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SPIREASALICIN, A NEW ACYLATED QUERCETIN GLYCOSIDE FROM *Spiraea salicifolia*

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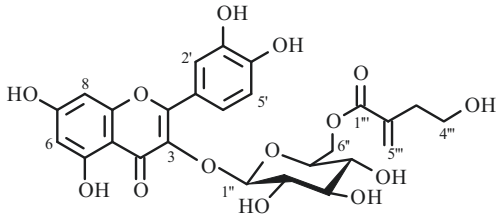
A new acylated quercetin glycoside and 36 known compounds were isolated from flowering runners of Spiraea salicifolia *L. (Rosaceae). The structures of the new glycoside quercetin-3-*O*-[6″-(4‴-hydroxy-2‴-methylenebutyroyl)]-*β*-D-glucopyranoside (spireasalicin, **1**) and the 36 known compounds were elucidated using UV, IR, and NMR spectroscopy and mass spectrometry. The biological screening of the isolated compounds showed that **1** inhibited* α*-glucosidase. The contents of several phenolic compounds in* S. salicifolia *organs were studied and found to be unevenly distributed in the separate plant parts.*

Keywords: *Spiraea salicifolia*, Rosaceae, quercetin-3-*O*-[6″-(4‴-hydroxy-2‴-methylenebutyroyl)]-*β*-D-glucopyranoside, spireasalicin, anti-glucosidase activity, HPLC.

Diabetes mellitus is a metabolic disorder characterized by abnormal post-prandial elevation of the blood glucose level. Recently, research on diabetes treatment has been directed more and more at the discovery of efficacious inhibitors of *α*-glucosidase, the key enzyme in carbohydrate metabolism. Previously, we showed that plants of the family Rosaceae (*Comarum*, *Filipendula*, *Potentilla*) are sources of phenolic compounds with anti-glucosidase activity [1–3]. A promising plant species of this family is *Spiraea salicifolia* L., which is used in Tibetan medicine (‘om bu) to treat diabetes-like conditions and gastrointestinal diseases [4]. Few chemical studies of this species were reported. The leaves are known to contain cinnamoyl glycosides [5] and lignans and triterpenes [6]. The biological activity of *S. salicifolia* has not been studied. Our screening studies established that extracts of *S. salicifolia* inhibited *α*-glucosidase [7]. Research on flowering runners of *S. salicifolia* isolated 37 compounds including the new quercetin glycoside **1**. Its anti-glucosidase activity was determined. The distribution of phenolic compounds in separate organs was studied.



1

According to HR-ESI-MS, **1** had the formula $C_{26}H_{26}O_{14}$ (561.318 $[M - H]^-$, calcd 561.480). The ESI-MS spectrum exhibited a quasi-molecular ion with *m*/*z* 561 that corresponded to deprotonated $[M - H]^-$ and ions with *m*/*z* 463 and 301 that were indicative of sequential cleavage of fragment A with *m*/*z* 98 and glucose $\{[(M - H) - 98 - 162]^-\}$. The principal products from acid hydrolysis of **1** were quercetin and D-glucose. The UV spectrum was typical of quercetin glycosides [8]. The IR spectrum of **1** showed bands for ester (ν_{max} 1710 cm^{-1}) and conjugated carbonyls (ν_{max} 1652 cm^{-1}) [9].

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TABLE 1. PMR (500 MHz) and ^{13}C NMR (125 MHz) Spectra of **1** (MeOH-d_4, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	–	158.4	6′	7.53 (1H, dd, J = 8.1, 2.0)	123.3
3	–	135.4	1″	5.10 (1H, d, J = 8.0)	101.6
4	–	179.5	2″	3.57 (1H, m)	76.1
5	–	161.3	3″	3.50 (1H, m)	78.7
6	6.19 (1H, d, J = 2.0)	100.2	4″	3.43 (1H, m)	71.8
7	–	165.4	5″	3.87 (1H, m)	75.2
8	6.30 (1H, d, J = 2.0)	95.0	6″	4.18 (1H, dd, J = 12.0, 6.1)	64.5
9	–	157.8		4.45 (1H, dd, J = 12.0, 2.1)	
10	–	106.3	1‴	–	169.2
1′	–	122.0	2‴	–	139.4
2′	7.92 (1H, d, J = 2.0)	112.7	3‴	2.43 (2H, t, J = 7.1)	35.1
3′	–	145.3	4‴	3.65 (2H, t, J = 7.1)	60.4
4′	–	146.2	5‴	5.68 (1H, s)	129.5
5′	6.91 (1H, d, J = 8.1)	116.0		6.15 (1H, s)	

UV spectroscopy with diagnostic additives found that **1** was characterized by unsubstituted hydroxyls on C-5, C-7, C-3′, and C-4′. Thus, fragment A was an acyl moiety on the carbohydrate substituent. The nature of the acyl moiety was determined by analyzing the alkaline hydrolysis products of **1**, which showed a compound with molecular mass 116 that was identified using NMR and MS data as 4-hydroxy-2-methylenebutyric acid [10].

PMR and ^{13}C NMR spectra of **1** were similar to those of quercetin-3-*O*-*β*-D-glucopyranoside (isoquercitrin) [11] with the exception of additional resonances in the ^{13}C NMR spectrum for ester carboxyl (δ 169.2), a pair of alkene C atoms >C=CH_2 (δ_C 139.4, 129.5), saturated –CH_2– (δ 35.1), and a terminal –CH_2OH (δ 60.4) [12]. The PMR spectrum also contained two additional strong-field resonances for saturated –CH_2–CH_2– (δ 2.43, 3.65) and weak-field vinyl =CH_2 resonances (δ 5.68, 6.15) [13] (Table 1). HMBC correlations between inequivalent H-6″ protons of glucopyranose (δ 4.18, 4.45) and the carboxyl of 4-hydroxy-2-methylenebutyric acid (δ 169.2) and a weak-field shift of the glucopyranose C-6″ resonance (δ 64.5) [8] were consistent with a substituted carbohydrate C-6″. Thus, the results elucidated the structure of **1** as quercetin-3-*O*-[6″-(4‴-hydroxy-2‴-methylenebutyroyl)]-*β*-D-glucopyranoside, which we called spireasalicin.

The 4-hydroxy-2-methylenebutyric acid moiety was also found in the natural glycosides 6-tuliposide A [6-*O*-(4′-hydroxy-2′-methylenebutyroyl)-*β*-D-glucopyranoside] from *S. thunbergii* Siebold ex Blume [14] and 1-*O*-cinnamoyl-6-*O*-(4″-hydroxy-2″-methylenebutyroyl)-*β*-D-glucopyranoside and 1-*O*-(4′-hydroxy-2′-methylenebutyroyl)-6-*O*-cinnamoyl-*β*-D-glucopyranoside from *S. thunbergii* [12] and *S. prunifolia* Siebold & Zucc. [15]. Until now, flavonoids containing this type of acyl substituent were not observed.

Besides **1**, the known compounds **2–37** were also isolated from runners of *S. salicifolia*. They were identified using UV, IR, and NMR spectroscopy and mass spectrometry as kaempferol-3-*O*-(6″-caffeoyl)-*β*-D-glucopyranoside (**2**), kaempferol-3-*O*-(6″-caffeoyl)-*β*-D-galactopyranoside (**3**), quercetin-3-*O*-(6″-caffeoyl)-*β*-D-glucopyranoside (**4**), quercetin-3-*O*-(6″-caffeoy)-*β*-D-galactopyranoside (**5**) [16], tiliroside (**6**) [17], helichrysoside (**7**) [18], astragalin (**8**), trifoliin (**9**), isoquercitrin (**10**), hyperoside (**11**), quercitrin (**12**), miquelianin (**13**), kaempferol-4′-glucoside (**14**), spireoside (**15**) [19], nicotiflorin (**16**), rutin (**17**) [11], caffeic acid (**18**), 3-*O*-caffeoylquinic acid (**19**) [20], 4-*O*-caffeoylquinic acid (**20**), 1-*O*-caffeoyl-*β*-D-glucopyranoside (**21**), cinnamic acid (**22**), *p*-coumaric acid (**23**), 4-methoxycinnamic acid (**24**) [5], 6-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside (**25**),6-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside (**26**), 6-*O*-*cis*-4-methoxycinnamoyl-*β*-D-glucopyranoside (**27**), 6-*O*-*trans*-4-methoxycinnamoyl-*β*-D-glucopyranoside (**28**), 1-*O*-*trans*-cinnamoyl-6-*O*-(4″-hydroxy-2″-methylenebutyroyl)-*β*-D-glucopyranoside (**29**) [12], protocatechoic acid (**30**), gallic acid (**31**) [20], 5-*O*-galloylquinic acid (**32**), (+)-catechin (**33**), (–)-epicatechin (**34**) [21], and procyanidins B_1 (**35**), B_2 (**36**), and C_1 (**37**) [22]. Previously, leaves of *S. salicifolia* afforced **11** and **22**–**24** [5]. Compounds **2**–**10**, **12**–**21**, and **25**–**37** were detected in this plant species for the first time.

TABLE 2. α-Glucosidase Activity 50% Inhibitory Concentration (IC_{50}) of Several Flavonoids from *S. salicifolia*, μg/mL ± SD

Carbohydrate part[a]	Aglycone[b]	
	kaempferol	quercetin
–	> 350	207.10 ± 8.69
3-*O*-Glc*p*	>350 (**8**)	273.57 ± 11.21 (**10**)
3-*O*-Gal*p*	>350 (**9**)	291.64 ± 11.95 (**11**)
3-*O*-(6″-HMB)-Glc*p*	–	254.31 ± 7.62 (**1**)
3-*O*-(6″-Caf)-Glc*p*	302.07 ± 12.38 (**2**)	42.97 ± 1.93 (**4**)
3-*O*-(6″-Caf)-Gal*p*	308.16 ± 13.25 (**3**)	40.69 ± 1.66 (**5**)
3-*O*-(6″-Cou)-Glc*p*	> 350 (**6**)	331.14 ± 14.23 (**7**)
Acarbose[c]	273.11 ± 12.28	

[a]Caf = caffeoyl; Cou = *p*-coumaroyl; Gal*p* = galactopyranose; Glc*p* = glucopyranose; HMB = 4′-hydroxy-2′-methylenebutyroyl; [b]compound number shown in parentheses; "–", not determined (compound missing); [c]reference compound.

An examination of the occurrence in the genus *Spiraea* of phenolic glycosides containing an acyl substituent on the carbohydrate found that flavonoid acylglycosides **2**, **3**, and **5** were also observed in flowers of *S. cantoniensis* Lour. [16]; **6**, in fruit of *S. formosana* Hayata [23] and runners of *S. canescens* D. Don [24]. Phenolic glycosides **25**–**29** were detected as constituents in the aerial part of *S. canescens* [24], *S. prunifolia* [15], and *S. thunbergii* [12]. Acylation of phenolic glycosides is probably a systematic chemical feature of the genus *Spiraea*.

Studies of the biological activities of the isolated compounds with respect to α-glucosidase showed that several flavonoids were very potent inhibitors of the enzyme (Table 2). The 50% inhibitory concentrations (IC_{50}) for phenylpropanoids **18**–**29**, benzoic acids **30**–**32**, catechins **33** and **34**, and procyanidins **35**–**37** were ≥400 μg/mL, which indicated that they were not efficacious. The degree of α-glucosidase inhibition increased from kaempferol to quercetin for both the aglycones and glycosides. This indicated that substitution of flavonoid ring B was very important for the manifestation of biological activity. The activities of the glycoside derivatives decreased if a carbohydrate moiety (glucose, galactose) was added to the flavonoid C-3 position. However, a C-6″ *p*-coumaroyl or caffeoyl substituent on the sugar helped to increase the inhibition efficacy with caffeoyl being the most potent. Quercetin glycosides **4** (IC_{50} 42.97 μg/mL) and **5** (IC_{50} 40.69 μg/mL) were the most efficacious α-glucosidase inhibitors. Their activities were significantly greater than that of the reference compound acarbose (IC_{50} 273.11 μg/mL). The efficacy of **1** (IC_{50} 254.31 μg/mL) as the 4-hydroxy-2-methylenebutyryl derivative of glycoside **10** was similar to that of the starting compound (IC_{50} 273.57 μg/mL) and acarbose.

HPLC-UV was used for quantitative characterization of *S. salicifolia* flowering runners as a source of phenolic α-glucosidase inhibitors. An analysis of *S. salicifolia* organs showed that the various compound groups were unevenly distributed in the plant (Table 3). Flowers of *S. salicifolia* were capable of accumulating flavonoids (42.40 mg/g) and less catechins and phenylpropanoids. The principal flavonoids of the flowers were quercitrin (**12**, 11.70 mg/g), hyperoside (**11**, 8.01 mg/g), and isoquercitrin (**10**, 7.43 mg/g). Quercetin derivatives were responsible for ~92% (38.91 mg/g) of the total flavonoids in flowers. Flavonoid monosides dominated over biosides. The contents of glucosides, galactosides, and rhamnosides were similar. The total content of acylated glycosides **2**, **3**, and **5** was 5.22 mg/g or 12.3% of the total flavonoids. The flavonoid concentrations in *S. salicifolia* leaves were lower (23.40 mg/g) with **10** (7.55 mg/g), **3** (5.53 mg/g), and **11** (4.56 mg/g) being dominant. The principal flavonoid species were distributed similarly in leaves and flowers. The flavonoid contents in *S. salicifolia* stems were the least at 5.37 mg/g. Glycoside **1** was found in *S. salicifolia* flowers and leaves. The accumulation of phenylpropanoids in *S. salicifolia* was greater in leaves (48.11 mg/g) than in flowers (21.86 mg/g). The principal compounds of this group in *S. salicifolia* leaves was 1-*O*-caffeoyl-β-D-glucopyranoside (**21**, 38.46 mg/g); in flowers, 3-*O*-caffeoylquinic acid (**19**, 13.42 mg/g). Benzoic acids (**30** and **31**) and derivative **32** were detected only in *S. salicifolia* flowers (2.19 mg/g), where (+)-catechin (**33**, 21.81 mg/g) and catechins in general (30.14 mg/g) also localized. Stems of *S. salicifolia* were characterized by larger concentrations of catechins (10.88 mg/g) than leaves (5.57 mg/g). In general, it is noteworthy that the various organs of *S. salicifolia* runners typically had high contents of phenolic compounds. However, flowers and leaves had the highest contents of the target group of acylated flavonoid glycosides (**1**–**3** and **5**) and could be recommended as a new medicinal raw material.

TABLE 3. Contents of Several Phenolic Compounds (PCs) in *S. salicifolia* Organs, mg/g of Air-dried Mass ± SD

Compound	Flowers	Leaves	Stems
	Flavonoids (Fl)		
1	< 0.01	< 0.01	
2	2.19 ± 0.04	0.86 ± 0.02	0.18 ± 0.00
3	1.30 ± 0.03	5.53 ± 0.11	< 0.01
5	1.73 ± 0.04	0.64 ± 0.02	0.21 ± 0.00
10	7.43 ± 0.14	7.55 ± 0.15	2.80 ± 0.05
11	8.01 ± 0.15	4.56 ± 0.09	1.22 ± 0.02
12	11.70 ± 0.26	1.08 ± 0.02	0.44 ± 0.01
13	1.12 ± 0.02	0.11 ± 0.00	< 0.01
15	1.91 ± 0.05	1.07 ± 0.02	0.17 ± 0.00
17	3.62 ± 0.08	< 0.01	0.16 ± 0.00
	Phenylpropanoids (PP)		
18	1.34 ± 0.04	1.51 ± 0.03	1.09 ± 0.02
19	13.42 ± 0.28	7.14 ± 0.14	2.14 ± 0.04
20	1.93 ± 0.05	0.25 ± 0.00	1.00 ± 0.02
21	4.82 ± 0.11	38.46 ± 0.76	2.25 ± 0.04
22	0.18 ± 0.00	0.48 ± 0.01	
24	0.17 ± 0.00	0.27 ± 0.00	
	Benzoic acids (BA)		
30	0.93 ± 0.02		
31	0.36 ± 0.01	< 0.01	< 0.01
32	0.90 ± 0.02	< 0.01	
	Catechins (C)		
33	21.81 ± 0.43	5.57 ± 0.11	6.95 ± 0.14
34	8.33 ± 0.16	< 0.01	3.93 ± 0.07
Σ_{Fl}	42.40	23.40	5.37
Σ_{PP}	21.86	48.11	6.48
Σ_{BA}	2.19	< 0.01	< 0.01
Σ_{C}	30.14	5.57	10.88
Σ_{PC}	96.59	77.08	22.73

EXPERIMENTAL

Runners of *S. salicifolia* were collected during flowering in the vicinity of Kyren (Tunkinskii District, Republic of Buryatia, Russia; July 20, 2015; 51°40′48″ N, 102°11′54″ E, 741 m above sea level). The species was determined by Dr. T. A. Aseeva (IGEB, SB, RAS). Raw material was dried in a convection oven (50°C) to moisture content ≤5%. Column chromatography (CC) was performed over SiO_2, RP-SiO_2, polyamide (Sigma-Aldrich, St. Louis, MO, USA), and Sephadex LH-20 (GE Healthcare, Little Chalfont, UK). Spectrophotometry was carried out on an SF-2000 spectrophotometer (OKB Spektr, St. Petersburg, Russia). MS analyses used LCMS-8050 TQ-mass spectrometer (Shimadzu, Columbia, MD, USA) with electrospray ionization (ESI) in negative-ion mode at capillary potential –30 to –60 V, field potential 5.0 kV, ion-source temperature 250°C, and molecular mass scan range 100–2000 Da. NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian, Palo Alto, CA, USA). Preparative HPLC was performed on a Summit liquid chromatograph (Dionex, Sunnyvale, CA, USA).

Extraction and Fractionation. A weighed portion of dried and ground raw material (1400 g) was extracted (2×) with EtOH (60%, 1:20) in an ultrasonic bath (100 W, 35 kHz) at 40°C for 2 h. The resulting extract was filtered off and concentrated in vacuo (40°C) to an aqueous residue that was extracted with hexane and *n*-BuOH. The solvents were removed to produce hexane (SF-1, 36 g), *n*-BuOH (SF-2, 283 g), and aqueous fractions (SF-3, 204 g). Anti-glucosidase activity of the fractions showed that fraction SF-2 had the greatest inhibitory activity with IC_{50} 163.27 ± 6.52 μg/mL. The activities of

SF-1 and SF-3 were <400 μg/mL. Fraction SF-2 (250 g) was investigated further by separation over polyamide (CC, 2.5 kg) with elution successively by H_2O (fraction SF-2/1, 26 g), EtOH (30%, SF-2/2, 49 g; 70%, SF-2/3, 134 g), and NH_3 (0.5%) in EtOH (90%, SF-2/4, 37 g). Fraction SF-2/1 was not investigated further because it was inactive. Fraction SF-2/2 (40 g) was chromatographed over SiO_2 (CC, 4 × 60 cm) using a EtOAc–Me_2CO gradient (100:0→60:40) to produce subfractions SF-2/2-01–SF-2/2-09. Subfractions SF-2/2-04 and SF-2/2-05 were rechromatographed over SiO_2 (CC, 2 × 30 cm) using a EtOAc–EtOH gradient (100:0→70:30) to isolate procyanidins B_1 (63 mg, **35**) [22], B_2 (40 mg, **36**) [22], and C_1 (15 mg, **37**) [22]. Analogous separation of subfraction SF-2/2-06 produced 1-*O*-caffeoyl-*β*-D-glucopyranoside (1264 mg, **21**) [5], (+)-catechin (107 mg, **33**) [21], and (–)-epicatechin (27 mg, **34**) [21]. Separation of subfractions SF-2/2-08 and SF-2/2-09 over SiO_2 (CC, 2 × 40 cm, Me_2CO–H_2O, 100:0→60:40) isolated rutin (142 mg, **17**) [11], protocatechoic acid (84 mg, **30**) [20], gallic acid (20 mg, **31**) [20], and 5-*O*-galloylquinic acid (54 mg, **32**) [21]. Fraction SF-2/3 (125 g) was chromatographed over SiO_2 (CC, 8 × 70 cm) using a hexane–EtOAc–Me_2CO gradient (100:0:0→60:40:0→0:70:30) to produce subfractions SF-2/3-01–SF-2/3-16.

Next, subfractions SF-2/3-01 and SF-2/3-02 were separated over SiO_2 (CC, 1 × 30 cm) using a hexane–EtOAc gradient (100:0→80:20) to isolate tiliroside (14 mg, **6**) [17], cinnamic acid (30 mg, **22**) [5], *p*-coumaric acid (10 mg, **23**) [5], and 4-methoxycinnamic acid (14 mg, **24**) [5]. Subfractions SF-2/3-03 and SF-2/3-04 were chromatographed over SiO_2 (CC, 2 × 30 cm, EtOH–H_2O, 95:5→70:30) and RP-SiO_2 (CC, 1 × 18 cm, H_2O–MeCN, 100:0→10:90) to isolate kaempferol-4′-glucoside (10 mg, **14**) [19], spireoside (207 mg, **15**) [19], 6-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside (52 mg, **25**) [12], and 6-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside (75 mg, **26**) [12]. Subfractions SF-2/3-06 and SF-2/3-07 were separated over SiO_2 (CC, 3 × 30 cm, hexane–EtOAc, 100:0→70:30), Sephadex G-10 (CC, 2 × 90 cm, EtOH–H_2O, 95:5→60:40), RP-SiO_2 (CC, 1 × 20 cm, H_2O–MeCN, 100:0→20:80), and preparative HPLC [LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm, Supelco, Bellefonte, PA, USA); mobile phase H_2O (A) and MeCN (B) in gradient mode (%B): 0–40 min, 40–60%; 40–60 min, 60–90%; flow rate 1 mL/min; column temperature 30°C; UV detector at 350 nm] to produce kaempferol-3-*O*-(6″-caffeoyl)-*β*-D-glucopyranoside (30 mg, **2**) [16], kaempferol-3-*O*-(6″-caffeoyl)-*β*-D-galactopyranoside (31 mg, **3**) [16], and helichrysoside [quercetin-3-*O*-(6″-*p*-coumaroyl)-*β*-D-glucopyranoside; 28 mg, **7**) [18].

Subfraction SF-2/3-08 was separated analogously to afford **1** (spireasalicin, 18 mg), 6-*O*-*cis*-4-methoxycinnamoyl-*β*-D-glucopyranoside (47 mg, **27**) [12], 6-*O*-*trans*-4-methoxycinnamoyl-*β*-D-glucopyranoside (29 mg, **28**) [12], and 1-*O*-*trans*-cinnamoyl-6-*O*-(4″-hydroxy-2″-methylenebutyroyl)-*β*-D-glucopyranoside (15 mg, **29**) [12]. Subfraction SF-2/3-09 yielded quercetin-3-*O*-(6″-caffeoyl)-*β*-D-glucopyranoside (14 mg, **4**) [16], quercetin-3-*O*-(6″-caffeoyl)-*β*-D-galactopyranoside (36 mg, **5**) [16], and nicotiflorin (25 mg, **16**) [11]. Subfractions SF-2/3-10–SF-2/3-15 were separated using CC over SiO_2 (2 × 30 cm, hexane–EtOAc, 85:15→60:40), Sephadex G-10 (2 × 90 cm, EtOH–H_2O, 95:5→50:50), and RP-SiO_2 (1 × 20 cm, H_2O–MeCN, 100:0→40:60) to produce astragalin (35 mg, **8**) [19], trifoliin (27 mg, **9**) [19], isoquercitrin (180 mg, **10**) [19], hyperoside (183 mg, **11**) [19], quercitrin (1541 mg, **12**) [19], and miquelianin (48 mg, **13**) [19]. Fraction SF-2/4 (35 g) was chromatographed over Sephadex G-10 (CC, 2 × 90 cm, EtOH–H_2O, 95:5→20:80) and RP-SiO_2 (CC, 1 × 20 cm, H_2O–MeCN, 100:0→40:60) to isolate caffeic acid (102 mg, **18**) [20], 3-*O*-caffeoylquinic acid (915 mg, **19**) [20], and 4-*O*-caffeoylquinic acid (26 mg, **20**) [5].

Spireasalicin (1), $C_{26}H_{26}O_{14}$. HR-ESI-MS *m*/*z* 561.318 ([M – H]$^-$; calcd 561.480). (–)ESI-MS *m*/*z*: 561 [M – H]$^-$, 463 [(M – H) – 98]$^-$, 301 [(M – H) – 98 – 162]$^-$. UV spectrum (MeOH, λ_{max}, nm): 256, 267 sh, 356; +$AlCl_3$ 276, 412; +$AlCl_3$/HCl 270, 298, 401; +NaOAc 270, 323, 395; +NaOAc/H_3BO_3 261, 300, 391; +NaOMe 271, 331, 411. IR spectrum (ν_{max}, cm^{-1}): 3425, 1710, 1652, 1602. Table 1 lists the PMR and ^{13}C NMR spectra.

Acid Hydrolysis of 1. Compound **1** (2 mg) was dissolved in TFA (5%, 10 mL) and heated at 110°C (2 h). The hydrolysate was concentrated *in vacuo* and dissolved in MeOH. The resulting hydrolysate was chromatographed over polyamide (CC, 20 g) with elution successively by H_2O (100 mL, eluate I) and EtOH (90%, 250 mL, eluate II). The resulting eluates were concentrated *in vacuo* and analyzed by HPLC (conditions 1, monosaccharides as 3-methyl-1-phenyl-2-pyrazolin-5-ones [25]; conditions 2, phenolic compounds). Glucose was detected in eluate I (t_R 12.53 min); quercetin, in eluate II (t_R 12.66 min). Eluate I was also analyzed as before to determine if the monosaccharides were D- or L-isomers [26].

Alkaline Hydrolysis of 1. Compound **1** (7 mg) was dissolved in NaOH (5 mL, 1 M) and thermostatted at 50°C (4 h). The resulting solution was neutralized with AcOH and extracted with Et_2O (3 × 30 mL). The organic layer was concentrated *in vacuo*. The resulting residue was dissolved in hexane and chromatographed over SiO_2 (CC, 1 × 10 cm) using a hexane–EtOAc gradient (100:0→70:30). The fraction eluted by hexane–EtOAc (85:15) afforded a compound (1.1 mg) that was identified by NMR and mass spectrometry as 4-hydroxy-2-methylenebutyric acid [10].

4-Hydroxy-2-methylenebutyric acid, $C_5H_8O_3$. HR-ESI-MS *m/z* 115.127 ([M – H]$^-$; calcd 115.109). ^{1}H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm, J/Hz): 6.18 (1H, s, =C$\underline{H}_2$), 5.63 (1H, s, =C$\underline{H}_2$), 3.58 (1H, t, J = 7.0, H-4), 2.40 (1H, t, J = 7.0, H-3). ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): 170.5 (C-1), 140.6 (C-2), 129.1 (=$\underline{C}H_2$), 60.9 (C-4), 34.8 (C-3).

HPLC. Conditions 1: ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG); mobile phase: CH_3COONH_4 (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 250 nm. Conditions 2: ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG); mobile phase $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B); gradient mode (%B): 0–9 min, 0–80%; 9–15 min, 80–100%; flow rate 100 μL/min; column temperature 35°C; UV detector at 270 nm. Quantitative analysis was performed on a Milichrom A-02 microcolumn liquid chromatograph (EcoNova, Novosibirsk, Russia); ProntoSIL-120-5-C18 AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG, Herisau, Switzerland); mobile phase: $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B); gradient mode (%B): 0–40 min, 5–80; 40–43 min, 100; flow rate 100 μL/min; column temperature 35°C; UV detector at 270 (benzoic acids, catechins), 320 (phenylpropanoids), and 356 nm (flavonoids). The contents of pure constituents were calculated from calibration curves constructed using commercial samples of isoquercitrin; hyperoside; quercitrin; miquelianin; spireoside; rutin; caffeic acid; 3-*O*- and 4-*O*-caffeoylquinic acids; cinnamic, 4-methoxycinnamic, protocatechoic, and gallic acids; (+)-catechin; and (–)-epicatechin (Sigma-Aldrich). Astragalin (Sigma-Aldrich) was used for analysis of kaempferol-3-*O*-(6″-caffeoyl)glucoside and kaempferol-3-*O*-(6″-caffeoyl)-*β*-D-galactopyranoside; hyperoside, quercetin-3-*O*-(6″-caffeoyl)-*β*-D-galactopyranoside; and caffeic acid, 1-*O*-caffeoyl-*β*-D-glucopyranoside; and gallic acid, 5-*O*-galloylquinic acid. The difference in molecular masses of the compounds was considered in the calculations when using external standards. The results were reported as the averages of three parallel determinations (±standard deviation, SD).

Anti-glucosidase activity of the fractions and compounds was studied by a microplate spectrophotometric method using *α*-glucosidase from *Saccharomyces cerevisiae* (3.2.1.20; type I; ≥10 U/mg; Sigma-Aldrich, St. Louis, MO, USA) [3]. Results were expressed as the IC_{50} (μg/mL) values. Solutions of the compounds in normal saline (0.9% NaCl) were used. The reference compound was acarbose (≥94%, Sigma-Aldrich).

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