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ANTHOCYAN GLYCOSIDE FROM *Hedera colchica*

M. D. Alaniya,* N. Sh. Kavtaradze, and A. V. Skhirtladze

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Hedera colchica is an evergreen vine [1]. Observations carried out by us on separate specimens of *H. colchica* showed that the color changes depending on the vegetative phase. Thus, the stems and leaves acquire a green color during active vegetation whereas they become reddish during winter. We collected samples of the plant in Tbilisi (Georgia) in January and July 2007 in order to explain the essence of this phenomenon.

The flavonol glycoside rutin has been previously isolated from *H. colchica* [2]. Rutin was not observed in the aqueous alcohol extract of a sample collected in January. It was found that the rutin content varies depending on the season. Rutin accumulated most (0.57%) in summer. In January, its content decreases to only traces whereas the anthocyan content changes in the opposite direction. Anthocyan pigment was completely absent in the summer sample whereas its content in the January sample reached 0.52%.

Aerial parts of the plant collected in January were extracted by the method proposed for isolating anthocyan pigments [3]. The extraction was performed using methanol containing conc. HCl (0.1%) at room temperature in the dark. The resulting extract was purified by adsorption chromatography over cellulose sorbent and using reversed-phase HPLC (Agilent HP 1100, Palo Alto, USA) with a UV detector and fixed wavelength of 520 nm. We used an XTerra column (300 × 7.8 mm) packed with Silica gel RP-18 adsorbent (5 μm). The mobile phase flow rate was 2 mL/min. Samples (5 mg) (the fraction containing anthocyan pigment produced by chromatography over cellulose) were dissolved in methanol (100 μL) and injected into the chromatograph. The mobile phase was binary solutions of CH_3CN and H_2O containing trifluoroacetic acid (0.1%, from 10:90 to 60:40). The duration was 60 min; retention time of anthocyan, 15.63 min.

A pure compound that in daylight on PC gave a pink spot; in UV light, a bright pinkish-red fluorescence; and in ammonia vapor and upon spraying with aqueous NaOH solution (1%), a bright blue color, was produced. IR spectrum (KBr, ν, cm^{-1}): 3300-3400 (OH), 1655 (C=O), 1615-1430 (Ar). UV spectrum (MeOH containing HCl, 0.01%, λ_{max}, nm): 520. Addition of methanolic $AlCl_3$ (5%) caused a bathochromic shift of 30 nm, which is typical of anthocyans containing free hydroxyls in the 3′- and 4′-positions [4].

Acid hydrolysis gave D-glucose, L-rhamnose, and the aglycon with mp >300°C (dec.). UV spectrum (MeOH, 0.01% HCl, λ_{max}, nm): 535; +$AlCl_3$, 565. This indicated the presence of an *o*-dihydroxy group in the side chain. Alkaline cleavage of the algycon formed phloroglucinol and protocatechoic acid. Chromatography by PC using *n*-BuOH:HCl (2 N) (1:1, upper phase) and CH_3CO_2H:HCl:H_2O (5:1:5), R_f 0.68 and 0.35, corresponded to cyanidin [4].

Stepwise acid hydrolysis of the glycoside by Hcl (4%) in methanol for 25-30 min gave an intermediate and L-rhamnose. The site of attachment of the sugar was determined by oxidative destruction of the glycoside [4, 6]. This formed a biose identical to rutinose [2] that was bonded to C_3 of the aglycon.

PMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 6.48 (1H, d, J = 0.8, H-4), 6.01 (1H, d, J = 2.1, H-6), 5.99 (1H, dd, J = 2.12, H-8), 7.09 (1H, d, J = 2.12, H-2′), 6.82 (1H, d, J = 8.0, H-5′), 6.98 (1H, dd, J = 2.14, 8.4, H-6′), 4.99 (1H, m, Glc H-1), 4.32 (1H, d, J = 1.68, Rha H-1), 1.32 (3H, d, J = 6.3, Rha CH_3).

The chemical shifts of the aromatic protons did not correlate with those for the flavylium cation because the isolated anthocyan glycoside was the hemiacetal form [5]. The PMR data given above did agree fully with those.

The chemical shifts and SSCC of the anomeric protons indicated that the sugar had the pyranose form.

Therefore, the compound was identified as cyanidin-3-*O*-β-D-rutinoside, which was found for the first time in *H. colchica*.

I. Kutateladze Institute of Pharmaceutical Chemistry, Georgia, 0159, Tbilisi, ul. P. Saradzhishvili, 36, fax (995) 32 52 00 23, e-mail: merialania@yahoo.com. Translated from Khimiya Prirodnykh Soedinenii, No. 5, p. 542, September-October, 2008. Original article submitted February 18, 2008.

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Our results agreed with literature data for a similar biosynthetic origin of flavonols and anthocyans [6].

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