PHENOLIC COMPOUNDS FROM *Carthamus tinctorius*

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The contents of quercetin, luteolin, apigenin, isorhamnetin, umbelliferone, and daphnoretin in common safflower and of acacetin in the flowers were determined based on the physical and chemical properties of the isolated substances.

Key words: *Carthamus tinctorius*, orthodihydroxy group, bathochromic shift, hydroxy groups.

Common safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae family. The genus comprises 19 species, of which 5 are represented in the flora of Azerbaidzhan [1]. Safflower is known from antiquity in India, Egypt, Iran and other Asian countries, and North Africa. This plant replaces sunflower in certain regions of Russia and arid regions of Asia. It is used to produce high-quality margarine; is found in dietetic products for patients with hypocholesterolemia, myocardial infarct, and atherosclerosis; and also possesses purgative and cholagogic activity. It is applied externally to heal burns and other skin diseases. Several medicinal preparations include safflower: safflower oil (USA), obesitol (England), Red Dragon pills (China) [2-4].

In spite of the wide use of the oil, the chemical composition of the plant is poorly studied. It is known that the oil content is 15-30% depending on the habitat. The non-drying oil contains up to 75% linoleic acid and up to 7% saturated fatty acids [2, 5, 6]. The chalcone glycosides carthamine and carthamidin-5-glucoside were found in the petals [5].

The aerial part of *C. lanata* contained bisabolane fucoside, 3-norbisabolane fucoside, intermediol β-D-fucopyranoside, and intermediol β-D-fucopyranoside α',α-methylbutyrate [7, 8]. The new quinochalcone C-glycoside-Ca^{2+} antogonist - tinctormine was also found [9]. The dyes carthamine ($C_{21}H_{22}O_{11}\cdot H_2O$) and safflower bile ($C_{24}H_{30}O_{15}$) were isolated from the bright saffron-yellow petals [10].

We studied phenolic compounds from the herb and flowers of safflower. Ground raw material was extracted with alcohol (70%). The extract was evaporated and chromatographed using *n*-butanol:acetic acid:water (4:1:2) in the first direction and acetic acid (15%) in the second direction. Analysis of the chromatograms in the visible and UV regions used specific reagents allow to detect in the herb and flowers up to 30 phenolic compounds. Some of them were hydroxycinnamic acids (6 compounds) and hydroxycoumarins (4-5 compounds), the rest were flavonoids.

The evaporated aqueous extract was treated successively with ether, $CHCl_3$, ethylacetate, and *n*-butanol. The ether and $CHCl_3$ extracts from the herb were separated over a polyamide sorbent column with elution by $CHCl_3$ and $CHCl_3$:alcohol mixtures of increasing alcohol concentration. Compounds **1**-**4** were isolated from the ether extract; **5** and **6**, from the $CHCl_3$ extract; and **7**, from the $CHCl_3$ extract of flowers.

Compounds **1**-**4** and **7** are yellow crystals that are very soluble in ethanol, methanol, and acetone and insoluble in water and $CHCl_3$.

The aglycons of the studied compounds were identified using their chromatographic mobilities in benzene:ethylacetate:acetic acid:water (50:50:1:1) and $CHCl_3$:acetic acid:water (13:6:1) and the Bryant cyanidin reaction.

IR spectra of the aglycons showed that the phenolic hydroxyls appear at 3385-3275 cm^{-1}; carbonyl of the γ-pyrone ring at 1670-1660 cm^{-1}. Aromatic rings give several strong bands at 1615-1510 cm^{-1}. Furthermore, an absorption band at 2980 cm^{-1} that is characteristic of methoxyl is found in the spectra of **4** and **7**.

Demethylation of **4** and **7** forms **1** and **3**, respectively. The absorption maxima of the long-wavelength band in the UV spectra of **1** and **4** are weaker than those of the short-wavelength one. This is characteristic of flavonols. They are almost equal in **2**, **3**, and **7**, which indicates they are flavones.

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The presence of free hydroxyls was established using ionizing and complexing reagents. Thus, addition of sodium acetate to starting solutions of the compounds produced a bathochromic shift of the long-wavelength maxima by 15-21 nm. This is consistent with a free hydroxyl on C-7 in the compounds. Sodium ethoxide causes a bathochromic shift of the long-wavelength maximum in **2** (53 nm) and **3** (45 nm) and has no effect on **7**. Compound **1** gives a hypsochromic shift of 50 nm and of 13 nm with boric acid and sodium acetate. This is consistent with the presence of an orthodihydroxy group.

Compound **2** gives an analogous shift of 24 nm with boric acid and sodium acetate. Adding zirconium chloride to starting solutions of **1**-**4** and **7** causes a bathochromic shift of the long-wavelength maxima by 17-91 nm. This disappears upon adding citric acid, which is typical for a free hydroxyl on C-5. For **1** and **4**, it decreases to 53 nm, which is due to the presence in them of hydroxyls on C-3 also.

Alkaline destruction of the compounds produced fluoroglucinol and acids: for **1** and **2**, 3,4-dihydroxybenzoic; for **3**, *p*-hydroxybenzoic; for **4**, 3′-methoxy-4′-hydroxybenzoic; for **7**, 4′-methoxybenzoic.

Qualitative reactions; physicochemical properties; UV, IR, and PMR spectra; alkaline destruction; constants of demethylated derivatives; and chromatographic comparison with authentic samples established that **1** is quercetin; **2**, luteolin; **3**, apigenin; **4**, isorhamnetin; and **7**, acacetin. The lack of melting point depressions of mixtures with authentic samples confirmed these assignments.

Compounds **5** and **6** were coumarin derivatives based on qualitative color reactions, i.e., the presence of blue fluorescence in UV light upon treatment of the chromatograms with alcoholic base (10%) and a yellowish-orange color in daylight after treatment of the chromatograms with diazotized sulfanilic acid. The strong blue fluorescence of **5** and **6**, which intensifies after exposure to ammonia vapor or alcoholic base, is consistent with a free hydroxyl on C-7 in the structure.

The presence in the UV spectrum of a strong absorption maximum at 320-340 nm and in the IR spectrum of an absorption band at 1710-1730 cm^{-1}, which is typical for stretching vibrations of a γ-pyrone carboxyl, confirm the correctness of assigning them as coumarin derivatives [11].

The presence in **5** of strong blue fluorescence and of an absorption band at 3200 cm^{-1} in the IR spectrum is consistent with the presence of a hydroxyl on C-7. Therefore, **5** should correspond to umbelliferone. The lack of melting-point depression of a mixture of **5** with umbelliferone indicated that they are identical.

A comparison of the properties of known coumarins and **6** obtained by us and its acetyl (mp 240-242°C) and methyl (mp 239-241°C) derivatives showed that it was similar to daphnoretin, which was first isolated from *Daphne odora* Thunb. and also from seeds of a Coronilla species [12]. This assumption was confirmed by thermal decomposition of **6** in a stream of H_2. Chromatography of the decomposition products detected scopoletin and umbelliferone (**5**). Thermal destruction confirmed that **6** isolated by us was identical to daphnoretin-6-methoxy-7-hydroxy-3,7′-dicoumarin ether.

EXPERIMENTAL

Melting points were determined on a Kofler block; UV spectra, on an SF-46 spectrophotometer; IR spectra, on a UR-20 instrument. PMR spectra were recorded on a Bruker WP-400 spectrophotometer (working frequency 400 MHz, DMSO-d_6 solvent).

Isolation of Phenolic Compounds. Air-dried safflower herb (2 kg) was extracted with alcohol (70%) at a 1:10 raw material:extractant ratio. The aqueous alcohol extract was evaporated to 2 L of aqueous solution and treated successively with diethylether, $CHCl_3$, ethylacetate, and *n*-butanol. The ether extract was evaporated and separated over a polyamide sorbent column (h = 60 cm, d = 4 cm) with elution by $CHCl_3$ and $CHCl_3$:alcohol mixtures with increasing alcohol concentrations. Fractions of 100-150 mL were collected. The qualitative compositions of the fractions were analyzed by paper chromatography using benzene:ethylacetate:acetic acid:water (50:50:1:1), $CHCl_3$:acetic acid:water (13:6:1), and acetic acid (50%). Similar fractions were combined and evaporated. Dry solids were crystallized from alcohol. The ether fraction of safflower afforded **1**-**4**.

The extract of safflower flowers (2 kg) was isolated analogously. The $CHCl_3$ extract of safflower herb afforded **5** and **6** by the same method; the $CHCl_3$ extract of flowers, **7**.

Quercetin (1). (3,5,7,3′,4′-pentahydroxyflavone), $C_{15}H_{10}O_7$, mp 313-315°C, UV spectrum (λ_{max}, nm): 256, 370; $+CH_3COONa$ 274, 385; $+CH_3COONa + H_3BO_3$ 264, 383; $+ZrO(NO_3)_2$ 270, 461; $+ ZrO(NO_3)_2$ + citric acid 265, 428; $+C_2H_5ONa$ 250, 320.

IR spectrum: 1665 (C=O), 3385-3300 (OH), 1612, 1560, 1518 cm^{-1} (aromatic C=C).

Luteolin (2). (5,7,3′,4′-tetrahydroxyflavone), $C_{15}H_{10}O_6$, mp 327-329°C, UV spectrum (λ_{max}, nm): 256, 265, 352; +CH_3COONa 272, 368; +CH_3COONa + H_3BO_3 265, 376; +$ZrO(NO_3)_2$ 280, 405; +$ZrO(NO_3)_2$ + citric acid 256, 352; +C_2H_5ONa 272, 405.

The IR spectrum has been reported [13].

PMR (δ, ppm, J/Hz): 6.35 (1H, d, J = 2.0, H-6), 6.50 (1H, d, J = 2.0, H-8), 6.10 (s, H-3), 6.83 (1H, d, J = 8.0, H-5′), 7.29 (br.s, H-2′), 7.32 (1H, s, H-6′).

Apigenin (3). (5,7,4′-trihydroxyflavone), $C_{15}H_{10}O_5$, mp 343-346°C, UV spectrum (λ_{max}, nm): 272, 343; +CH_3COONa 275, 365; +CH_3COONa + H_3BO_3 272, 345; +$ZrO(NO_3)_2$ 310, 355; +$ZrO(NO_3)_2$ + citric acid 270, 345; +C_2H_5ONa 280, 410.

The IR spectrum has been reported [13].

Isorhamnetin (4). (3,5,7,4′-tetrahydroxy-3′-methoxyflavone), $C_{16}H_{12}O_7$, mp 317-319°C, UV spectrum (λ_{max}, nm): 255, 266, 372; +CH_3COONa 275, 385; +CH_3COONa + H_3BO_3 256, 370; +$AlCl_3$ 265, 430; +C_2H_5ONa 255, 334.

IR spectrum: 1658 (C=O), 3455 (OH), 2895 (CH_3), 1605, 1565, 1505 cm^{-1} (aromatic C=C).

Umbelliferone (5). (7-hydroxycoumarin), $C_9H_6O_3$, mp 228-230°C, UV spectrum (λ_{max}, nm): 218, 251, 323; +C_2H_5ONa 231, 370.

The IR spectrum has been reported [14].

Daphnoretin (6). (6-methoxy-7-hydroxy-3,7′-dicoumaric ether), $C_{19}H_{12}O_7$, mp 254-256°C, UV spectrum (λ_{max}, nm): 228, 265, 325, 345; +C_2H_5ONa 240, 280, 368, 393.

The IR spectrum has been reported [14].

Acacetin (7). (5,7-dihydroxy-4′-methoxyflavone), $C_{16}H_{12}O_5$, mp 268-270°C, UV spectrum (λ_{max}, nm): 270, 328; +CH_3COONa 275, 380; +CH_3COONa + H_3BO_3 272, 338; +$ZrO(NO_3)_2$ 277, 245; +$ZrO(NO_3)_2$ + citric acid 270, 328; +C_2H_5ONa 280, 377.

The IR spectrum has been reported [13].

Demethylation of 4 and 7. Compounds **4** and **7** (10 mg each) were dissolved in acetic anhydride (5 mL), treated gradually with HI (d = 1.7), refluxed gently for 2 h, cooled, and diluted into sodium thiosulfate solution (5 mL). Light yellow precipitates of the demethylation products of **4** and **7** were recrystallized twice from aqueous ethanol to afford from **4** quercetin (**1**), mp 312-315°C, and from **7** apigenin (**2**), mp 342-345°C.

Daphnoretin Acetate. A solution of **7** (10 mg) in absolute pyridine (3-5 mL) was treated with acetic anhydride (3.5 mL), left for 3 d, and poured into a crystallizer containing cold water. The resulting crystals were recrystallized from methanol to afford daphnoretin acetate, mp 240-242°C.

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