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NEW *C*,*O*-GLYCOSYLFLAVONES FROM THE GENUS *Silene*

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Chromatographic separation of extracts from the aerial parts of three Silene *species (Caryophyllaceae) isolated 26 flavonoids including the four new* C,O*-glycosylflavones acacetin-6-*C*-(2″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside (sileneside D, **1**) from* S. jeniseensis *Willd., apigenin-6-*C*-(2″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside (sileneside E, **2**) and genkwanin-6-*C*-(2″-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside (sileneside F, **3**) from* S. italica *(L.) Pers., and apigenin-6-*C*-(2″-*O*-β-D-xylopyranosyl)- β-D-glucopyranoside-7-*O*-(6‴-*O*-feruloyl)-β-D-glucopyranoside (sileneside G, **4**) from* S. dioica *(L.) Clairv. The structures of **1**–**4** were studied using UV, IR, and NMR spectroscopy and mass spectrometry.*

Keywords: *Silene jeniseensis*, *Silene italica*, *Silene dioica*, Caryophyllaceae, *C*,*O*-glycosylflavones, sileneside.

Plants of the family Caryophyllaceae are capable of accumulating flavonoid glycosides with the most common form being *C*,*O*-glycosylflavones [1]. The flavonoid compositions of three *Silene* species introduced to Baikal Region, i.e., *S. jeniseensis* Willd., *S. italica* (L.) Pers., and *S. dioica* (L.) Clairv., were investigated during the course of our continuing studies on flavonoids of this family. Wild specimens of them had previously yielded *C*,*O*-glycosylflavones [2–4]. Herein, the flavonoid compositions of the three cultivated species *S. jeniseensis*, *S. italica*, and *S. dioica* are reported and the structures of four new compounds **1**–**4** are studied.

Fractionation of the EtOH extract of the aerial part of *S. jeniseensis* produced EtOAc and BuOH fractions that were separated by column chromatography (CC) over polyamide Sephadex LH-20, and reversed-phase SiO_2 and by preparative HPLC to isolate 11 compounds, including new glycosylflavone **1** with molecular formula $C_{34}H_{42}O_{20}$ according to mass spectrometry (*m*/*z* 771.6024 for $[M + H]^+$) and ^{13}C NMR spectroscopy. The UV spectrum of **1** contained bands at 271 and 339 nm that were characteristic of flavones. The fragmentation pattern in ESI-MS spectra was indicative of two *O*-bound hexose fragments (*m*/*z* 771 $[M + H]^+ \rightarrow 609.447$) and one *C*-bound hexose (*m*/*z* 357, 329, 327, 299) [5]. Hydrolysis in H_2SO_4 (30%)–AcOH formed acacetin and D-glucose while TFA (2 M) gave isocytisoside (acacetin-6-*C*-glucoside) [6] and D-glucose.

The PMR spectrum exhibited two singlets at 6.65 (H-3) and 6.41 ppm (H-8), 2H doublets at 7.83 (H-2′, 6′) and 6.73 ppm (H-3′, 5′), and a methoxy resonance at 3.76 (3H, s) that were characteristic of acacetin [7]. Resonances of three anomeric protons at 4.91 (1H, d, J = 9.0 Hz), 4.31 (1H, d, J = 7.0 Hz), and 5.11 (1H, d, J = 7.0 Hz) correlated in the HSQC spectrum with three resonances of anomeric C atoms at 72.4, 104.3, and 101.4 ppm, respectively, indicating that they belonged to a *C*-bound *β*-glucopyranose and two *O*-bound *β*-glucopyranoses.

Analyses of ^{13}C NMR and DEPT spectra allowed a resonance for a quaternary C atom at 110.9 ppm to be attributed to C-6 of the aglycon bound by a C–C bond to a glucose, which was consistent with correlations between resonances for H-1″ (δ_H 4.91, 6-*C*-glucose) and C-6 (δ_C 110.9) in the HMBC spectrum. A weak-field shift of the C-2″ resonance (δ_C 80.0) and correlations in the HMBC spectrum between H-1‴ (δ_H 4.31, 2″-*O*-glucose) and C-2″ indicated that C-2″ had an *O*-bound glucose. A weak-field shift of the C-7 resonance (δ_C 162.9) and correlations between H-1⁗ (δ_H 5.11, 7-*O*-glucose) and C-7 indicated that the C-7 hydroxyl was substituted by glucose. Thus, **1** was an isocytisoside derivative with two additional *O*-bound glucose residues in the 7- and 2″-positions or acacetin-6-*C*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside-7-*O*-*β*-D-glucopyranoside (isocytisoside-7,2″-di-*O*-glucoside), which we called sileneside D. Known *O*-glucosides of isocytisoside include the 7-*O*-glucoside from *Gentiana pyrenaica* L. (Gentianaceae) [8] and the 2″-*O*-glucoside from *Securigera securidaca* (L.) Degen & Dorfl. (Fabaceae) [9].

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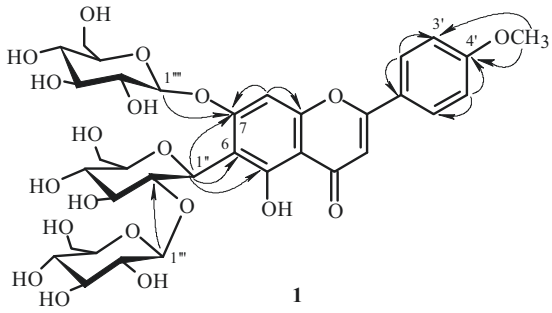
TABLE 1. PMR Spectra of **1** and **2** (500 MHz, DMSO-d_6, 298 K, δ, ppm, J/Hz)

H atom	**1**	**2**
	Acacetin	Apigenin
3	6.65 (1H, s)	6.62 (1H, s)
8	6.41 (1H, s)	
2′, 6′	7.83 (2H, d, J = 8.2)	7.93 (2H, d, J = 8.0)
3′, 5′	6.73 (2H, d, J = 8.2)	6.97 (2H, d, J = 8.0)
5-OH	13.31 (1H, br.s)	13.45 (1H, br.s)
7-OH		10.79 (1H, br.s)
4′-OH		10.25 (1H, br.s)
4′-OCH_3	3.76 (3H, s)	
	6-*C*-*β*-D-Glc*p*	
1″	4.91 (1H, d, J = 9.0)	4.85 (1H, d, J = 9.0)
2″	4.49 (1H, m)	4.52 (1H, m)
3″	3.59 (1H, m)	3.61 (1H, m)
4″	3.24–3.29 (2H, m)	3.31–3.35 (2H, m)
5″		
6″	3.73 (1H, dd, J = 2.9, 11.0, H_A); 3.32 (1H, m, H_B)	3.77 (1H, dd, J = 2.7, 11.2, H_A); 3.37 (1H, m, H_B)
	2″-*O*-*β*-D-Glc*p*	
1‴	4.31 (1H, d, J = 7.0)	4.34 (1H, d, J = 7.2)
2‴	2.95–3.20 (4H, m)	2.99–3.27 (4H, m)
3‴		
4‴		
5‴		
6‴	3.89 (1H, m, H_A); 3.61 (1H, m, H_B)	3.94 (1H, m, H_A); 3.64 (1H, m, H_B)
	7-*O*-*β*-D-Glc*p*	8-*C*-*α*-L-Ara*p*
1⁗	5.11 (1H, d, J = 7.0)	4.97 (1H, d, J = 9.0)
2⁗	3.33–3.51 (4H, m)	4.49 (1H, m)
3⁗		3.53 (1H, m)
4⁗		3.91 (1H, m)
5⁗		3.97 (1H, m, H_A); 3.68 (1H, m, H_B)
6⁗	3.94 (1H, dd, J = 2.9, 11.4, H_A); 3.67 (1H, d, J = 11.4, H_B)	



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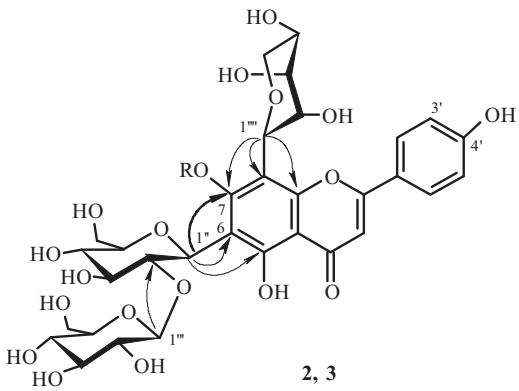


2, 3

2: R = H; **3:** R = CH_3

Known compounds from *S. jeniseensis* included the *C*,*O*- and *C*-glycosylflavones isocytisoside (**5**) [6], isomargariten (isocytisoside-2″-*O*-rhamnoside, **6**) [7], isoscoparin-2″-*O*-rhamnoside (**7**) [10], isovitexin-2″-*O*-rhamnoside (**8**) [11], isoorientin-7-*O*-rutinoside (**9**) [12], lutonarin (isoorientin-7-*O*-glucoside, **10**) [13], isoscoparin-7-*O*-rutinoside (**11**) [12], and isoorientin-2″-*O*-rhamnoside (**12**) [14], and the pyrone glucoside dianthoside [15] that were isolated for the first time from it in addition to the flavonoid isoorientin (**13**) [16] that is known from this species.

The extract of *S. italica* was fractionated and chromatographed to isolate nine known compounds and two new flavonoids (**2** and **3**). The molecular formula of **2** was determined as $C_{32}H_{38}O_{19}$ according to mass spectrometry (*m*/*z* 727.2210 for $[M + H]^+$) and ^{13}C NMR spectroscopy. The UV spectrum of **2** was indicative of a flavone.

TABLE 2. PMR Spectra of **3** and **4** (500 MHz, DMSO-d_6, 298 K, δ, ppm, J/Hz)

H atom	**3**	**4**
	Genkwanin	Apigenin
3	6.75 (1H, s)	6.70 (1H, s)
8		6.48 (1H, s)
2′, 6′	7.97 (2H, d, J = 8.1)	7.91 (2H, d, J = 8.0)
3′, 5′	7.05 (2H, d, J = 8.1)	6.95 (2H, d, J = 8.0)
5-OH	13.36 (1H, br.s)	13.42 (1H, br.s)
4′-OH	10.16 (1H, br.s)	10.22 (1H, br.s)
7-OCH_3	3.89 (3H, s)	
		6-*C*-*β*-D-Glc*p*
1″	4.87 (1H, d, J = 9.0)	4.83 (1H, d, J = 9.2)
2″	4.54 (1H, m)	4.45 (1H, m)
3″	3.58 (1H, m)	3.57 (1H, m)
4″	3.25–3.37 (2H, m)	3.15–3.22 (2H, m)
5″		
6″	3.72 (1H, dd, J = 2.5, 11.0, H_A); 3.39 (1H, m, H_B)	3.75 (1H, dd, J = 2.8, 11.0, H_A); 3.36 (1H, m, H_B)
	2″-*O*-*β*-D-Glc*p*	2″-*O*-*β*-D-Xyl*p*
1‴	4.30 (1H, d, J = 7.1)	4.98 (1H, d, J = 7.2)
2‴		3.12 (1H, m)
3‴	2.82–3.24 (4H, m)	3.24–3.34 (2H, m)
4‴		
5‴		3.62 (1H, m, H_A); 2.91 (1H, m, H_B)
6‴	3.97 (1H, m, H_A); 3.61 (1H, m, H_B)	
	8-*C*-*α*-L-Ara*p*	7-*O*-*β*-D-Glc*p*
1⁗	4.92 (1H, d, J = 9.0)	5.14 (1H, d, J = 7.1)
2⁗	4.52 (1H, m)	3.38–3.53 (4H, m)
3⁗	3.55 (1H, m)	
4⁗	3.94 (1H, m)	
5⁗	3.99 (1H, m, H_A); 3.67 (1H, m, H_B)	
6⁗		4.38 (1H, dd, J = 2.9, 12.0, H_A); 4.10 (1H, dd, J = 7.0, 12.0, H_B)
		6⁗-*O*-Feruloyl
2⁗′		7.07 (1H, d, J = 1.9)
5⁗′		6.65 (1H, d, J = 8.1)
6⁗′		6.77 (1H, dd, J = 8.1, 2.1)
7⁗′		7.21 (1H, d, J = 15.7)
8⁗′		6.09 (1H, d, J = 15.7)
3⁗′-OCH_3		3.83 (3H, s)
4⁗′-OH		9.30 (1H, s)

Total hydrolysis produced apigenin, D-glucose, and L-arabinose. The hydrolysis products in TFA (2 M) were schaftoside (apigenin-6-*C*-glucoside-8-*C*-arabinoside) [17] and D-glucose. The glucose was *O*-bound according to mass spectrometry (ESI-MS, *m*/*z* 727 $[M + H]^+ \rightarrow 565$). Fragmentation of a di-*C*-glycosyl daughter (*m*/*z* 565) was typical of schaftoside [5].

NMR spectroscopy confirmed that **2** was a schaftoside derivative [17] containing an additional *β*-glucopyranose. A weak-field shift of the C-2″ resonance (δ_C 80.3) of the 6-*C*-glucose and correlations in the HMBC spectrum between resonances for glucose H-1‴ (δ_H 4.34) and C-2″ indicated that this position was substituted. Therefore, **2** was apigenin-6-*C*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside-8-*C*-*α*-L-arabinopyranoside (schaftoside-2″-*O*-glucoside), which was called sileneside E. Schaftoside-6″-*O*-glucoside was isomeric to **2** and was isolated earlier from *Stellaria holostea* L. (Caryophyllaceae) [18].

Compound **3** with molecular formula $C_{33}H_{40}O_{19}$ (*m*/*z* 741.4215 for $[M + H]^+$) was hydrolyzed in H_2SO_4 (30%)–AcOH to genkwanin, D-glucose, and L-arabinose. Use of TFA (2 M) formed genkwanin-6-*C*-glucoside-8-*C*-arabinoside [3] and D-glucose. Fragmentation of **3** in mass spectra showed ions caused by loss of an *O*-bound glucose (*m*/*z* 741→579) and those typical of di-*C*-glycosylflavones (*m*/*z* 519, 489, 459, 429, 401, 399, 371, 369, 341) [5].

TABLE 3. ^{13}C NMR Spectra of **1**–**4** (125 MHz, DMSO-d_6, 298 K, δ, ppm)

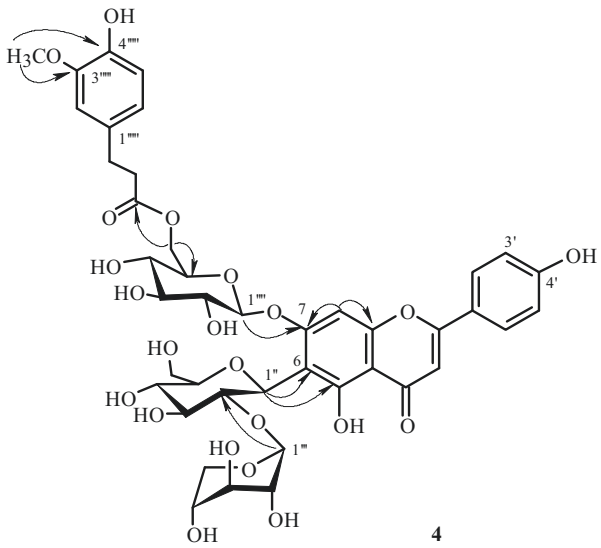
C atom	**1**	**2**	**3**	**4**
	Acacetin	Apigenin	Genkwanin	Apigenin
2	164.0	164.1	163.9	164.3
3	103.5	103.5	103.1	103.9
4	182.1	182.1	181.7	182.4
5	160.4	160.7	160.3	160.2
6	110.9	110.2	108.4	110.7
7	162.9	161.7	164.5	162.7
8	94.6	104.8	103.5	94.5
9	156.0	154.3	155.7	156.3
10	105.2	105.0	104.2	105.6
1′	123.0	122.1	121.8	122.4
2′, 6′	128.2	129.3	128.7	129.1
3′, 5′	114.1	115.7	115.9	116.2
4′	162.5	161.0	161.2	161.3
4′-OCH_3	55.1			
7-OCH_3			56.5	
			6-*C*-*β*-D-Glc*p*	
1″	72.4	72.0	72.6	72.1
2″	80.0	80.3	80.5	80.1
3″	77.2	77.0	77.1	77.3
4″	71.3	70.8	71.0	71.5
5″	81.5	81.7	81.6	81.2
6″	61.9	61.5	61.2	62.2
	2″-*O*-*β*-D-Glc*p*	2″-*O*-*β*-D-Glc*p*	2″-*O*-*β*-D-Glc*p*	2″-*O*-*β*-D-Xyl*p*
1‴	104.3	104.7	104.2	105.9
2‴	74.3	74.1	74.0	75.3
3‴	77.7	77.9	77.7	76.2
4‴	70.1	70.4	70.2	70.3
5‴	76.1	76.3	76.5	66.3
6‴	60.2	60.4	60.1	
	7-*O*-β-*D*-Glc*p*	8-*C*-*α*-L-Ara*p*	8-*C*-α-*L*-Ara*p*	7-*O*-*β*-D-Glc*p*
1⁗	101.4	74.9	74.6	101.9
2⁗	73.9	68.5	68.9	74.0
3⁗	76.9	75.7	75.3	76.7
4⁗	69.7	69.2	69.3	69.8
5⁗	76.3	71.4	71.6	74.4
6⁗	59.8			64.7
				6⁗-*O*-Feruloyl
1⁗′				125.1
2⁗′				110.2
3⁗′				147.7
4⁗′				149.2
5⁗′				115.4
6⁗′				123.7
7⁗′				144.5
8⁗′				114.3
9⁗′				166.0
3⁗′-OCH_3				55.1

NMR spectra were similar to that of sileneside E (**2**) except for additional resonances for a methoxy group [δ_H 3.89 (3H, s)/ δ_C 56.5] on C-7 according to correlations in the HMBC spectrum between the methoxyl protons (δ_H 3.89) and the aglycon C-7 (δ_C 164.5). Thus, **3** was a methoxylated derivative of sileneside E and had the structure genkwanin-6-*C*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside-8-*C*-*α*-L-arabinopyranoside (sileneside F).

A cultivated specimen of *S. italica* also contained isoorientin (**13**) [16], isoscoparin (**14**) [19], isovitexin (**15**) [20], genkwanin-6-*C*-glucoside-8-*C*-arabinoside (**16**) [3], schaftoside (apigenin-6-*C*-glucoside-8-*C*-arabonoside, **17**) [17], isovitexin-2″-*O*-arabinoside (**18**) [20], carlinoside (luteolin-6-*C*-glucoside-8-*C*-arabinoside, **19**) [21], meloside A (isovitexin-2″-*O*-glucoside, **20**) [22], and saponarin (**21**) [23]. Compounds **13–19** and **21** were previously described from wild *S. italica* [3] while **20** was observed for the first time in this species.

The EtOH extract of the aerial part of *S. dioica* was fractionated and chromatographed (CC over polyamide, Sephadex LH-20, and RP-SiO_2 and prep. HPLC) to afford new glycoside **4** and seven known compounds including **20**, **21**, divarioside (**22**) [24], saponarin-6‴-*O*-ferulate (**23**) [25], isovitexin-7,2″-di-*O*-glucoside (**24**) [26], isovitexin-7-*O*-xyloside-2″-*O*-glucoside (**25**) [4], and isovitexin-7-*O*-glucoside-2″-*O*-xyloside (**26**) [4]. Compounds **20**, **21**, **24–26** were identified earlier in specimens of *S. dioica* growing in Europe [4] while **22** and **23** were observed for the first time.

Compound **4** according to mass spectrometric and ^{13}C NMR spectroscopic data had molecular formula $C_{42}H_{46}O_{22}$ (*m*/*z* 903.4216 for $[M + H]^+$). The total hydrolysis products of **4** contained apigenin, D-glucose, D-xylose, and ferulic acid [27]. The UV spectrum exhibited a hypsochromic shift of the long-wavelength band relative to that of apigenin (λ_{max} 335→327 nm). The IR spectrum showed bands for a substituted carboxylic group (1652, 1717 cm^{-1}) [24]. Acid hydrolysis in TFA (2 M) produced isovitexin (**15**), ferulic acid, D-glucose, and D-xylose.



4

The mass spectrum (ESI-MS) gave peaks for fragments formed by loss of *O*-bound xylose ($C_5H_8O_4$), ferulic acid ($C_{10}H_8O_3$), and glucose ($C_6H_{10}O_5$) and fragmentation of an ion with *m*/*z* 433 that was characteristic of isovitexin (*m*/*z* 343, 315, 313, 285) [5]. Enzymatic hydrolysis of **4** by β-glucosidase gave 6-*O*-feruloylglucose [28] and isovitexin-2″-*O*-xyloside [29]. Use of β-xylosidase formed saponarin-6‴-*O*-ferulate (**23**) [25]. An analysis of NMR spectroscopic data showed that **4** had a structure similar to that of the known acylated glycosylflavone saponarin-6‴-*O*-ferulate (**23**) [25] except for additional resonances for an *O*-bound β-D-xylopyranose. A weak-field shift of the C-2″ resonance of 6-*C*-glucose (δ_C 80.1) and HMBC spectral data demonstrating correlations between resonances for xylose H-1‴ (δ_H 4.98) and C-2″ indicated that the xylose was attached to C-2″ of the 6-*C*-glucose.

The studies established that **4** was apigenin-6-*C*-(2″-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside-7-*O*-(6⁗-*O*-feruloyl)-β-D-glucopyranoside (saponarin-2″-*O*-xyloside-6⁗-*O*-ferulate), which was called sileneside G. Alkaline hydrolysis of sileneside G formed ferulic acid and glycoside **4a**, which was identified as saponarin-2″-*O*-β-D-xylopyranoside [apigenin-6-*C*-(2″-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside-7-*O*-β-D-glucopyranoside], which was also a new compound. Saponarin *O*-glycosides acylated by cinnamic acids were observed earlier in Caryophyllaceae species, including *Melandrium divaricatum* Fenzl. (divariosides A and B) [24] and *Gypsophila vaccaria* (L.) Sm. (vaccarins E and F) [23].

EXPERIMENTAL

Plant specimens of the three *Silene* species were grown on open ground at the experimental plot of IGEB, SB, RAS (50°51′14.23″ N, 107°38′14.94″ E, 612 m above sea level) from authenticated seeds collected in nature of *S. jeniseensis* in

the vicinity of Nilova Pustyn′ (Tunkinskii District, Republic of Buryatia, Russia; July 21, 2015; 51°51′54″ N, 101°43′24″ E, 2233 m above sea level; No. CA/BUR-0715/61-11) and *S. italica*, in the vicinity of Mestia (Mestiiskii municipality, Samegrelo-Verkhnyaya Svanetiya Province, Georgia; July 14, 2017; 43°2′35.88″ N, 42°42′41.23″ E, 1435 m above sea level; No. CA/GEO-0717/41-06). Seeds of *S. dioica* (Graham's Delight variety) were obtained from the botanical garden of Plant World Gardens & Nursery (Plant World Devon Ltd., Devon, UK). The aerial parts of the plants were collected during flowering, dried in a convection oven (45°C) to <5% moisture, and milled (1–2 mm).

Spectrophotometric studies used an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia); mass spectrometric studies, an LCMS-8050 TQ-mass spectrometer (Shimadzu, Columbia, MD, USA) as described before [30]. NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian, Palo Alto, CA, USA). Preparative HPLC used a Summit liquid chromatograph (Dionex, Sunnyvale, CA, USA); LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm; Supelco, Bellefonte, PA, USA); mobile phase H_2O (A) and MeCN (B); flow rate 1 mL/min; column temperature 30°C; and UV detector at λ 330 nm. Analytical HPLC used a 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).

Isolation of 1, 5–13, and Dianthoside from *S. jeniseensis*. The aerial part of *S. jeniseensis* (970 g) was sequentially extracted with EtOH (80% and 50%; 1:12) in an ultrasonic bath (100 W, 35 kHz) at 55°C (2.5 h). The combined EtOH extract was concentrated to dryness (184.3 g), suspended in H_2O (1 L), and extracted with hexane, EtOAc, and BuOH. The EtOAc fraction (58 g) was separated over polyamide for CC (3 kg) that was eluted by H_2O and EtOH (60%). The fraction eluted by 60% EtOH was separated over Sephadex LH-20 (CC, 90 × 3 cm, MeOH–H_2O eluent, 90:10→0:100) and RP-SiO_2 (CC, 60 × 3 cm, H_2O–MeCN eluent, 60:40→20:80) and by prep. HPLC [gradient mode (%B): 0–60 min, 40–60%; 60–120 min, 60–80%] to isolate isocytisoside (acacetin-6-*C*-glucoside, 11 mg, **5**) [6], isomargariten (isocytisoside-2″-*O*-rhamnoside, 14 mg, **6**) [7], isoscoparin-2″-*O*-rhamnoside (21 mg, **7**) [10], and isovitexin-2″-*O*-rhamnoside (19 mg, **8**) [11]. The BuOH fraction (106 g) was separated by CC over polyamide, Sephadex LH-20, and RP-SiO_2 as described above and by prep. HPLC [gradient mode (%B): 0–20 min, 5–17%; 20–60 min, 17–25%; 60–90 min, 25–35%] to isolate **1** (22 mg), isoorientin-7-*O*-rutinoside (10 mg, **9**) [12], lutonarin (isoorientin-7-*O*-glucoside, 25 mg, **10**) [13], isoscoparin-7-*O*-rutinoside (17 mg, **11**) [12], isoorientin-2″-*O*-rhamnoside (76 mg, **12**) [14], isoorientin (8 mg, **13**) [16], and dianthoside (maltol-*O*-glucoside, 64 mg) [15].

Sileneside D (1). $C_{34}H_{42}O_{20}$. UV spectrum (50% MeOH, λ_{max}, nm): 271, 339. HR-ESI-MS, *m*/*z* 771.6024 (calcd for $C_{34}H_{43}O_{20}$, 771.6418). ESI-MS, *m*/*z*: 771 $[M + H]^+$; MS^2 [771]: 609 $[(M + H) - C_6H_{10}O_5]^+$, 447 $[(M + H) - 2 \times C_6H_{10}O_5]^+$; MS^3 [447]: 357 $[(M + H) - 2 \times C_6H_{10}O_5 - C_3H_6O_3]^+$, 329 $[(M + H) - 2 \times C_6H_{10}O_5 - C_3H_6O_3 - CO]^+$, 327 $[(M + H) - 2 \times C_6H_{10}O_5 - C_4H_8O_4]^+$, 299 $[(M + H) - 2 \times C_6H_{10}O_5 - C_4H_8O_4 - CO]^+$. Table 1 lists the PMR spectrum (500 MHz, DMSO-d_6, δ, ppm). Table 3 lists the ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm).

Isolation of 2, 3, and 13–21 from *S. italica*. The aerial part of *S. italica* (720 g) afforded EtOAc and BuOH fractions that were separated as described above. The EtOH fraction produced isoscoparin (6 mg, **14**) [19], isovitexin (5 mg, **15**) [20], and genkwanin-6-*C*-glucoside-8-*C*-arabinoside (12 mg, **16**) [3]. The BuOH fraction gave **2** (18 mg), **3** (11 mg), **13** (11 mg) [16], schaftoside (apigenin-6-*C*-glucoside-8-*C*-arabinoside, 85 mg, **17**) [17], isovitexin-2″-*O*-arabinoside (14 mg, **18**) [20], carlinoside (luteolin-6-*C*-glucoside-8-*C*-arabinoside, 10 mg, **19**) [21], meloside A (isovitexin-2″-*O*-glucoside, 7 g, **20**) [22], and saponarin (isovitexin-7-*O*-glucoside, 9 mg, **21**) [23].

Sileneside E (2). $C_{32}H_{38}O_{19}$. UV spectrum (50% MeOH, λ_{max}, nm): 270, 337. HR-ESI-MS, *m*/*z*: 727.2210 (calcd for $C_{32}H_{39}O_{19}$, 727.5924). ESI-MS, *m*/*z*: 727 $[M + H]^+$; MS^2 [727]: 565 $[(M + H) - C_6H_{10}O_5]^+$; MS^3 [565]: 505 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2]^+$, 475 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3]^+$, 445 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4]^+$, 415 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3 - C_2H_4O_2]^+$, 387 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3 - C_2H_4O_2 - CO]^+$, 385 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2 - C_4H_8O_4]^+$, 357 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2 - C_4H_8O_4 - CO]^+$, 355 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4 - C_3H_6O_3]^+$, 327 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4 - C_3H_6O_3 - CO]^+$. Table 1 lists the PMR spectrum (500 MHz, DMSO-d_6, δ, ppm). Table 3 lists the ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm).

Sileneside F (3). $C_{33}H_{40}O_{19}$. UV spectrum (50% MeOH, λ_{max}, nm): 268, 338. HR-ESI-MS, *m*/*z*: 741.4215 (calcd for $C_{33}H_{41}O_{19}$, 741.6176). ESI-MS, *m*/*z*: 741 $[M + H]^+$; MS^2 [741]: 579 $[(M + H) - C_6H_{10}O_5]^+$; MS^3 [579]: 519 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2]^+$, 489 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3]^+$, 459 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4]^+$, 429 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3 - C_2H_4O_2]^+$, 401 $[(M + H) - C_6H_{10}O_5 - C_3H_6O_3 - C_2H_4O_2 - CO]^+$, 399 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2 - C_4H_8O_4]^+$, 371 $[(M + H) - C_6H_{10}O_5 - C_2H_4O_2 - C_4H_8O_4 - CO]^+$, 369 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4 - C_3H_6O_3]^+$, 341 $[(M + H) - C_6H_{10}O_5 - C_4H_8O_4 - C_3H_6O_3 - CO]^+$. Table 2 lists the PMR spectrum (500 MHz, DMSO-d_6, δ, ppm). Table 3 lists the ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm).

Isolation of 4 and 20–26 from *S. dioica*. The aerial part of *S. dioica* (320 g) was extracted (3×) with EtOH (60%, 1:15) in an ultrasonic bath (100 W, 35 kHz) at 50°C (2 h). The obtained extract (86.4 g) was concentrated to dryness under vacuum, suspended in H_2O (250 mL), and extracted with hexane, EtOAc, and BuOH. The EtOAc fraction (19.2 g) was separated over polyamide for CC (kg) with elution by H_2O and EtOH (20, 40, and 60%). The fraction eluted by 60% EtOH was separated over Sephadex LH-20 (CC, 80 × 2 cm, MeOH–H_2O eluent, 90:10→0:100) and RP-SiO_2 (CC, 40 × 2 cm, H_2O–MeCN eluent, 70:30→20:80) and by prep. HPLC [isocratic mode (%B): 0–60 min, 65%] to give **4** (52 mg), divarioside B (saponarin-2″-*O*-glucoside-6‴-*O*-feerulate, 46 mg, **22**) [24], and saponarin-6‴-*O*-ferulate (17 mg, **23**) [25]. The BuOH fraction was separated analogously over polyamide, Sephadex LH-20, and RP-SiO_2 and by prep. HPLC [gradient mode (%B): 0–30 min, 5–15%; 30–60 min, 15–40%; 60–80 min, 40–45%] to isolate **20** (9 mg), **21** (4 mg), isovitexin-7,2″-di-*O*-glucoside (11 mg, **24**) [26], isovitexin-7-*O*-xyloside-2″-*O*-glucoside (19 mg, **25**) [4], and isovitexin-7-*O*-glucoside-2″-*O*-xyloside (21 mg, **26**) [4].

Sileneside G (4). $C_{42}H_{46}O_{22}$. UV spectrum (MeOH, λ_{max}, nm): 272, 327. IR spectrum (ν, cm^{-1}): 1652, 1717. HR-ESI-MS, *m*/*z*: 903.4216 (calcd for $C_{42}H_{47}O_{22}$, 903.7478). ESI-MS, *m*/*z*: 903 $[M + H]^+$; MS^2 [903]: 771 $[(M + H) – C_5H_8O_4]^+$, 595 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3]^+$, 433 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3 – C_6H_{10}O_5]^+$; MS^3 [433]: 343 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3 – C_6H_{10}O_5 – C_3H_6O_3]^+$, 315 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3 – C_6H_{10}O_5 – C_3H_6O_3 – CO]^+$, 313 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3 – C_6H_{10}O_5 – C_4H_8O_4]^+$, 285 $[(M + H) – C_5H_8O_4 – C_{10}H_8O_3 – C_6H_{10}O_5 – C_4H_8O_4 – CO]^+$. Table 2 lists the PMR spectrum (500 MHz, DMSO-d_6, δ, ppm). Table 3 lists the ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm).

Total Hydrolysis. A weighed portion (5 mg) of compound was mixed with a mixture (5 mL) of H_2SO_4 (30%) and AcOH (30%) (1:1) thermostatted at 95°C for 10 h, neutralized with $CaCO_3$, and centrifuged. The supernatant was separated over polyamide (5 g) with elution by H_2O (eluate 1) and MeOH (80%, eluate 2). Monosaccharides in eluate 1 were derivatized with 3-methyl-1-phenyl-2-pyrazolin-5-one [31] and analyzed by anal. HPLC (conditions 1). Monosaccharides were assigned to D- and L-series after reductive amination with L-tryptophan [32] using anal. HPLC (conditions 2). Non-carbohydrate hydrolysis products (eluate 2) were analyzed by GC-MS [33] and NMR spectroscopy. Hydrolysis of **1** produced acacetin [34] and D-glucose; of **2**, apigenin [34], D-glucose, and L-arabinose; of **3**, genkwanin [34], D-glucose, and L-arabinose; of **4**, apigenin, ferulic acid [27], D-glucose, and D-xylose.

Acid hydrolysis with TFA was performed in TFA (2 M) at 120°C. The hydrolysate was separated over polyamide using the previously reported method [24]. The hydrolysis products of **1** contained isocytisoside (**5**) [6] and D-glucose; of **2**, schaftoside (**17**) [17] and D-glucose; of **3**, genkwanin-6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside (**16**) [3], and D-glucose; of **4**, isovitexin (**15**) [20], ferulic acid, D-glucose, and D-xylose.

Hydrolysis by β-glucosidase used β-glucosidase from *Amygdalus* (3.2.1.21, 30 U/mg, No. G4511; Sigma-Aldrich) as described earlier [24]. The hydrolysis products were analyzed by HPLC, mass spectrometry, and NMR spectroscopy [24]. The hydrolysis products of **4** were 6-*O*-feruloylglucose [28] and isovitexin-2″-*O*-xyloside [29].

Hydrolysis of 4 by β-Xylosidase. A weighed portion of **4** (10 mg) was dissolved in DMSO (150 μL). The volume was adjusted to 5 mL using sodium succinate solution (50 mM, pH 5.3). The mixture was treated with β-xylosidase (2 U) from *Selenomonas ruminantium* (3.2.1.37, 115 U/mg; Megazyme Ltd., Bray, Ireland). The reaction mixture was incubated at 40°C for 10 h, heated at 95°C (15 min), and centrifuged (6,000 rpm, 15 min). The hydrolysate was separated over polyamide (5 g) with elution by H_2O (50 mL) and EtOH (60%, 100 mL). The EtOH eluate afforded saponarin-6‴-*O*-ferulate (4 mg, **23**), which was identified using ^{13}C NMR spectroscopy and mass spectrometry [25].

Alkaline hydrolysis of 4 with NaOH used the previously reported method [24]. The hydrolysate of **4** contained ferulic acid [27] and saponarin-2″-*O*-β-D-xylopyranoside (**4a**) according to GC-MS [33] and NMR spectroscopy.

Saponarin-2″-*O*-β-D-xylopyranoside (4a), $C_{32}H_{38}O_{19}$. UV spectrum (MeOH, λ_{max}, nm): 271, 334. HR-ESI-MS, *m*/*z* 727.7202 (calcd for $C_{32}H_{39}O_{19}$, 727.5924). ESI-MS, *m*/*z*: 727 $[M + H]^+$; MS^2 [727]: 595 $[(M + H) – C_5H_8O_4]^+$, 433 $[(M + H) – C_5H_8O_4 – C_6H_{10}O_5]^+$; MS^3 [433]: 343 $[(M + H) – C_5H_8O_4 – C_6H_{10}O_5 – C_3H_6O_3]^+$, 315 $[(M + H) – C_5H_8O_4 – C_6H_{10}O_5 – C_3H_6O_3 – CO]^+$, 313 $[(M + H) – C_5H_8O_4 – C_6H_{10}O_5 – C_4H_8O_4]^+$, 285 $[(M + H) – C_5H_8O_4 – C_6H_{10}O_5 – C_4H_8O_4 – CO]^+$. ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): apigenin – 6.74 (1H, s, H-3), 6.51 (1H, s, H-8), 7.90 (2H, d, J = 8.1, H-2′, 6′), 6.99 (2H, d, J = 8.1, H-3′, 5′), 13.38 (1H, br.s, 5-OH), 10.29 (1H, br.s, 4′-OH); 6-*C*-β-*D*-glucopyranose – 4.87 (1H, d, J = 9.0, H-1″), 4.41 (1H, m, H-2″), 3.59 (1H, m, H-3″), 3.17–3.25 (2H, m, H-4″, 5″), 3.79 (1H, dd, J = 3.0, 11.4, H_A-6″), 3.37 (1H, m, H_B-6″); 2″-*O*-β-*D*-xylopyranose – 4.96 (1H, d, J = 7.0, H-1‴), 3.10 (1H, m, H-2‴), 3.27–3.35 (2H, m, H-3‴, 4‴), 3.62 (1H, m, H_A-5‴), 2.93 (1H, m, H_B-5‴); 7-*O*-β-*D*-glucopyranose – 5.09 (1H, d, J = 7.1, H-1⁗), 3.39–3.57 (4H, d, H-2⁗–5⁗), 3.92 (1H, dd, J = 3.1, 11.6, H_A-6⁗), 3.65 (1H, m, H_B-6⁗). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): apigenin – 164.1 (C-2), 103.6 (C-3), 182.4 (C-4), 160.1 (C-5), 110.4 (C-6), 162.3 (C-7), 94.7 (C-8), 156.4 (C-9), 105.9 (C-10),

122.1 (C-1′), 129.3 (C-2′, 6′), 115.8 (C-3′, 5′), 161.5 (C-4′); 6-*C*-β-*D*-glucopyranose – 72.3 (C-1″), 79.8 (C-2″), 77.6 (C-3″), 71.6 (C-4″), 81.0 (C-5″), 62.0 (C-6″); 2″-*O*-β-*D*-xylopyranose – 105.4 (C-1‴), 75.1 (C-2‴), 76.0 (C-3‴), 70.2 (C-4‴), 66.1 (C-5‴); 7-*O*-β-*D*-glucopyranose – 101.5 (C-1⁗), 74.1 (C-2⁗), 76.7 (C-3⁗), 69.7 (C-4⁗), 76.4 (C-5⁗), 61.2 (C-6⁗).

Analytical HPLC. Conditions 1: mobile phase: NH_4OAc (100 mM, pH 4.5) (A) and MeCN (B); gradient mode (%B): 0–20 min, 20–26%; flow rate 150 μL/min; column temperature 35°C; UV detector at λ 250 nm. Conditions 2: mobile phase: NaH_2PO_4 (10 mM), $Na_2B_4O_7$ (50 mM), 1:1 (pH 9.6); isocratic mode, flow rate 200 μL/min; column temperature 35°C; UV detector at λ 220 nm.

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