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CAROTINOIDS AND AMINO ACIDS FROM *Allium rotundum*

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Carotinoids are some of the principal representatives of natural yellow-red-orange pigments that are synthesized by vegetative and generative organs of plants, microorganisms, and certain animals during their growth and development [1]. Studies of *Allium rotundum* (Alliaceae) at the Department of Pharmacognosy, Tbilisi State Medical University, isolated steroidal sapogenins [2] and saponins [3], phenolic compounds [4], and carotinoids, the study of which is of great phenomenological interest.

Steroidal sapogenins, saponins, and phenolic compounds were extracted successively from the EtOH extract of inflorescences and upper flower spikes. The contents of the mother liquors were combined, washed with EtOH (80°), and extracted with CCl_4, which was then distilled. The solid was purified of chlorophyll over inactivated Al_2O_3 and eluted by EtOH (40°) with added EtOAc (1%) [5]. The fraction containing the whole carotinoid complex was isolated in 1.9% yield calculated for total air-dried raw material.

Total carotinoids were separated by chromatography over a column of silica gel (L 40/100, Czech. Rep.) using a mobile phase of $CHCl_3$:petroleum ether (1:10). Column performancee was monitored by TLC on Silufol UV-254 plates and Al_2O_3 using *n*-hexane:Et_2O (7:3, 1), *n*-hexane:acetone (96:4, 8:3, 2), and heptane:MEK (5:3, 3). Carotinoids were detected visually by their color. Colorless fractions were colored using iodine vapor [6].

Fractions containing pure compounds were evaporated. The resulting compounds were recrystallized in polar solvents such as MeOH and acetone to isolate six compounds of carotinoid nature.

The compounds were identified by qualitative reactions from the characteristic color in H_2SO_4, melting point, specific rotation, absorption spectra in the visible and UV ranges (200–700 nm), and chromatography on TLC plates in the presence of authentic samples [7–9].

The quantitative content of carotinoids was determined spectrophotometrically using published specific extinction coefficients [8, 10].

Compound **1**, yellow crystals (MeOH), mp 179–180°C, $[\alpha]_D^{20}$ 0° (*c* 0.92, $CHCl_3$), UV spectrum (λ_{max}, nm): ($CHCl_3$) 466.3, 497.5; (C_6H_{14}) 424.2, 451.0, 482.4. β-Carotene appeared on TLC plates (Silufol UV-254) at the level of an authentic sample [9]. The relative content was 38% of the total carotinoid mass.

Compound **2** was violaxanthin (5,6,5′,6′-diepoxyxanthin). It crystallized from MeOH as yellowish-orange prisms, dissolved in alcohol, did not dissolve in water, mp 166–167°C. UV spectrum (λ_{max}, nm): ($CHCl_3$) 423.9, 551.2, 481.9; (C_6H_{14}) 443.0, 471.9 [9]. Relative content, 7.8%.

Compound **3** was identified by us as flavoxanthin (5,8-epoxylutein). It crystallized from MeOH as gold brush-like prisms, mp 182–183°C, $[\alpha]_D^{20}$ +189.9° (*c* 1.0, C_6H_6). UV spectrum (λ_{max}, nm): ($CHCl_3$) 430.0, 459.1; (C_6H_{14}) 421.1, 450.4; (C_6H_6) 432.2, 481.0; (CS_2) 450.2, 480.5 [9]. It was isolated in 10.2% yield of total carotinoids.

Compound **4** was brick-red crystals (MeOH), mp 190–192°C, $[\alpha]_D^{20}$ +161.2° (*c* 1.0, $CHCl_3$). A solution in conc. H_2SO_4 acquired a green color that gradually turned blue. UV spectrum (λ_{max}, nm): ($CHCl_3$) 428.1, 454.9, 486.3; (C_6H_{14}) 420.3, 446.8, 449.4. The chromatographic mobility was analogous to lutein (xanthophyll-3,3′-dioxy-α-carotene) [9]. The relative content was 16.5%.

Compound **5** crystallized as orangish-red needles from benzene:petroleum ether, mp 160–161°C, $[\alpha]_D^{20}$ 0° (*c* 0.98, $CHCl_3$). UV spectrum (λ_{max}, nm): ($CHCl_3$) 439.3, 473.9, 510.0; (C_6H_{14}) 432.1, 462.2, 493.8 [9]. It appeared on Silufol UV-254 plates at the level of an authentic sample of rubixanthin (3-hydroxy-γ-carotene), a mixed sample with which did not show melting-point depression. It made up 16.1% of total carotinoids.

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Compound **6** was yellow needle-like crystals, mp 207–208°C (MeOH), $[\alpha]_D^{20}$ –(40–50)° (*c* 1.0, $CHCl_3$). UV spectrum (λ_{max}, nm): ($CHCl_3$) 429.3, 461.9, 494.0; (C_6H_{14}) 450.7, 482.1, 520.4 [9]. Treatment with conc. H_2SO_4 produced a dark blue color. The compound was identified as zeaxanthin (3,3′-dihydroxy-β-carotene). The relative content was 9.1%.

In addition to the aforementioned carotinoids, the aqueous alcohol extract of inflorescences yielded eight free amino acids such as serine, 3.8 (mg% calculated per air-dried weight of raw material); valine, 2.9; aspartic acid, 9.1; methionine, 1.8; histidine, 1.4; α-alanine, 5.2; lysine, 3.1; and proline, 2.8.

Amino acids were identified qualitatively using repeated paper chromatography [10, 11] of the alcohol extract together with authentic samples and the system *n*-BuOH:HOAc:H_2O (4:1:5). Quantitative determination used an AAA 400 amino-acid analyzer (Czech. Rep.) with built-in control program and data processing (GLP).

All these carotinoids and amino acids were isolated and described from *A. rotundum* for the first time.

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