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PHENOLIC COMPOUNDS FROM *Filipendula ulmaria*

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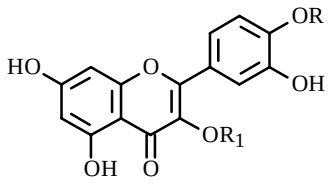
*A chromatographically inseparable crystalline mixture of the previously undescribed quercetin-4′-*O*-β-galactopyranoside and the known quercetin-3-*O*-β-glucopyranoside (isoquercitrin) in a 7:5 ratio was isolated from the extract of the aerial part of* Filipendula ulmaria *(L.) Maxim.*

Key words: *Filipendula ulmaria* (L.) Maxim., phenolic compounds, flavonoids, quercetin-4′-*O*-*β*-galactopyranoside.

Queen of the meadow [*Filipendula ulmaria* (L.) Maxim., Rosaceae] is a dominant plant in damp and swampy meadows of European Russia and Western and Eastern Siberia [1]. This species has a wide distribution, in which it is encountered in various ecological conditions [2]. Flowers of queen of the meadow are permitted for use in medical practice [3] as an anti-inflammatory, astringent, and wound-healing agent. Extracts of this plant are known to decrease capillary permeability of blood vessels and to exhibit distinct anticoagulant, antiulcer, antidiabetic, and anticancer effects [4, 5].

It has been known for some time that its chemical components include isosalicin (2-hydroxyphenylmethyl-*β*-D-glucopyranoside) [6]. Two phenolic glycosides have also been described. One of these was identified as monotropitin (gaulterin), the 6-(*β*-D-xylopyranosido)-*β*-D-glucopyranoside of methylsalicylate; the other, spirein (salicylaldehyde primveroside) [7].

Seven flavonoids and their glycosides, quercetin, rutin, hyperoside, avicularin, spireoside, quercetin-3-*O*-glucuronide, and kaempferol-4′-*O*-glucoside, were detected by HPLC in various parts of plants of two *Filipendula* species, *F. ulmaria* and *F. denudata* (J. and C. Presl.) [8]. Two-dimensional TLC showed that the herb and roots of *F. ulmaria* and *F. hexapetala* contain 20 phenolic acids, of which gallic, *p*-coumaric, and vanillic dominated [9].



1 - 4

1: R = R_1 = H
2: R = H, R_1 = *α*-Ara*f*
3: R = *β*-Gal*p*, R_1 = H
4: R = H, R_1 = *β*-Glc*p*

We investigated the aerial part of *F. ulmaria* during flowering. The aqueous ethanol extract was evaporated in vacuo until the ethanol was completely removed. The residual was extracted successively with chloroform and ethylacetate. Removal of solvents afforded the corresponding extracts in yields of 0.7 and 6.8% per air-dried raw material.

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TABLE 1. ^{13}C NMR Spectra of a Crystalline Mixture of Quercetin 4′-*O*-Galactopyranoside (**3**) and Quercetin 3-*O*-Glucopyranoside (**4**, Isoquercitrin) (DMSO-d_6, 0 = TMS, δ, ppm)

C atom	**1***	**3**	**4**
2	146.9	146.82 s	156.34 s
3	135.5	136.45 s	133.13 s
4	175.8	176.10 s	177.53 s
5	160.7	160.76 s	161.27 s
6	98.2	98.31 d	98.71 d
7	163.9	164.10 s	164.16 s
8	93.3	93.57 d	93.57 d
9	156.2	156.28 s	156.28 s
10	103.1	103.15 s	103.96 s
2′	115.3[a]	115.97 d	115.23 d
1′	122.1	125.13 s	122.05 s
3′	145.0	145.95 s	144.88 s
4′	147.6	146.37 s	148.52 s
5′	115.6[a]	115.18 d	115.81 d
6′	120.0	119.58 d	121.12 d

[a]Literature data [16] are given for comparison.

TABLE 2. ^{13}C NMR Spectra of the Carbohydrate Parts of **3** and **4** (DMSO-d_6, 0 = TMS, δ, ppm)

Galactose[a]	**3**	**4**	Glucose[a]
102.3	101.78	101.35	101.4
75.8	75.88	77.32	77.5
73.4	73.20	75.98	78.6
71.3	71.23	73.71	74.3
68.0	67.96	69.81	70.3
60.8	60.73	60.17	61.3

[a]Literature data [16] are given for comparison.

Separation of the ethylacetate extract over polyamide with subsequent rechromatography over silica gel isolated successively salicylic acid, an as yet unidentified crystalline compound, the mass spectrum of which was somewhat similar to that of the known fungal metabolite puberulic (1,3,6-cycloheptatrien-3,4,6-trihydroxy-5-oxo-1-carboxylic) acid [10, 11], quercetin (**1**), avicularin (quercetin 3-*O*-α-arabofuranoside) (**2**) [12], and another crystalline flavonoid glycoside that gives a single spot for TLC on silufol but two spots for paper chromatography (PC). One of these two spots corresponded to that of standard isoquercitrin (quercetin 3-*O*-β-glucopyranoside) whereas the second spot could not be matched with any standard.

Acid hydrolysis of the isolated two-component glycoside produced quercetin, glucose, and galactose. The second glycoside in this crystalline inseparable pair is not the known quercetin 3-*O*-β-galactoside (hyperoside) [13] because it differs on PC from an authentic hyperoside sample. We used NMR spectra to determine the structure of the second glycoside in the crystalline pair of quercetin glycosides (Tables 1 and 2).

Spectra recorded in Py-d_6 and DMSO-d_6 showed that the mixture components were two quercetin mono-*O*-glycosides in a 7:5 ratio. The large doublet splitting of the signal for the anomeric proton in the PMR spectrum (8 Hz) indicated that they were β-glucosides [14] (the J value for the α-configuration of the anomeric center is known [15] to be zero or insignificant).

TABLE 3. Chemical Shifts and Spin—Spin Coupling Constants (J/Hz) in PMR spectra of **1** and a Crystalline Mixture of **3** and **4** (DMSO-d_6, 0 = TMS)

H atom	**1**	**3**	**4**
6	6.18 (d, 2.0)	6.20 (d, 2.0)	6.21 (d, 2.0)
8	6.40 (d, 2.0)	6.45 (d, 2.0)	6.42 (d, 2.0)
2′	7.67 (d, 2.0)	7.70 (d, 2.0)	7.57 (d, 2.0)
5′	6.88 (d, 9.0)	7.26 (d, 9.0)	6.84 (d, 9.0)
6′	7.54 (dd, 9.0; 2.0)	7.62 (dd, 9.0; 2.0)	7.65 (dd, 9.0; 2.0)

One of these, as established by PC, was quercetin 3-*O*-glucopyranoside and was the minor component of the mixture. Therefore, the second (principal) glycoside was quercetin *O*-*β*-galactopyranoside with the galactose attached to the O atom on C-4′. This was confirmed by the positions of the signals for C-5 and C-7 in the ^{13}C NMR spectrum, which were unchanged compared with those in the spectrum of starting **1**, the observation of a distinct *ortho*-effect for the H-5′ signal (and an unchanged signal for H-2′ compared with the same spectrum) in the PMR spectrum, and finally the observation of the same effect on the flavone ring C atoms as for adding an isomeric sugar, glucose, to the C-4′ position [16].

We found no information in the literature on the occurrence in plants of quercetin 4′-*O*-*β*-galactopyranoside (**1**).

EXPERIMENTAL

Melting points were determined on a Boetius apparatus. IR spectra were obtained on a Vector 22 instrument; NMR spectra, on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C); mass spectra (EI, 70 eV), in a Finnigan MAT 8200 instrument. Column chromatography was performed over SiO_2 (Chemapol 40/100) with elution by mixtures of petroleum ether and ethylacetate with an increasing (from 0 to 60%) content of the latter. TLC used Silufol plates with development by spraying with vanillin solution (1%) in H_2SO_4. PC used FN-4 and Leningrad M paper.

Starting raw materal was collected in Tomsk region during flowering and dried in air.

Extraction of Raw Material and Isolation of Fractions. A weighed portion (0.6 kg) of raw material was extracted with hot aqueous ethanol (70%, 3 × 4.8 L). The extracts were filtered and evaporated to an aqueous residual that was treated successively in a separatory funnel with chloroform (4 × 0.2 L) and ethylacetate (4 × 0.2 L). Solvents were evaporated to afford chloroform (0.42 g, 0.7%) and ethylacetate (40.8 g, 6.8%) fractions.

Chromatography of the latter over polyamide with subsequent rechromatography over silica gel (chloroform eluent with gradually increasing methanol content) isolated salicylic acid {0.035 g, white needle-like crystals, mp 144-146°C, λ_{max} 275 nm (MeOH), R_f 0.66 (here and henceforth on Silufol UV-254 plates using $CHCl_3$:CH_3OH, 8:2), $[M]^+$ 138} and a white microcrystalline powder (0.025 g, mp 152-154°C, λ_{max} 231 and 300 nm, R_f 0.35). Its mass spectrum exhibited peaks with *m*/*z* 125, 153, 170, and 198. According to the mass spectrometric database "Databaze/wiley7n.1," this mass spectrum is similar to that of puberulic acid. However, this isolated compound could not be clearly identified owing to the presence of difficultly separated impurities.

Further elution of the column afforded successively gallic acid (3,4,5-trihydroxybenzoic acid, 100 mg, mp 238-240°C), flavonoid **1** (0.08 g, identified by the lack of melting-point depression of a mixed sample with authentic **1**), glycoside **2** (0.03 g, mp 218-219°C, lit. [12] mp 217°C), and a crystalline mixture of **3** and **4** (2.30 g, 7:5).

Mixture of Glycosides 3 and 4. Light yellow crystals, mp 219-221°C (CH_3OH:$CHCl_3$, 4:6). UV spectrum (λ_{max}, CH_3OH, nm): 256 and 365. TLC on Silufol gave a single spot: PC (30% AcOH eluent), two spots with R_f 0.27 and 0.56. The compound with R_f 0.56 corresponded to isoquercitrin (comparison with an authentic sample, whereas a standard hyperoside sample had R_f 0.67 under the same conditions). Tables 1 and 2 give the ^{13}C NMR spectra of the crystalline mixture; Table 3, the PMR spectrum.

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