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ANTHOCYANINS FROM FRUIT OF TWO SPECIES OF THE GENUS *Rosa*

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We investigated the composition of anthocyanins from fruit of *Rosa spinosissima* L. and *R. hracziana* Tamam. (Rosaceae Juss.) growing near Bata-Bat, Shakhbuz Region, Republic of Azerbaidzhan. Fruit was collected on Sept. 16, 2009, during ripening.

Freshly collected fruit (100 g each) was frozen with liquid N_2 and pulverized. Pulp was separated from stones. Pulp (50 g each) was ground and extracted at room temperature for 2 d twice with EtOH (95%) and once with MeOH containing HCl (1%). The extracts were combined, filtered, and evaporated to dryness *in vacuo* at 30°C. The dry solid was treated with $CHCl_3$ and then dissolved in MeOH (50 mL) containing HCl (0.5%). A part of the extract was hydrolyzed by HCl (2 N, 1:1 ratio) on a boiling-water bath for 60 min under reflux [1].

The hydrolysate was cooled. Aglycones were extracted by isoamyl alcohol for 20 h (shaking periodically). The isoamyl extract was studied by 2D chromatography on FN16 paper using Formic and Forestal systems [2]. Two aglycones were detected in the chromatogram. Preparative column chromatography using the Formic system isolated two pure aglycones. Aglycone-1 with R_f 0.32 (Formic) and 0.48 (Forestal) gave absorption maxima with fuchsine dye at 276 and 535 nm (MeOH containing 0.01% HCl). Adding $AlCl_3$ produced a bathochromic shift of 20 nm. Aglycone-2 with R_f 0.12 and 0.31 (in the same systems, respectively) gave absorption maxima with purple dye at 277 and 546 nm. Addition of $AlCl_3$ produced a bathochromic shift of 22 nm. Based on the chromatographic and spectral data and a comparison with authentic samples and the literature [2, 3], the first aglycone was identified as cyanidin; the second, delphinidin.

Preparative 2D paper chromatography using *n*-BuOH:AcOH:H_2O (4:1:2) (first direction) and AcOH:HCl (conc.):H_2O (15:3:82) (second direction) showed that pulp of *R. spinosissima* fruit contained three; of *R. hracziana*, four anthocyanin glycosides. The first three components were identical in both species. Therefore, total anthocyanins from fruit pulp of *R. hracziana* was analyzed in detail.

Preparative column chromatography (acidic cellulose powder) [4] and rechromatography on paper produced four pure anthocyanins. The pure anthocyanins were studied by chromatography and spectroscopy [2, 3, 5]. The anthocyanins were hydrolyzed as described above by neutralizing the hydrolysate with ion-exchange resin. Paper chromatography with authentic sugar samples identified D-glucose in hydrolysates of all glycosides. The chromatographic mobilities of anthocyanins **1** and **2** suggested that they were monoglucosides; **3** and **4**, diglucosides. This agreed with results of stepwise acid analysis. The aglycone and sugar were cleaved after stepwise hydrolysis of anthocyanins **1** and **2** for 20 min. However, anthocyanins **3** and **4** formed four components. The first component corresponded to the starting glucoside; the second and third, to a monoglucoside; the fourth, the aglycone. The second spot did not fluoresce. Its R_f value indicated that it was the 3-glucoside. The third spot did fluoresce in UV light with emission analogous to the starting glucoside. It corresponded to the 5-glucoside according to fluorescence and chromatographic mobility. Oxidation of the anthocyanins with H_2O_2 produced glucose and confirmed that it was bonded to C_3 in **1** and **2** and to C_3 and C_5 in **3** and **4** [1–3, 5, 6].

Thus, cyanidin-3-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, and delphinidin-3,5-diglucoside were identified for the first time in total anthocyanins from fruit pulp of *R. hracziana* using chromatography, spectroscopy, total and stepwise hydrolysis, oxidation by H_2O_2, and comparison with authentic samples. All components with the exception of delphinidin-3,5-diglucoside were found in *R. spinosissima* anthocyanidins.

The monoglucosides of cyanidin and delphinidin dominated quantitatively the anthocyanin complex from fruit of both *Rosa* species.

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