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NEW *C*,*O*-GLYCOSYLFLAVONES FROM *Melandrium divaricatum*

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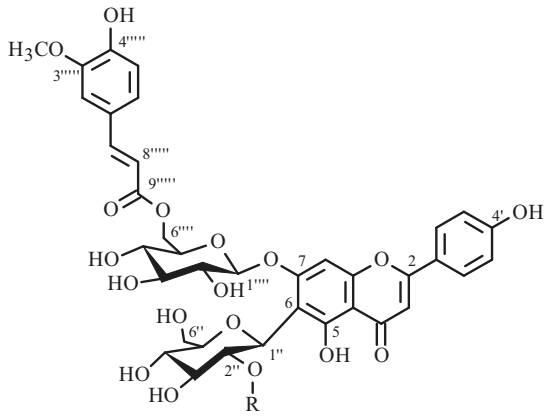
The aerial part of Melandrium divaricatum *Fenzl (Caryophyllaceae) afforded 11 glycosylflavones including two new compounds that were characterized using UV, IR, and NMR spectroscopy and mass spectrometry as apigenin-6-*C*-(2″-*O*-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside-7-*O*-(6‴-*O*-feruloyl)-β-*D*-glucopyranoside (divarioside A, **1**) and apigenin-6-*C*-(2″-*O*-*D*-glucopyranosyl)- β-*D*-glucopyranoside-7-*O*-(6‴-*O*-feruloyl)- β-*D*-glucopyranoside (divarioside B, **2**).*

Keywords: *Melandrium divaricatum*, *C*,*O*-glycosylflavones, divarioside, HPLC.

Melandrium species (Caryophyllaceae) are capable of accumulating apigenin and luteolin derivatives including their *C*-, *O*-, and *C*,*O*-glycosides, which have been observed in *M. album* (Mill.) Garcke (*Silene latifolia* Poir.) [1]; *M. dioicum* (L.) Coss. & Germ. [*S. dioica* (L.) Clariv.] [2]; and *M. vespertinum* Fr. [*S. latifolia* subsp. *alba* (Mill.) Greuter et Burdet] [3]. The biennial species *M. divaricatum* Fenzl (*M. balansae* Boiss., *M. pratense* Roehl.) occurs broadly in thinned forests of Turkey and the western Caucasus [4]. However, the chemical composition of this species is unreported. We studied the flavonoid composition of *M. divaricatum* growing in Georgia and characterized two new compounds.

Chromatographic separation of the EtOAc and BuOH fractions from *M. divaricatum* herb afforded 11 glycosylflavones that were identified using UV, IR, and NMR spectroscopy and mass spectrometry as saponarin-2″-*O*-glucoside (**3**) [5], saponarin-2″-*O*-rhamnoside (**4**) [6], saponarin (**5**) [7], isovitexin-2″-*O*-glucoside (**6**) [8], isovitexin-2″-*O*-rhamnoside (**7**) [9], isovitexin (**8**) [7], saponarin 6‴-*O*-ferulate (**9**) [7], cosmosiin (**10**) [10], cosmosiin 6″-*O*-ferulate (**11**) [10], and two new compounds **1** and **2**.



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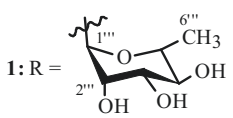

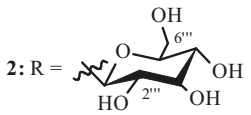

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TABLE 1. PMR Spectra (500 MHz, DMSO-d_6, 298 K, δ, ppm, J/Hz) of **1**–**4**

H atom	Divarioside A (**1**)	Divarioside B (**2**)	Saponarin-2″-*O*-glucoside (**3**)	Saponarin-2″-*O*-rhamnoside (**4**)
		Apigenin		
3	6.73 (1H, s)	6.72 (1H, s)	6.78 (1H, s)	6.72 (1H, s)
8	6.50 (1H, s)	6.51 (1H, s)	6.54 (1H, s)	6.52 (1H, s)
2′, 6′	7.88 (2H, d, J = 8.5)	7.88 (2H, d, J = 8.3)	7.91 (2H, d, J = 8.6)	7.90 (2H, d, J = 8.5)
3′, 5′	6.95 (2H, d, J = 8.5)	6.92 (2H, d, J = 8.3)	6.97 (2H, d, J = 8.6)	6.94 (2H, d, J = 8.5)
5-OH	13.41 (1H, br.s)	13.45 (1H, br.s)	13.40 (1H, br.s)	13.44 (1H, br.s)
4′-O<u>H</u>	10.40 (1H, br.s)	10.38 (1H, br.s)	10.43 (1H, br.s)	10.41 (1H, br.s)
		6-*C*-*β*-D-Glucopyranose		
1″	4.76 (1H, d, J = 9.2)	4.79 (1H, d, J = 9.5)	4.78 (1H, d, J = 9.5)	4.70 (1H, d, J = 9.4)
2″	4.41 (1H, m)	4.47 (1H, t, J = 9.5)	4.45 (1H, t, J = 9.5)	4.39 (1H, m)
3″	3.39 (1H, m)	3.54 (1H, m)	3.56 (1H, m)	3.40 (1H, m)
4″	3.25 (1H, m)	3.24 (1H, m)	3.20 (1H, m)	3.20 (1H, m)
5″	3.21 (1H, m)	3.14 (1H, m)	3.17 (1H, m)	3.17 (1H, m)
$6''_A$	3.79 (1H, m)	3.79 (1H, dd, J = 3.3, 11.4)	3.75 (1H, dd, J = 3.6, 11.6)	3.76 (1H, m)
$6''_B$	3.35 (1H, m)	3.40 (1H, m)	3.38 (1H, m)	3.36 (1H, m)
	2″-*O*-*α*-L-Rhamnopyranose	2″-*O*-*β*-D-Glucopyranose	2″-*O*-*β*-D-Glucopyranose	2″-*O*-*α*-L-Rhamnopyranose
1‴	5.04 (1H, s)	4.25 (1H, d, J = 7.4)	4.21 (1H, d, J = 7.6)	5.02 (1H, s)
2‴	3.63 (1H, m)	3.04 (1H, m)	3.07 (1H, m)	3.62 (1H, m)
3‴	3.11 (1H, m)	3.27 (1H, m)	3.25 (1H, m)	3.12 (1H, m)
4‴	2.96 (1H, m)	3.01 (1H, m)	3.03 (1H, m)	2.90 (1H, m)
5‴	2.51 (1H, m)	2.98 (1H, m)	3.00 (1H, m)	2.53 (1H, m)
$6'''_A$	0.54 (1H, d, J = 5.4)	3.87 (1H, m)	3.85 (1H, m)	0.52 (1H, d, J = 5.6)
$6'''_B$		3.60 (1H, m)	3.62 (1H, m)	
		7-*O*-*β*-D-Glucopyranose		
1⁗	5.12 (1H, d, J = 7.1)	5.11 (1H, d, J = 7.0)	5.06 (1H, д, J = 7.1)	5.08 (1H, d, J = 7.0)
2⁗	3.28 (1H, m)	3.31 (1H, m)	3.30 (1H, m)	3.27 (1H, m)
3⁗	3.53 (1H, m)	3.52 (1H, m)	3.52 (1H, m)	3.50 (1H, m)
4⁗	3.42 (1H, m)	3.45 (1H, m)	3.47 (1H, m)	3.44 (1H, m)
5⁗	3.31 (1H, m)	3.38 (1H, m)	3.35 (1H, m)	3.32 (1H, m)
$6'''_A$	4.37 (1H, dd, J = 3.1, 11.4)	4.41 (1H, dd, J = 2.8, 11.8)	3.89 (1H, dd, J = 3.5, 11.5)	3.94 (1H, dd, J = 3.2, 11.7)
$6'''_B$	4.10 (1H, dd, J = 6.4, 11.4)	4.12 (1H, dd, J = 6.8, 11.8)	3.69 (1H, d, J = 11.5)	3.71 (1H, d, J = 11.7)
		6⁗-*O*-Feruloyl		
2⁗′	7.04 (1H, d, J = 2.0)	7.01 (1H, d, J = 1.7)		
5⁗′	6.63 (1H, d, J = 8.1)	6.61 (1H, d, J = 8.0)		
6⁗′	6.81 (1H, dd, J = 8.1, 2.0)	6.75 (1H, dd, J = 8.0, 2.0)		
7⁗′	7.21 (1H, d, J = 15.9)	7.18 (1H, d, J = 15.8)		
8⁗′	6.10 (1H, d, J = 15.9)	6.12 (1H, d, J = 15.8)		
3⁗′-OC<u>H</u>$_3$	3.82 (3H, s)	3.80 (3H, s)		
4⁗′-O<u>H</u>	9.42 (1H, s)	9.38 (1H, s)		

Compound **1** was a pale-yellow powder with mp 192–194°C. Its solutions had negative optical rotation ($[\alpha]_D^{20}$ –92.4°). Its UV spectrum showed bands at 271 and 333 nm that were characteristic of flavones [11]. Its IR spectrum exhibited peaks at 1646 and 1726 cm^{-1} that were indicative of esterified carboxyl [12]. Acid hydrolysis of **1** produced isovitexin (**8**), D-glucose, L-rhamnose, and ferulic acid. Alkaline hydrolysis of **1** produced saponarin-2″-*O*-rhamnoside (**4**) [6] and ferulic acid.

The HR-ESI-MS spectrum of **1** contained a peak for deprotonated $[M - H]^-$ with *m/z* 915.826 (calcd for $C_{43}H_{47}O_{22}$, 915.811), which indicated the molecular formula was $C_{43}H_{48}O_{22}$. ESI-MS^2 spectra of the fragment with *m/z* 915 $[M - H]^-$ gave daughter ions resulting from loss of *O*-bound rhamnose ($C_6H_{10}O_4$), glucose ($C_6H_{10}O_5$), and ferulic acid ($C_{10}H_8O_3$) with *m/z* 769 $[(M - H) - C_6H_{10}O_4]^-$; 739 $[(M - H) - C_{10}H_8O_3]^-$; 593 $[(M - H) - C_6H_{10}O_4 - C_{10}H_8O_3]^-$; 577 $[(M - H) - C_6H_{10}O_5 - C_{10}H_8O_3]^-$; and 431 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3]^-$ [13]. Further fragmentation of the ion with *m/z* 431 was characteristic of flavone-*C*-glycosides and formed ions with *m/z* 341 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3]^-$, 313 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3 - CO]^-$, 311 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4]^-$, and 283 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4 - CO]^-$ [14].

TABLE 2. ^{13}C NMR Spectra (125 MHz, DMSO-d_6, 298 K, δ, ppm) of **1**–**4**

C atom	Divarioside A (**1**)	Divarioside B (**2**)	Saponarin-2″-*O*-glucoside (**3**)	Saponarin-2″-*O*-rhamnoside (**4**)
			Apigenin	
2	164.5	164.7	164.8	164.6
3	103.6	103.7	103.7	103.2
4	182.0	182.3	182.1	182.4
5	160.4	160.1	160.2	160.7
6	110.4	110.5	110.9	110.3
7	162.7	162.5	162.9	162.3
8	94.5	94.3	94.7	94.6
9	156.4	156.7	156.9	156.2
10	105.7	105.9	105.7	105.5
1′	122.0	122.6	122.1	122.5
2′, 6′	128.5	128.9	128.4	128.7
3′, 5′	116.7	116.5	116.3	116.4
4′	161.2	161.4	161.7	161.4
			6-*C*-β-D-Glucopyranose	
1″	72.0	71.8	71.9	72.1
2″	79.9	80.2	80.3	79.6
3″	77.6	76.6	78.4	77.8
4″	70.8	71.3	71.0	71.0
5″	81.1	80.8	80.7	81.0
6″	62.0	62.0	62.1	62.2
	2″-*O*-α-L-Rhamnopyranose	2″-*O*-β-D-Glucopyranose	2″-*O*-β-D-Glucopyranose	2″-*O*-α-L-Rhamnopyranose
1‴	100.1	104.2	104.5	99.8
2‴	71.4	74.2	74.4	71.6
3‴	71.0	77.4	77.3	71.2
4‴	72.4	70.3	70.5	72.5
5‴	68.4	76.4	76.6	68.5
6‴	17.7	60.4	60.5	17.5
			7-*O*-β-D-Glucopyranose	
1⁗	101.3	101.6	101.5	101.2
2⁗	73.7	73.9	74.0	74.1
3⁗	76.7	76.9	76.9	76.8
4⁗	70.1	69.9	69.8	69.7
5⁗	74.3	74.6	76.3	76.1
6⁗	64.6	64.3	60.1	60.0
		6⁗-*O*-Feruloyl		
1′′′′′	125.6	125.4		
2′′′′′	110.1	110.4		
3′′′′′	147.4	147.5		
4′′′′′	149.2	149.4		
5′′′′′	115.3	115.6		
6′′′′′	123.1	123.3		
7′′′′′	144.6	144.3		
8′′′′′	114.2	114.1		
9′′′′′	166.9	166.4		
3′′′′′-O$\underline{C}$H$_3$	55.6	55.3		

A comparison of PMR and ^{13}C NMR spectra of **1** and saponarin-2″-*O*-rhamnoside (**4**) showed that they were similar except for additional resonances in spectra of **1** for an acyl substituent, i.e., ferulic acid (Tables 1 and 2). Weak-field shifts of the resonances for H-6⁗ of 7-*O*-glucopyranose in **1** relative to those in **4** (δ_H 3.71, 3.94→4.10, 4.37) and a shift of the C-6⁗ resonance (δ_C 60.0→64.6) indicated that the substituent was on this atom. This was confirmed by HMBC spectra in which correlations were observed between resonances for H-6⁗ of 7-*O*-glucopyranose (δ_H 4.10, 4.37) and ferulic acid carbonyl C-9′′′′′ (δ_C 166.9) (Table 3).

TABLE 3. Correlations in HMBC Spectra of **1** and **2**

H atom	C atom **1**/**2**	H atom	C atom **1**/**2**
3	2, 4, 10, 1′	$6''''_A$, $6''''_B$	5′′′′, 9′′′′
8	6, 7, 9, 10	2′′′′	1′′′′, 3′′′′, 4′′′′, 6′′′′, 7′′′′
2′, 6′	2, 1′, 3′, 5′	5′′′′	1′′′′, 3′′′′, 4′′′′, 6′′′′
3′, 5′	1′, 2′, 4′, 6′	6′′′′	1′′′′, 2′′′′, 4′′′′, 5′′′′, 7′′′′
5-OH	5, 6, 10	7′′′′	1′′′′, 2′′′′, 6′′′′, 8′′′′, 9′′′′
4′-OH	3′, 4′, 5′	8′′′′	1′′′′, 7′′′′, 9′′′′
1′′	5, 6, 7, 2′′	3′′′′-OCH_3	3′′′′
1′′′	2′′	4′′′′-OH	3′′′′, 4′′′′, 5′′′′
1′′′′	7		

Enzymatic hydrolysis of **1** by β-glucosidase produced isovitexin-2′′-*O*-rhamnoside (**7**) [9] and 6-*O*-feruloylglucose [15]. Treatment of **1** with α-rhamnosidase gave a hydrolysate in which saponarin-6′′′-*O*-ferulate (**9**) [7] was identified. This was also indicative of a feruloyl fragment on C-6′′′′ of 7-*O*-glucopyranose.

Thus, the studies determined the structure of **1** as apigenin-6-*C*-(2′′-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside-7-*O*-(6′′′′-*O*-feruloyl)-β-D-glucopyranoside (saponarin-2′′-*O*-rhamnoside-6′′′′-*O*-ferulate), which we called divarioside A.

Compound **2** was a pale-yellow powder with mp 184–186°C and optical rotation $[\alpha]_D^{20}$ –33.0°. Its UV spectrum contained bands at 270 and 333 nm. Its IR spectrum showed peaks at 1650 and 1718 cm^{-1} (esterified carboxyl). The acid-hydrolysis products of **2** were isovitexin (**8**), D-glucose, and ferulic acid. The last was cleaved during alkaline hydrolysis to give saponarin-2′′-*O*-glucoside (**3**) [5]. HR-ESI-MS gave a molecular formula of $C_{43}H_{48}O_{23}$ (*m*/*z* 931.789 $[M - H]^-$; calcd for $C_{43}H_{47}O_{23}$, 931.810). ESI-MS spectra of daughter fragments taken in negative-ion mode exhibited peaks due to cleavage of *O*-bound glucose and ferulic acid with *m*/*z* 769, 755, 593, and 431 and peaks with *m*/*z* 341, 313, 311, and 283 that were typical of apigenin *C*-glycosides. PMR and ^{13}C NMR spectra of **2** differed from those of **1** by resonances due to 2′′-*O*-β-D-glucopyranose and were similar to spectra of saponarin-2′′-*O*-glucoside (**3**) (Tables 1 and 2).

HMBC spectroscopy (Table 3) and enzymatic hydrolysis by β-glucosidase indicated that the feruloyl moiety was positioned on C-6′′′′ of the 7-*O*-glucopyranose. The results indicated that **2** was apigenin-6-*C*-(2′′-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside-7-*O*-(6′′′′-*O*-feruloyl)-β-D-glucopyranoside (saponarin-2′′-*O*-glucoside-6′′′′-*O*-ferulate), which we called divarioside B.

Previously, isovitexin derivatives acylated by ferulic acid were isolated from *Cerastium arvense* L. (Caryophyllaceae), isovitexin-2′′-*O*-ferulate [16]; *M. vespertinum* Fr. (Caryophyllaceae), saponarin 6′′′-*O*-ferulate (**9**) [17]; *Gentiana punctata* L. (Gentianaceae), isosaponarin-2′′-*O*-ferulate [18]; and *Cucumis sativus* L. (Cucurbitaceae), isovitexin-2′′-*O*-glucoside-6′′′-*O*-ferulate and isovitexin-4′,2′′-di-*O*-glucoside-6′′′-*O*-ferulate [19]. Isovitexin *O*-glycosides **3**–**9** were found in other *Melandrium* species, including *M. album* and *M. vespertinum* [3], and were probably a chemical signature of the genus.

EXPERIMENTAL

General comments have been published [20, 21]. The aerial part of *M. divaricatum* was collected during flowering in the vicinity of Lebarde resort (Samegrelo-Verkhnyaya Svaneti, Georgia; May 10, 2015; 42°44′36.14″ N, 42°29′15.99″ E; 1767 m above sea level). A specimen of the raw material is preserved in the Herbarium of the IGEB, SB, RAS (No. Ca/ae-15/05-07/2217). The species was determined by Dr. T. A. Aseeva (IGEB, SB, RAS). Raw material was dried in a convection oven (45°C) to <5% moisture.

Extraction and Fractionation. Milled raw material (720 g) was extracted (3×) with EtOH (70%) at 70°C for 2 h in an ultrasonic bath (100 W, 35 kHz). The EtOH extracts were combined and evaporated to dryness under vacuum. The dry residue was suspended in H_2O (2 L) and extracted with hexane, EtOAc, and *n*-BuOH. Concentration of the EtOAc fraction produced a precipitate that was purified by column chromatography (CC) over Sephadex LH-20 (2 × 50 cm, eluent EtOH–H_2O, 80:20→30:70) to give **1** (620 mg). The EtOAc fraction after removal of **1** (46 g) was chromatographed over polyamide (CC, 5 × 40 cm, eluent H_2O–EtOH, 100:0→90:10). Fractions eluted by 30–50% EtOH were separated again by prep. HPLC [LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm; Supelco, Bellefonte, PA, USA); mobile phase H_2O (A) and

MeCN (B); flow rate (ν) 1 mL/min; column temperature 30°C; gradient mode (%B): 0–60 min, 10–40%, 60–80 min, 40–60%] to afford **1** (62 mg; t_R prep. HPLC, 30–34 min) and cosmosiin (apigenin-7-*O*-*β*-D-glucopyranoside, 12 mg, **10**; t_R prep. HPLC, 36–38 min) [10]. Fractions eluted by 80–90% EtOH were separated by prep. HPLC [gradient mode (%B): 0–60 min, 50–80%] to give cosmosiin 6″-*O*-ferulate (18 mg) [apigenin-7-*O*-(6″-*O*-feruloyl)-*β*-D-glucopyranoside, **11**; t_R prep. HPLC, 40–42 min] [10]. The BuOH fraction (108 g) was separated using CC over polyamide (6 × 50 cm, eluent H_2O–EtOH, 100:0→90:10). Fractions eluted by 10–20% EtOH were separated by prep. HPLC [gradient mode (%B): 0–60 min, 0–30%, 60–80 min, 30–40%] to produce saponarin-2″-*O*-glucoside [apigenin-6-*C*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside-7-*O*-*β*-D-glucopyranoside, 27 mg, **3**; t_R prep. HPLC, 20–22 min] [5]; saponarin-2″-*O*-rhamnoside [apigenin-6-*C*-(2″-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside-7-*O*-*β*-D-glucopyranoside, 16 mg, **4**; t_R prep. HPLC, 23–24 min] [6]; and saponarin (apigenin-6-*C*-*β*-D-glucopyranoside-7-*O*-*β*-D-glucopyranoside, 22 mg, **5**; t_R prep. HPLC, 24–25 min) [7]. The fraction eluted by 30% EtOH was recrystallized and rechromatographed over Sephadex LH-20 (CC, 2 × 70 cm, eluent EtOH–H_2O, 80:20→30:70) to give isovitexin-2″-*O*-glucoside (37 mg) [apigenin-6-*C*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside, **6**] [8]. Fractions eluted by 50–70% EtOH were separated using prep. HPLC [gradient mode (%B): 0–60 min, 10–40%, 60–100 min, 40–80%] and CC over Sephadex LH-20 (1 × 50 cm, eluent EtOH–H_2O, 90:10→30:70) to isolate **2** (31 mg; t_R prep. HPLC, 30–32 min), isovitexin-2″-*O*-rhamnoside [apigenin-6-*C*-(2″-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside, 11 mg, **7**; t_R prep. HPLC, 28–29 min] [9], isovitexin (apigenin-6-*C*-*β*-D-glucopyranoside, 14 mg, **8**; t_R prep. HPLC, 29–30 min) [7]; and saponarin 6‴-*O*-ferulate [apigenin-6-*C*-*β*-D-glucopyranoside-7-*O*-(6‴-*O*-feruloyl)-D-glucopyranoside, 18 mg, **9**; t_R prep. HPLC, 44–48 min] [7].

Divarioside A (1). $C_{43}H_{48}O_{22}$, mp 192–194°C, $[\alpha]_D^{20}$ –92.4° (*c* 0.11, MeOH). UV spectrum (MeOH, λ_{max}, nm): 271, 333. IR spectrum (ν, cm^{-1}): 1646, 1726. HR-ESI-MS, *m/z* 915.826 $[M - H]^-$ (calcd 915.811 for $C_{43}H_{47}O_{22}$). ESI-MS, *m/z*: 915 $[M - H]^-$; MS^2 [915]: 769 $[(M - H) - C_6H_{10}O_4]^-$, 739 $[(M - H) - C_{10}H_8O_3]^-$, 593 $[(M - H) - C_6H_{10}O_4 - C_{10}H_8O_3]^-$, 577 $[(M - H) - C_6H_{10}O_5 - C_{10}H_8O_3]^-$, 431 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3]^-$; MS^3 [431]: 341 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3]^-$, 313 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3 - CO]^-$, 311 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4]^-$, 283 $[(M - H) - C_6H_{10}O_4 - C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4 - CO]^-$. Table 1 lists the PMR spectrum (500 MHz, MeOH-d_4, δ, ppm). Table 2 lists the ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm).

Divarioside B (2). $C_{43}H_{48}O_{23}$, mp 184–186°C, $[\alpha]_D^{20}$ –33.0° (*c* 0.10, MeOH). UV spectrum (MeOH, λ_{max}, nm): 270, 333. IR spectrum (ν, cm^{-1}): 1650, 1718. HR-ESI-MS, *m/z* 931.789 $[M - H]^-$ (calcd 931.810 for $C_{43}H_{47}O_{23}$). ESI-MS, *m/z*: 931 $[M - H]^-$; MS^2 [931]: 769 $[(M - H) - C_6H_{10}O_5]^-$, 755 $[(M - H) - C_{10}H_8O_3]^-$, 593 $[(M - H) - C_6H_{10}O_5 - C_{10}H_8O_3]^-$, 431 $[(M - H) - 2 \times C_6H_{10}O_5 - C_{10}H_8O_3]^-$; MS^3 [431]: 341 $[(M - H) - 2 \times C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3]^-$, 313 $[(M - H) - 2 \times C_6H_{10}O_5 - C_{10}H_8O_3 - C_3H_6O_3 - CO]^-$, 311 $[(M - H) - 2 \times C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4]^-$, 283 $[(M - H) - 2 \times C_6H_{10}O_5 - C_{10}H_8O_3 - C_4H_8O_4 - CO]^-$. Table 1 lists the PMR spectrum (500 MHz, MeOH-d_4, δ, ppm). Table 2 lists the ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm).

Acid Hydrolysis of 1 and 2. A weighed portion (7 mg) was heated with TFA (2 M, 4 mL) at 120°C for 2 h. The hydrolysate was evaporated with MeOH to dryness under vacuum. The dry residue was dissolved in EtOH (50%, 2 mL) and chromatographed over polyamide (3 g) with elution sequentially by H_2O (50 mL, eluate I) and EtOH (70%, 100 mL, eluate II). Monosaccharides were isolated by derivatizing a portion of eluate I with 3-methyl-1-phenyl-2-pyrazolin-5-one as before [22] and analyzing by analytical HPLC (conditions 1). Monosaccharides in eluate I were assigned as D- and L-isomers using reductive amination with L-tryptophan [23] followed by analytical HPLC (conditions 2). Eluate II was analyzed using ^{13}C NMR spectroscopy and mass spectrometry. The hydrolysate of **1** contained isovitexin (**8**), D-glucose, L-rhamnose, and ferulic acid; of **2**, **8**, D-glucose, and ferulic acid.

Alkaline Hydrolysis of 1 and 2. A weighed portion (5 mg) was dissolved in MeOH (2 mL); treated with NaOH (2 M, 1 mL); incubated at 30°C for 40 min; neutralized with HCl (2 M); placed on an RP-SiO_2 cartridge (5 g) that was preconditioned with H_2O; and eluted with H_2O until the eluate was neutral, MeCN (40%, 50 mL eluate I), and MeCN (90%, eluate II). The eluates were analyzed by HPLC (conditions 3) and mass spectrometry. The hydrolysate of **1** contained saponarin-2″-*O*-rhamnoside (**4**, eluate I) [6] and ferulic acid (eluate II); of **2**, saponarin-2″-*O*-glucoside (**3**, eluate I) [5] and ferulic acid (eluate II).

Hydrolysis of 1 and 2 by *β*-Glucosidase. A weighed portion (5 mg) was dissolved in DMSO (100 μL), adjusted to 2 mL using MeOH (30%), and treated with *β*-glucosidase [3.2.1.21, 30 U/mg, Sigma-Aldrich; 2 U in 500 μL of phosphate buffer (100 mM, pH 5.0)]. The reaction mixture was incubated at 37°C for 10 h, heated to 95°C (15 min), and centrifuged (6,000 rpm, 15 min). The supernatant was chromatographed over polyamide (5 g) with elution by H_2O (50 mL), EtOH (30%, 100 mL, eluate I), and EtOH (70%, 100 mL, eluate II). The eluates were analyzed by HPLC (conditions 3), ^{13}C NMR

spectroscopy, and mass spectrometry. The hydrolysate of **1** contained 6-*O*-feruloylglucose (eluate I), which was identified by comparison with an authentic sample (Synthose Inc., Concord, Ontario, Canada) and the literature [15], and isovitexin-2″-*O*-rhamnoside (**7**, eluate II) [9]; of **2**, 6-*O*-feruloylglucose (eluate I) and isovitexin-2″-*O*-glucoside (**6**, eluate II) [8].

Hydrolysis of 1 by α-Rhamnosidase. A weighed portion of **1** (5 mg) was dissolved in DMSO (100 μL), adjusted to 2 mL using MeOH (20%), treated with α-rhamnosidase [naringinase from *Thermomicrobia* sp., 3.2.1.40, 5 U/mg, Prokazyme, Vinlandsleid, Reykjavik, Iceland; 1 U in 300 μL of phosphate buffer (100 mM, pH 7.5)], incubated at 65°C for 2 h, heated to 95°C (15 min), and centrifuged (6,000 rpm, 15 min). The supernatant was chromatographed over polyamide (3 g) with elution by H_2O (50 mL) and EtOH (60%, 100 mL). The EtOH eluate was analyzed by HPLC (conditions 3), ^{13}C NMR spectroscopy, and mass spectrometry to identify saponarin 6‴-*O*-ferulate (**9**) [7].

Analytical HPLC. Conditions 1: Milichrom A-02 chromatograph (EcoNova, Novosibirsk, Russia) equipped with a ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm; Metrohm AG, Herisau, Switzerland); mobile phase CH_3COONH_4 (100 mM, pH 4.5) (A) and MeCN (B); gradient mode (%B): 0–20 min, 20–26%; ν 150 μL/min; column temperature 35°C; UV detector at 250 nm. Retention times of monosaccharide derivatives with 3-methyl-1-phenyl-2-pyrazolin-5-one (t_R, min): rhamnose 9.02; glucose 12.52, galactose 13.54, fucose 16.48. Conditions 2: Milichrom A-02 chromatograph; mobile phase NaH_2PO_4 (10 mM) and $Na_2B_4O_7$ (50 mM), 1:1 (pH 9.6); isocratic mode; ν 200 μL/min; column temperature 35°C; UV detector at 220 nm. Retention times of monosaccharide derivatives with L-tryptophan (t_{R-}, min): D-glucose 8.32, L-glucose 8.67, D-rhamnose 29.64, L-rhamnose 30.74. Conditions 3: LC-20 Prominence chromatograph (Shimadzu, Columbia, MD, USA) equipped with a GLC Mastro C18 column (2.1 × 150 mm, ∅ 3 μm; Shimadzu, Kyoto, Japan); mobile phase H_2O (A) and MeCN (B); gradient mode (%B): 0–10 min, 10–20%, 10–30 min, 20–100%; ν 200 μL/min; column temperature 30°C; UV detector at 330 nm. Retention times of flavonoids (t_R, min): **3** 10.48, **4** 12.04, **5** 12.39, **6** 13.14, **7** 14.75, **2** 14.86, **8** 15.02, **1** 15.54, **9** 15.79, **10** 16.02, **11** 19.61, 6-*O*-feruloylglucose 21.34, ferulic acid 23.18, and apigenin 25.84.

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