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FLAVONOIDS OF *Carthamus tinctorius* FLOWERS

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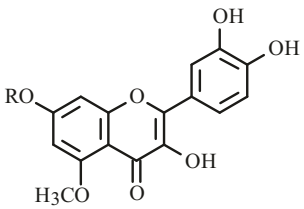
*The known flavonoids luteolin, cinaroside, 5-*O*-methylluteolin, azaleatin (3,7,3′,4′-tetrahydroxy-5-methoxyflavone), and the new natural product 3,7,3′,4′-tetrahydroxy-5-methoxyflavone 7-*O*-β-D-glucopyranoside (safloroside) were isolated from* Carthamus tinctorius *flowers and characterized by PMR and UV spectroscopy and mass spectrometry.*

Keywords: dyer's saffron, *Carthamus tinctorius*, flowers, flavonoids, luteolin, cinaroside, 5-*O*-methylluteolin, azaleatin (3,7,3′,4′-tetrahydroxy-5-methoxyflavone), safloroside (azaleatin 7-*O*-*β*-D-glucopyranoside).

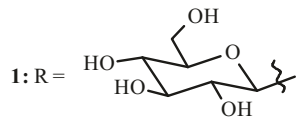
Dyer's saffron (*Carthamus tinctorius* L., Asteraceae) is a known oil crop, the seeds of which contain up to 40% fatty oil that is used as a component in the production of drugs, biologically active additives (BAAs), and cosmetics [1, 2]. We studied earlier the fatty-acid composition of fatty oil from seeds of *C. tinctorius* [3]. However, the flowers of this plant, which have antioxidant, hepatoprotective, anti-inflammatory, neurotropic, and other properties, are also interesting as a source of drugs [4]. In our opinion, the flavonoids are of interest with respect to the manifestation of antioxidant and hepatoprotective activity by *C. tinctorius* flowers. According to the literature [4–7], flowers of this plant contain acacetin, luteolin, quercetin, acacetin-7-*O*-*β*-D-glucuronide, acacetin-6-*C*-*β*-D-glucuronyl-8-*C*-*β*-D-glucuronide, luteolin-7-*O*-*β*-D-glucopyranoside, luteolin-7-*O*-*β*-D-glucuronide, luteolin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucuronide, kaempferol-3-*O*-rutinoside, glycosides of 6-hydroxykaempferol, quercetin-3-*O*-*β*-D-glucopyranoside, quercetin-7-*O*-*β*-D-glucuronide, quercetin-7-*O*-(6″-*O*-acetyl)-*β*-D-glucuronide, and rutin.

Herein we present results from a study of the flavonoid composition of *C. tinctorius* flowers that were cultivated in Samara Region.

We studied flowers of *C. tinctorius* that was cultivated at Tulaikov Samara State Agricultural Research Institute, Russian Academy of Agricultural Sciences (Bezenchuk, Samara Region). Chromatographic separation of the evaporated aqueous EtOH extract of *C. tinctorius* flowers and subsequent rechromatography of the obtained fractions isolated five (**1–5**) flavonoid compounds.



1, 2



2: R = H

The PMR spectrum of **1** exhibited resonances for aromatic protons H-2′ at 7.78 ppm (1H, d, J = 2.5 Hz), H-6′ at 7.75 (1H, dd, J = 2.5, 9 Hz), H-5′ at 6.95 (1H, d, J = 9 Hz), H-8 at 6.86 (1H, d, J = 2.5 Hz), and H-6 at 6.43 ppm (1H, d, J = 2.5 Hz). The observation of a 3H singlet at 3.83 ppm indicated that **1** contained a methoxy that was assigned to ring A based on mass spectral data. A peak for an ion with *m*/*z* 168 corresponded to an $[A + H]^+$ fragment. This conclusion was confirmed by UV spectral data. The 5-OH was identified as the methylation site because the resonance of this group was missing in PMR spectra of **1** and aglycon **2** that was prepared by acid hydrolysis and also isolated pure from *C. tinctorius* flowers. We identified **2** as 3,7,3′,4′-tetrahydroxy-5-methoxyflavone (azaleatin) [8]. The *β*-configuration of the glycoside bond in **1** was confirmed by a doublet at 5.05 ppm with SSCC 7 Hz in the PMR spectrum that belonged to the glucose anomeric proton.

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UV spectral data indicated that the 7-OH was glycosylated. Thus, a bathochromic shift of the short-wavelength band was not observed in the electronic spectrum in the presence of NaOAc [9] whereas this phenomenon did appear in aglycon **2**.

The PMR spectrum of **3**, in contrast with that of **2**, contained at 6.04 ppm an additional 1H singlet for the C-3 proton. This together with UV and mass spectral data, which showed peaks for ions with *m*/*z* 300 and 168 that corresponded to the molecular ion and an $[A + H]^+$ fragment, allowed **3** to be identified as 5-*O*-methylluteolin [10].

Compounds **4** and **5** were identified as 5,7,3′,4′-tetrahydroxyflavone (luteolin) and 5,7,3′,4′-tetrahydroxyflavone 7-*O*-*β*-D-glucopyranoside (cinaroside) [11], which were isolated previously from *C. tinctorius* flowers [4].

Thus, five flavonoids were isolated from *C. tinctorius* flowers and characterized by PMR and UV spectroscopy and mass spectrometry. Among these, azaleatin (3,7,3′,4′-tetrahydroxy-5-methoxyflavone) and 5-*O*-methylluteolin were described for the first time from this plant and azaleatin 7-*O*-*β*-D-glucopyranoside, which we called safloroside, was a new natural product.

EXPERIMENTAL

PMR spectra were taken on Bruker AM 300 (300 MHz) instruments. Mass spectra were recorded on a Kratos MS-30 mass spectrometer. UV spectra were recorded using a Specord 40 spectrophotometer (Analytik Jena).

Extraction and Isolation. Air-dried *C. tinctorius* flowers (100 g) that were collected in August 2013 were extracted with EtOH (70%) first twice at room temperature for 24 h and then with heating on a boiling-water bath for 30 min. The combined aqueous EtOH extract was evaporated *in vacuo* to 50 mL, mixed with L 40/100 silica gel (30 g), and dried. The dried powder (dry extract + silica gel) was placed on a layer of silica gel (8 cm diameter, 5 cm high) formed from a suspension in $CHCl_3$. The chromatography column was eluted by $CHCl_3$ and $CHCl_3$–EtOH (99:1, 98:2, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 70:30, 60:40, and 50:50). The separation of the compounds was monitored by TLC on PTSKh-AF-A-UF Sorbfil plates using $CHCl_3$–EtOH (9:1), $CHCl_3$–EtOH–H_2O (26:16:3), and *n*-BuOH–AcOH(glacial)–H_2O (4:1:2).

Fractions containing dominant **1** were combined. The resulting precipitate was separated and recrystallized from EtOH to afford **1** in 0.1 mass% yield (of air-dried raw material). Fractions containing **2–5** were placed on Woelm polyamide for further purification. Dry powder (extract + polyamide) was placed onto a chromatography column (5 cm high, 4 cm diameter) and eluted with H_2O and aqueous EtOH (20%, 40, 70, 96) to afford **2** and **3** (eluent 96% EtOH), **4** (eluent 70% EtOH), and **5** (eluent 40% EtOH), which were additionally purified by recrystallization from aqueous EtOH.

Acid hydrolysis of **1** used HCl (2%) at 100°C for 30 min. Cleavage of glycoside **5** used more forcing conditions (HCl, 10%, 100°C, 2 h). Precipitates obtained after cooling the reaction mixture were rinsed with purified H_2O and recrystallized from aqueous EtOH. Glycosides **1** and **5** afforded aglycons **2** and **4**.

3,7,3′,4′-Tetrahydroxy-5-methoxyflavone 7-*O*-*β*-D-glucopyranoside (1), yellow crystalline compound, $C_{22}H_{22}O_{12}$, mp 230–233°C (aqueous EtOH). Mass spectrum (70 eV, 200°C, *m/z*, %): 316 (M^+ of the aglycon, 100%), 168 (2), 153 (12), 137 (8). UV spectrum (λ_{max}, nm): 260, 274 sh, 371; + NaOAc 260, 274 sh, 371; + NaOAc + H_3BO_3 260, 378; +A1C1$_3$ 274, 432. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.78 (d, J = 2.5, H-2′), 7.75 (1H, dd, J = 2.5, 9, H-6′), 6.95 (1H, d, J = 9, H-5′), 6.86 (1H, d, J = 2.5, H-8), 6.43 (1H, d, J = 2.5, H-6), 5.05 (d, J = 7, H-1″ Glc), 3.83 (3H, s, CH_3O), 3.1–3.8 (6H, m, Glc).

3,7,3′,4′-Tetrahydroxy-5-methoxyflavone (2), yellow crystalline compound, $C_{16}H_{12}O_7$, mp 282–285°C (aqueous EtOH). Mass spectrum (70 eV, 200°C, *m/z*, %): 316 (M^+, 100 %), 168 (8), 167 (25), 137 (34). UV spectrum (λ_{max}, nm): 260, 274 sh., 371; + NaOAc 265, 277 sh, 372; + NaOAc + H_3BO_3 260, 378; +A1C1$_3$ 274, 432. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.78 (d, J = 2.5, H-2′), 7.75 (1H, dd, J = 2.5, 9, H-6′), 6.95 (1H, d, J = 9, H-5′), 6.86 (1H, d, J = 2.5, H-8), 6.43 (1H, d, J = 2.5, H-6), 3.82 (3H, s, CH_3O).

7,3′,4′-Trihydroxy-5-methoxyflavone (3), yellow amorphous compound, $C_{16}H_{12}O_6$. UV spectrum (λ_{max}, nm): 256, 266 sh, 350; +NaOAc 260, 268 sh, 390; +$AlCl_3$, 278, 330, 355, 400. Mass spectrum (70 eV, 200°C, *m/z*, %): 300 (M^+, 100%), 168 (4), 167 (7), 153 (14), 137 (23). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.60 (d, J = 2, H-2′), 7.55 (dd, J = 9, 2, H-6′), 7.14 (d, J = 9, H-5′), 6.82 (s, H-3), 6.80 (d, J = 2, H-8), 6.70 (d, J = 2, H-6), 3.83 (3H, s, CH_3O).

5,7,3′,4′-Tetrahydroxyflavone (4), yellow crystals, $C_{15}H_{10}O_6$, mp 227–230°C (aqueous EtOH). UV spectrum (EtOH, λ_{max}, nm): 256, 266 sh, 358; +NaOAc 259, 268 sh, 390; +$AlCl_3$ 278, 330, 355, 404. Mass spectrum (70 eV, 200°C, *m/z*, %): 286 (M^+, 100%), 153 (17), 137 (30). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.60 (1H, s, 5-OH), 7.40 (dd, J = 9, 2, H-6′), 7.37 (d, J = 2, H-2′), 6.88 (d, J = 9, H-5′), 6.65 (s, H-3), 6.42 (d, J = 2, H-8), 6.18 (d, J = 2, H-6).

5,7,3′,4′-Tetrahydroxyflavone 7-*O*-*β*-D-glucopyranoside (5), light-yellow crystals, $C_{21}H_{20}O_{11}$, mp 232–234°C (aqueous EtOH). UV spectrum (EtOH, λ_{max}, nm): 257, 266 sh, 352; +NaOAc 258, 268 sh, 380; +$AlCl_3$ 276, 330, 350, 394.

Mass spectrum (70 eV, 200°C, *m/z*, %): 286 (M^+ of the aglycon, 100%), 153 (52), 137 (46). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.98 (1H, s, 5-OH), 7.45 (dd, J = 9, 2, H-6′), 7.41 (d, J = 2, H-2′), 6.91 (d, J = 9, H-5′), 6.78 (d, J = 2, H-8), 6.73 (d, J = 2, H-6), 6.42 (s, H-3), 5.03 (d, J = 7.2, H-1′), 3.1–3.9 (6H, m, Glc).

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