correlation between the Api-1-H and the Glc-6-C (δ_C 68.7). The NOESY experiment of **2** showed NOE correlations between the same proton pairs as those of **1**. Consequently, the structure of everlastoside B (**2**) was determined.

Everlastoside C (**3**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{28}$ –53.2° in pyridine). In the positive-ion FAB-MS of **3**, a quasimolecular ion peak was observed at *m/z* 405 $(M+Na)^+$, and high-resolution FAB-MS analysis revealed the molecular formula of **3** to be $C_{16}H_{30}O_{10}$. Acid hydrolysis of **3** with 1.0 M HCl liberated 3-methyl-1-butanol,[23] D-apiose and D-glucose, which were identified by HPLC analysis. The ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[18] of **3** showed signals assignable to 3-methyl-1-butanol part [δ 0.78 (6H, d, J = 6.4 Hz, 4, 5-H_3), 1.48 (2H, m, 2-H_2), 1.70 (1H, m, 3-H), 3.64 (1H, m, 1-H)] together with glucopyranosyl and apiofuranosyl moieties [δ 4.72 (1H, d, J = 7.3 Hz, Glc-1-H), 5.75 (1H, br s, Api-1-H)]. Furthermore, in the HMBC experiment of **3**, long-range correlations were observed between the Api-1-H and the Glc-6-C (δ_C 68.9) and between the Glc-1-H and the 1-carbon (δ_C 68.6). Consequently, the structure of everlastoside C (**3**) was elucidated to be as shown.

Everlastosides D (**4**) and E (**5**) were obtained as white powders with negative optical rotations (**4**: $[\alpha]_D^{26}$ –71.3°; **5**: $[\alpha]_D^{23}$ –70.5°, both in MeOH). The molecular formulas of **4** ($C_{17}H_{30}O_{10}$) and **5** ($C_{19}H_{28}O_{11}$) were determined from the positive-ion FAB-MS [**4**: *m/z* 417 $(M+Na)^+$, **5**: *m/z* 455 $(M+Na)^+$] and high-

resolution FAB-MS measurements. Acid hydrolysis of **4** and **5** with 1.0 M HCl liberated (*Z*)-3-hexenol[23] (from **4**), *p*-methoxybenzyl alcohol[23] (from **5**), D-apiose and D-glucose, which were identified by HPLC analysis, respectively. The ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[18] of **4** indicated the presence of the following functions: a (*Z*)-3-hexenol part [δ 0.97 (3H, t, J = 6.8 Hz, 6-H_3), 2.07 (1H, br q, J = *ca.* 7 Hz, 5-H_2), 2.38 (2H, dd-like, J = *ca.* 8, 8 Hz, 2-H_2), 3.54, 3.83 (1H each, both m, 1-H_2), 5.39 (1H, m, 3-H), 5.44 (1H, m, 4-H)] together with a glucopyranosyl and an apiofuranosyl moieties [δ 4.25 (1H, d, J = 8.0 Hz, Glc-1-H), 5.00 (1H, d, J = 2.1 Hz, Api-1-H)]. On the other hand, the proton and carbon signals in the ^{1}H-(CD_3OD) and ^{13}C-NMR (Table 2) spectra[18] of **5** were superimposable with those for **4** except for the aglycone part [δ 3.77 (3H, s, -OCH_3), 4.58, 4.80 (1H each, both d, J = 11.3 Hz, 7-H_2), 6.88, 7.34 (2H each, both d, J = 8.6 Hz, 3,5, 2,6-H)]. In the HMBC experiment of **4** and **5**, long-range correlations were observed between the following proton and carbon pairs: [**4**: Api-1-H and Glc-6-C and Glc-1-H and 1-C; **5**: Api-1-H and Glc-6-C and Glc-1-H and 7-C]. Consequently, the structures of everlastosides D (**4**) and E (**5**) were determined. In conclusion, five new glycosides, everlastosides A—E (**1**—**5**), were isolated from the flowers of *H. arenarium* and their structures were determined on the basis of chemical and physicochemical evidence.

Table 2. ^{13}C-NMR (125 MHz) Data of **3**—**5**

	3	**4**	**5**
Position	**δ_C**	**δ_C**	**δ_C**
1	68.6	70.5	130.7
2	38.9	28.9	131.0
3	25.0	125.9	114.6
4	22.6*	134.4	160.8
5	22.7*	21.5	114.6
6		14.6	131.0
7			71.7
OCH_3			55.6
Glc			
1'	104.4	104.3	102.8
2'	75.0	75.5	75.0
3'	78.5	77.9	78.0
4'	71.6	71.8	71.6
5'	77.1	76.8	76.8
6'	68.9	68.7	68.6
Api			
1''	111.1	110.9	110.9
2''	77.8	78.0	78.0
3''	80.5	80.5	80.5
4''	74.9	75.0	74.9
5''	65.5	65.5	65.5

Measured in CD_3OD, *may be interchangeable

EXPERIMENTAL

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; IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA600 (600 MHz), JNM-LA500 (500 MHz), and EX-270 (270 MHz) and spectrometers; ^{13}C-NMR spectra, JEOL JNM-ECA600 (150 MHz), JNM-LA500 (125 MHz), and EX-270 (68 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors. HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 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: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-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

This item was described in a previous report.[1]

Extraction and Isolation

The dried flowers of *H. arenarium* (3.0 kg) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (593.8 g, 19.8%). The methanolic extract (543.8 g) was partitioned between an EtOAc–H_2O (1:1, v/v) mixture, and removal of the solvents *in vacuo* yielded an EtOAc-soluble fraction (210.0 g, 7.6%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (3.0 kg, H_2O → MeOH) to give H_2O-eluted fraction (237.2 g, 8.6%) and MeOH-eluted fraction (88.6 g, 3.2%). The MeOH-eluted fraction (68.6 g) was subjected to normal-phase silica gel column chromatography [2.5 kg, $CHCl_3$–MeOH–H_2O (20:3:1 → 10:3:1 → 7:3:1, lower layer → 6:4:1, v/v/v) → MeOH] to give 12 fractions [Fr. 1 (0.85 g), Fr. 2 (1.20 g), Fr. 3 (0.90 g), Fr. 4 (1.80 g), Fr. 5 (6.40 g), Fr. 6 (11.00 g), Fr. 7 (5.40 g), Fr. 8 (4.00 g), Fr. 9 (7.10 g), Fr. 10 (5.80 g), Fr. 11 (6.10 g), and Fr. 12 (17.10 g)] as reported previously.[1]
Fraction 4 (1.80 g) was subjected to reversed-phase silica gel column chromatography [70 g, MeOH–H_2O (15:85 → 80:20, v/v) → MeOH] and HPLC [MeOH–H_2O (30:70, v/v)] to give everlastoside A (**1**, 2.3 mg, 0.0005%) together with 7-hydroxy-5-methoxyphthalide 7-*O*-β-D-glucopyranoside (476.8 mg, 0.12%).[1]
Fraction 7 (5.40 g) was subjected by reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (15:85 → 70:30, v/v) → MeOH] and HPLC [MeOH–H_2O (10:90—40:60, v/v)] to furnish everlastosides B (**2**, 12.9 mg, 0.0032%), C (**3**, 16.9 mg, 0.0042%), D (**4**, 6.2 mg, 0.0015%), and E (**5**, 23.0 mg, 0.0060%) together with (2*S*)-helichrysin (223.0 mg, 0.055%), (2*R*)-helichrysin (17.0 mg, 0.0042%), chalconaringenin 2'-*O*-β-D-glucopyranoside (305.5 mg, 0.076%), quercetin 3-*O*-β-D-glucopyranoside (40.0 mg, 0.010%), (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside (10.0 mg, 0.0025%), oricinol β-D-glucopyranoside (12.2 mg, 0.0035%), phenethyl alcohol β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (6.2 mg, 0.0015%), icariside D_1 (90.0 mg, 0.017%), and adenosine (22.0 mg, 0.0055%).[1]

Everlastoside A (**1**): a white powder, $[\alpha]_D^{27}$ –14.6° (*c* 0.15, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{30}O_9Na$ $(M+Na)^+$: 425.1787. Found: 425.1780. IR (KBr): 3450, 1736, 1718, 1458, 1064 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1, (600 MHz, CD_3OD) δ: 1.16, 1.44, 2.15 (3H each, all s, 11, 13, 10-H_3), 1.68 (1H, m, 2β-H), 1.70, 1.81 (1H each, both m, 7-H_2), 1.72 (1H, m, 2α-H), 1.81 (1H, m, 6-H), 1.85 (1H, dd, J = 6.8, 13.7 Hz, 4β-H), 2.23 (1H, dd, J = 6.2, 13.7 Hz, 4α-H), 3.86 (1H, m, 3-H), 4.30 (1H, d, J = 7.6 Hz, 1'-H). ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 1, (150 MHz, CD_3OD) δ_C: 19.3 (7-C), 19.8 (11-C), 24.2 (13-C), 29.7 (10-C), 35.0 (4-C), 35.3 (2-C), 42.5 (8-C), 47.1 (1-C), 54.3 (7-C), 62.4 (6'-C), 71.4 (4'-C), 73.1 (3-C), 74.9 (2'-C), 77.7 (5'-C), 77.8 (3'-C), 87.1 (5-C), 102.8 (1'-C), 181.4 (12-C), 210.1 (9-C). Positive-ion FAB-MS *m/z*: 425 $(M+Na)^+$.

Everlastoside B (**2**): a white powder, $[\alpha]_D^{26}$ –33.6° (*c* 0.86, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{24}H_{38}O_{13}Na$ $(M+Na)^+$ 557.2210. Found: 557.2205. IR (KBr): 3430, 1736, 1718, 1458, 1064 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: given in Table 1, (600 MHz, CD_3OD) δ: 1.17, 1.44, 2.15 (3H each, all s, 11, 13, 10-H_3), 1.68 (1H, m, 2β-H), 1.72, 1.80 (1H each, both m, 7-H_2), 1.72 (1H, m, 2α-H), 1.81 (1H, m, 6-H), 1.90 (1H, dd, *J* = 8.1, 13.5 Hz, 4β-H), 2.22 (1H, dd, *J* = 5.4, 13.5 Hz, 4α-H), 3.84 (1H, m, 3-H), 4.30 (1H, d, *J* = 7.6 Hz, 1'-H), 4.98 (1H, d, *J* = 2.5 Hz, 1''-H). ^{13}C-NMR (150 MHz, , pyridine-d_5) δ_C: given in Table 1, (150 MHz, CD_3OD) δ_C: 19.4 (7-C), 20.0 (11-C), 24.3 (13-C), 29.9 (10-C), 35.3 (4-C), 35.5 (2-C), 42.6 (8-C), 47.1 (1-C), 54.3 (7-C), 65.6 (5''-C), 68.7 (6'-C), 71.7 (4'-C), 73.6 (3-C), 74.9 (2'-C), 74.9 (4''-C), 76.8 (5'-C), 77.9 (3'-C), 78.0 (2''-C), 80.5 (3''-C), 87.4 (5-C), 103.2 (1'-C), 110.9 (1''-C), 181.7 (12-C), 210.1 (9-C). Positive-ion FAB-MS *m/z*: 557 $(M+Na)^+$.

Everlastoside C (**3**): a white powder, $[\alpha]_D^{28}$ –53.2° (*c* 1.10, pyridine). High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{30}O_{10}Na$ $(M+Na)^+$ 405.1737. Found: 405.1743. IR (KBr): 3560, 1057 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.78 (6H, d, *J* = 6.4 Hz, 4, 5-H_3), 1.48 (2H, m, 2-H_2), 1.70 (1H, m, 3-H), 3.64 (1H, m, 1-H), 3.97 (3H, m, Glc-2, 4, 5-H), 4.11, 4.17 (1H each, both m, Api-5-H_2), 4.17 (1H, m, Glc-3-H), 4.17, 4.72 (1H each, both m, Glc-6-H_2), 4.30, 4.53 (1H each, both d, *J* = 9.2 Hz, Api-4-H_2), 4.72 (1H, d, *J* = 7.3 Hz, Glc-1-H), 5.75 (1H, br s, Api-1-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 405 $(M+Na)^+$.

Everlastoside D (**4**): a white powder, $[\alpha]_D^{26}$ –71.3° (*c* 0.40, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{17}H_{30}O_{10}Na$ $(M+Na)^+$ 417.1737. Found: 417.1743. IR (KBr): 3420, 1655, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.97 (3H, t, *J* = 6.8 Hz, 6-H_3), 2.07 (1H, br q, *J* = *ca.* 7 Hz, 5-H_2), 2.38 (2H, dd-like, *J* = *ca.* 8, 8 Hz, 2-H_2), 3.16, 3.97 (1H each, both m, Api-4-H_2), 3.27 (1H, m, Glc-4-H), 3.38 (1H, m, Glc-5-H), 3.54, 3.83 (1H each, both m, 1-H_2), 3.57 (2H, br s, Api-5-H_2), 3.58, 3.97 (1H each, both m, Glc-6-H_2), 3.76 (1H, m, Glc-2-H), 3.90 (1H, d, *J* = 2.1 Hz, Api-2-H), 4.25 (1H, d, *J* = 8.0 Hz, Glc-1-H), 5.00 (1H, d, *J* = 2.1 Hz, Api-1-H), 5.39 (1H, m, 3-H), 5.44 (1H, m, 4-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 417 $(M+Na)^+$.

Everlastoside E (**5**): a white powder, $[\alpha]_D^{23}$ –70.5° (*c* 0.35, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{28}O_{11}Na$ $(M+Na)^+$ 455.1529. Found: 455.1526. UV [MeOH, nm (log ε)]: 226 (3.82). IR (KBr): 3568, 1560, 1508, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.22, 3.99 (1H each, both m, Api-4-H_2), 3.22 (1H, m, Glc-2-H), 3.28 (2H, m, Glc-3, 4-H), 3.30 (1H, m, Glc-5-H), 3.60 (2H, m, Api-5-H_2), 3.77 (3H, s, -OCH_3), 3.94 (1H, d, *J* = 2.5 Hz, Api-2-H), 3.99 (2H, m, Glc-6-H_2), 4.28 (1H, d, *J* = 7.0 Hz, Glc-1-H), 4.58, 4.80 (1H each, both d, *J* = 11.3 Hz, 7-H_2), 5.05 (1H, d, *J* = 2.5 Hz, Api-1-H), 6.88, 7.34 (2H each, both d, *J* = 8.6 Hz, 3,5, 2,6-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 455 $(M+Na)^+$.

Acid Hydrolysis of 1—5

A solution of **1**—**5** (each 1.0 mg) in 1 M HCl (1.0 mL) was heated at 80 °C for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and then the resin was removed by filtration. Then the reaction mixture was extracted with EtOAc. The EtOAc-soluble fraction was subjected to HPLC analysis under the following conditions, respectively: HPLC column, Cosmosil 5C_{18}-

MS-II, 4.6 mm i.d. × 250 mm; detection, RI; mobile phase, MeOH-H_2O [(a) 35:65 or (b) 40:60, v/v]; flow rate 1.0 mL/min]. 3-Methyl-1-butanol[23] [from **3**, t_R 18.4 min, condition (a)], (*Z*)-3-hexenol[23] [from **4**, t_R 12.4 min, condition (a)], and *p*-methoxybenzyl alcohol[23] [from **5**, t_R 9.9 min, condition (b)] presents in the EtOAc-soluble fraction were identified by comparison of their retention times with those of authentic samples. On the other hand, the aqueous layers were subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN-H_2O (85:15, v/v); flow rate 0.8 mL/min]. Identification of D-apiose[24] (**i**, from **2**—**5**) and D-glucose (**ii**, from **1**—**5**) present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R: (**i**) 6.6 min (positive optical rotation) and (**ii**) 13.9 min (positive optical rotation), respectively.

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