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FLAVONOL MONO- AND DIGLYCOSIDES FROM LEAVES OF *Caragana spinosa*

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We continued research on the chemical composition of *Caragana spinosa* (L.) Vahl ex Hornem. [1]. It was found earlier that leaves of this species accumulate characteristically flavonol glycosides. The goal of the present work was to study in depth the composition of the flavonol glycosides from leaves of *C. spinosa*.

Leaves of *C. spinosa* were collected during flowering in the vicinity of Nizhnii Ubukun (Selenginskii Region, Republic of Buryatiya; 23 Jun., 2010; 51°52′54″ N, 106°89′35″ E). The species was determined by Cand. G. V. Chekhirova (IGEB, SB, RAS). Specimens of the plant are preserved in the herbarium of the IGEB, SB, RAS (No. Fb/s-24/17-02/1006).

Extraction and Fractionation. Ground leaves of *C. spinosa* (1.1 kg) were extracted successively with EtOH (80, 60, and 40%) on a boiling-water bath (1:10 × 5, 3 h). The combined extracts were concentrated to an aqueous residue that was extracted with $CHCl_3$, EtOAc, and BuOH. The BuOH fraction (154 g) was placed on a column of Sephadex LH-20 (10 × 150 cm) and eluted by a MeOH:H_2O gradient (100:0→40:60). Fractions of similar composition were combined and rechromatographed under analogous conditions over columns (2 × 120 cm). Pure compounds were isolated using recrystallization, preparative TLC (SiO_2, EtOAc:CH_2Cl_2:AcOH:HCOOH:H_2O, 10:2.5:1:1:1, two- and three-fold elutions), ascending CC on short SiO_2 columns (1 × 20 cm, EtOAc:AcOH:HCOOH:H_2O, 10:1:1:2), and preparative HPLC.

The separation isolated eight compounds: nicotiflorin (kaempferol-3-*O*-rutinoside, 311 mg, **1**) [2]; narcissin (isorhamnetin-3-*O*-rutinoside, 1.37 mg, **2**) [3]; rutin (quercetin-3-*O*-rutinoside, 2.74 g, **3**) [2]; myricetin-3-*O*-rutinoside (49 mg, **4**) [3]; quercetin-3-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside (15 mg, **5**), isorhamnetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (37 mg, **6**) [4]; quercetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (40 mg, **7**) [5]; and quercetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside (61 mg, **8**) [6].

Quercetin-3-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside (5), $C_{27}H_{30}O_{17}$. UV spectrum (MeOH, λ_{max}, nm): 255, 264sh, 355. FAB^+-MS (*m*/*z*): 627 $[M + H]^+$, 465 $[(M + H) – Glc]^+$, 303 $[(M + H) – 2×Glc]^+$. ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): 62.0 (2″-Glc, C-6), 68.2 (3-Glc, C-6″), 71.2 (3-Glc, C-4″), 71.9 (2″-Glc, C-4‴), 75.7 (2″-Glc, C-2‴), 77.0 (3-Glc, C-5″), 77.4 (2″-Glc, C-5‴), 77.7 (2″-Glc, C-3‴), 78.2 (3-Glc, C-3″), 81.5 (3-Glc, C-2″), 94.2 (C-8), 99.6 (C-6), 103.5 (3-Glc, C-1″), 104.7 (2″-Glc, C-1‴), 105.4 (C-10), 115.6 (C-5′), 116.3 (C-2′), 122.0 (C-6′), 122.4 (C-1′), 135.2 (C-3), 144.8 (C-3′), 148.3 (C-4′), 156.5 (C-2), 157.8 (C-9), 162.1 (C-5), 166.2 (C-7), 178.5 (C-4).

Isorhamnetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (6), $C_{34}H_{42}O_{21}$. UV spectrum (MeOH, λ_{max}, nm): 255, 266sh, 354. FAB^+-MS (*m*/*z*): 787 $[M + H]^+$, 641 $[(M + H) – Rha]^+$, 479 $[(M + H) – Rha – Glc]^+$, 317 $[(M + H) – Rha – 2 × Glc]^+$. ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): 17.9 (6″- Rha, C-6‴), 57.3 (3′-O<u>C</u>H$_3$), 62.3 (2″-Glc, C-6‴), 68.1 (3-Glc, C-6″), 68.9 (6″-Rha, C-5‴), 71.0 (3-Glc, C-4″), 71.5 (2″-Glc, C-4‴), 72.3 (6″-Rha, C-2‴), 73.5 (6″-Rha, C-4‴), 75.4 (2″-Glc, C-2‴), 77.0 (3-Glc, C-5″), 77.4 (2″-Glc, C-5‴), 77.7 (2″-Glc, C3‴), 78.0 (3-Glc, C-3″), 81.7 (3-Glc, C-2″), 94.4 (C-8), 99.3 (C-6), 102.0 (6″-Rha, C-1‴), 103.7 (3-Glc, C-1″), 104.9 (2″-Glc, C-1‴), 105.6 (C-10), 114.3 (C-2′), 115.8 (C-5′), 122.2 (C-6′), 122.7 (C-1′), 132.5 (C-3), 148.6 (C-4′), 151.3 (C-3′), 156.7 (C-2), 157.7 (C-9), 162.2 (C-5), 165.9 (C-7), 178.4 (C-4).

Quercetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (7), $C_{33}H_{40}O_{21}$. UV spectrum (MeOH, λ_{max}, nm): 255, 267sh, 354. FAB^+-MS (*m*/*z*): 773 $[M + H]^+$, 627 $[(M + H) – Rha]^+$, 465 $[(M + H) – Rha – Glc]^+$, 303 $[(M + H) – Rha – 2 × Glc]^+$. ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): 17.8 (6″-Rha, C-6‴), 62.2 (2″-Glc, C-6‴) 68.0 (3-Glc, C-6″), 68.8 (6″-Rha, C-5‴), 71.1 (3-Glc, C-4″), 71.6 (2″-Glc, C-4‴), 72.3 (6″-Rha, C-2‴), 72.7

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(6″-Rha, C-3‴″), 73.6 (6″-Rha, C-4‴″), 75.4 (2″-Glc, C-2‴), 76.9 (3-Glc, C-5″), 77.2 (2″-Glc, C-3‴), 77.6 (2″-Glc, C-5‴), 77.9 (3-Glc, C-3″), 81.3 (3-Glc, C-2″), 94.6 (C-8), 99.5 (C-6), 102.3 (6″-Rha, C-1‴″), 103.2 (3-Glc, C-1″), 104.8 (2″-Glc, C- 1‴), 105.7 (C-10), 115.7 (C-5′), 116.3 (C-2′), 121.8 (C-6′), 122.3 (C-1′), 135.2 (C-3), 144.8 (C-3′), 148.6 (C-4′) 156.3 (C-2), 157.9 (C-9), 162.0 (C-5), 166.0 (C-7), 178.6 (C-4).

Quercetin-3-*O*-β-glucopyranosyl-(1→2)-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside-7-*O*-α-rhamnopyranoside (8), $C_{39}H_{50}O_{25}$. UV spectrum (MeOH, λ_{max}, nm): 255, 303, 357; +$AlCl_3$: 270, 306, 430; +$AlCl_3$/HCl: 270, 362, 401; +NaOMe: 270, 324, 411; +NaOAc: 257, 270, 391, 432; +NaOAc/H_3BO_3: 255, 272. FAB^+-MS (*m*/*z*): 919 $[M + H]^+$, 773 $[(M + H) – Rha]^+$, 611 $[(M + H) – Rha – Glc]^+$, 465 $[(M + H) – 2 \times Rha – Glc]^+$, 303 $[(M + H) – 2 \times Rha – 2 \times Glc]^+$. PMR spectrum (500 MHz, MeOH-d_4, δ, ppm, J/Hz): 1.02 (d, J = 6.1, 6″-Rha, H-6‴″), 1.29 (d, J = 6.1, 7-Rha, H-6⁗″), 3.20–3.30 (m, 6″-Rha, H-4‴″, 7-Rha, H-4⁗″), 3.35–3.90 (m), 4.50 (d, J = 1.7, 6″-Rha, H-1‴″), 4.78 (d, J = 7.6, 2″-Glc, H-1‴), 5.30 (d, J = 7.6, 3-Glc, H-1″), 5.55 (br.s, 7-Rha, H-1⁗″), 6.21 (d, J = 2.0, H-6), 6.41 (d, J = 2.0, H-8), 6.87 (d, J = 8.5, H-5′), 7.60 (dd, J = 8.5, 2.0, H-6′), 7.68 (d, J = 2.0, H-2′). ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): 17.7 (6″-Rha, C-6‴″; 7-Rha, C-6⁗″), 62.0 (2″-Glc, C-6‴), 68.4 (3-Glc, C-6″), 68.9 (6″-Rha, C-5‴″), 69.3 (7-Rha, C-5⁗″), 71.2 (3-Glc, C-4″), 71.7 (2″-Glc, C-4‴), 72.4 (6″-Rha, C-2‴″; 7-Rha, C-2⁗″), 72.9 (6″-Rha, C-3‴″; 7-Rha, C-3⁗″), 73.0 (7-Rha, C-4⁗″), 73.5 (6″-Rha, C-4‴″), 75.6 (2″-Glc, C-2‴), 77.1 (3-Glc, C-5″), 77.5 (2″-Glc, C-5‴), 77.8 (2″-Glc, C-3‴), 78.0 (3-Glc, C-3″), 81.5 (3-Glc, C-2″), 94.7 (C-8), 98.4 (C-6), 99.0 (7-Rha, C-1⁗″), 102.4 (6″-Rha, C-1‴″), 103.7 (3-Glc, C-1″), 104.8 (2″-Glc, C-1‴), 105.6 (C-10), 115.8 (C-5′), 116.4 (C-2′), 122.0 (C-6′), 122.6 (C-1′), 135.2 (C-3), 144.9 (C-3′), 148.7 (C-4′), 156.7 (C-2), 158.2 (C-9), 162.2 (C-5), 162.6 (C-7), 178.8 (C-4).

Compounds **1–3** were isolated earlier from runners of *C. spinosa* [1, 7, 8]. The occurrence of **4–8** was established for the first time in this species and the genus *Caragana*.

TLC was performed on Sorbfil PTSKh-AF silica-gel plates (Imid Ltd.); CC, over Sephadex LH-20 (Pharmacia); silica gel (100/400, Woelm). Spectrophotometric studies used an SF-2000 spectrophotometer (OKB Spektr). MS analysis used an MAT 8200 high-resolution mass spectrometer (Finnigan). PMR and ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian). Spectroscopic analysis with complexing additives was carried out according to recommendations [9]. Total hydrolysis of the compounds (5 mg) used TFA (5%) in MeOH (110°C, 4 h) after which the reaction mixture was concentrated *in vacuo* and analyzed by HPLC (conditions 1, flavonoids; conditions 2, carbohydrates). Analytical HPLC was performed on a Millichrom A-02 microcolumn liquid chromatograph (EcoNova) using conditions 1 [ProntoSIL-120-5-C18 column, Metrohm AG, 75 × 2 mm, 5 μm; mobile phase (4.1 M $LiClO_4$ in 0.1 M $HClO_4$):H_2O, 5:95 (A) and MeCN (B), gradient regime 5–40% B (0–15 min), 40–100% B (15–25 min), 100% B (25–30 min), flow rate 1.0 mL/min; 35°C; UV detector, λ 330 nm] and conditions 2 (Separon 5-NH_2 column, Tessek Ltd., 80 × 2 mm, 5 μm; isocratic regime, mobile phase MeCN:H_2O, 3:1, flow rate 0.1 mL/min; 25°C; UV detector, λ 190 nm). Preparative HPLC used a Summit liquid chromatograph (Dionex), LiChrospher PR-18 column (Merck, 250 × 10 mm, 10 μm), isocratic regime (mobile phase MeOH:H_2O, 1:2, flow rate 4.5 mL/min), 30°C, and UVD 170S UV detector (λ 330 nm).

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