Chemistry of Natural Compounds, Vol. 45, No. 2, 2009

FLAVONOIDS AND ANTHOCYANS FROM *Alhagi pseudoalhagi*

E. N. Novruzov, G. A. Abdullaeva,
L. A. Shamsizade, and N. Sh. Mustafaev*

UDC 547.581.972

The plant *Alhagi pseudoalhagi* is relatively little studied. Acidic sulfatidylated polygalactones, tanning agents [1], catechins, and proanthocyanidines [2] have been observed in its aerial part. It was found that tincture prepared from runners exhibits diuretic, litholytic [3], hypoazotemic, antioxidant [4], and cholegogic activity [5].

Herein we present data on the flavonoid composition of the aerial part and the qualitative composition and quantitative content of anthocyans from flowers of *A. pseudoalhagi* (*Fabaceae* Lindl.) growing in the Republic of Azerbaidzhan [6]. Material was collected near Baku (Ramana) during budding (June 2007). Spectrophotometry [7] showed that the aerial part of the plant contained 2.95% flavonoids (per dry weight) [8].

Pure flavonoids were isolated from ground air-dried aerial part by exhaustive extraction with ethanol on a water bath at 70°C. The extract was filtered and condensed in vacuo to a small volume. The evaporated extract was diluted with water and treated successively with $CHCl_3$, ether, EtOAc, and *n*-BuOH. The ether extract contained two compounds; EtOAc, 3; BuOH, 2.

Preparative paper chromatography (Whatman-3) of the ether extract isolated two compounds from MeOH with mp 312°C, which were identified as quercetin (**1**) and **2** with mp 300-303°C. UV spectrum (MeOH, λ_{max}, nm): 256 and 276 nm. Alkaline destruction of **2** produced phloroglucinol and vanillinic acid. Analyses suggested that **2** was identical to isorhamnetin.

The EtOAc extract was separated using column chromatography over polyamide (Woelm) with elution by CH_3OH:$CHCl_3$ and CH_3OH:H_2O. Compounds **3** and **4** were isolated from the $CHCl_3$:CH_3OH (90:10) and CH_3OH:H_2O (95:5) fractions.

Compound **3** was yellow needles (from aqueous CH_3OH), mp 179-180°C. UV spectrum (MeOH, λ_{max}, nm): 258 and 360 nm. The algycon was obtained in 42% yield by hydrolysis with H_2SO_4 (5%) in ethanol (50%). The aglycon was identified as isorhamnetin. Paper chromatography of the hydrolysate detected D-glucose and L-rhamnose. The sugar in **3** was bonded to the C_3 position according to brown fluorescence of a spot in UV light and a positive zirconium-citrate sample. The bathochromic shift of the long-wavelength absorption maximum in CH_3COONa by 12 nm compared with that in EtOH indicated that C_7 was unsubstituted [9]. Thus, **3** was identified as isorhamnetin 3-rutinoside.

Compound **4**, mp 184-185°C. UV spectrum (MeOH, λ_{max}, nm): 258 and 364 nm. Addition of $AlCl_3$ and CH_3COOH and CH_3COONa + H_3BO_3 gave a bathochromic shift, indicating that free hydroxyls were located in the 5, 7, 3′, and 4′ positions. Hydrolysis of **4** by H_2SO_4 (2%) produced quercetin in 47% yield. The hydrolysate of **4** contained D-glucose and L-rhamnose.

Based on these data, **4** was characterized as 5,7,3′,4′-tetrahydroxyflavon-3-β-D-rutinoside (rutin).

Fresh petals were separated from the bases, homogenized with MeOH (1:1 ratio), and left to stand for 2 h in order to analyze the anthocyans. The homogenate was passed through a laboratory screw press. The solids were treated three times with MeOH containing HCl (1%) for 2 h. The extracts were combined, filtered, and condensed in vacuo to an aqueous residue that was treated successively with ether and EtOAc. Two-dimensional paper chromatography (FN 16) using CH_3CO_2H:HCl:H_2O (15:3:82, system I) and *n*-BuOH:CH_3CO_2H:H_2O (4:1:2, system II) detected three anthocyans in the purified extract.

Total purified anthocyans were separated by distribution chromatography over a column packed with cellulose activiated by HCl (1%) using system I in descending mode. The column showed three clearly resolved bands. The adsorbent was

Institute of Botany, National Academy of Sciences of Azerbaidzhan, Baku, AZ1073, Patamdarskoe Shosse, 40, e-mail: mustafayevn02@yahoo.co.uk. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 213-214, March-April, 2009. Original article submitted November 27, 2008.

0009-3130/09/4502-0249 ©2009 Springer Science+Business Media, Inc.

removed from the column. The layers were separated. Anthocyans were eluted by MeOH containing HCl (1%) and precipitated by Et_2O. The precipitates of pure anthocyans were rechromatographed on paper using system II to afford three pure anthocyans that were arbitrarily designated anthocyan **5**, **6**, and **7**.

Anthocyan **5** was fine dark-violet crystals, R_f 0.20 and 0.42 (systems I and II, respectively). It was purple in daylight and dull purple in UV light. UV spectrum [MeOH containing HCl (0.01%), λ_{max}, nm): 535 nm; $+AlCl_3$ (5%), 546 nm; E_{440}/E_{max} = 18%. Acid hydrolysis formed the aglycon with R_f 0.32 (system III, CH_3CO_2H:HCl:H_2O, 30:3:10). UV spectrum [MeOH containing HCl (0.01%), λ_{max}, nm): 546 nm; $+AlCl_3$, 568 nm; E_{440}/E_{max} = 18%. The aglycon was identified as delphinidin using chromatography and UV spectra. The sugar part of the hydrolysate contained D-glucose. The aglycon:sugar ratio was 1:1. The bonding site of the carbohydrate was found from the hypochromic shift of λ_{max} of the glycoside compared with the maximum of the aglycon (535 and 546 nm). This effect was consistent with a carbohydrate in the C_3 position [10].

Anthocyan **6** was fine dark-red crystals with R_f 0.17 and 0.28 (systems I and II, respectively). UV spectrum [MeOH containing HCl (0.01%), λ_{max}, nm): 525 nm; $+AlCl_3$, 540 nm; E_{440}/E_{max} = 13%. Acid hydrolysis produced the aglycon with R_f 0.48 (system III). UV spectrum [MeOH containing HCl (0.01%), λ_{max}, nm): 535 nm; $+AlCl_3$, 543 nm; E_{440}/E_{max} = 18%. The aglycon was cyanidin according to these data. The sugar part of the hydrolysate contained D-glucose. The aglycon:sugar ratio was 1:2. Stepwise hydrolysis formed first an anthocyan with R_f 0.38 and 0.43 (systems I and II). Further hydrolysis cleaved the aglycon cyanidin and D-glucose.

Anthocyan **7** was microcrystalline dark-violet powder with a metallic sparkle and R_f 0.32 and 0.15 (systems I and II, respectively). It was purple in daylight and dark-purple in UV light. UV spectrum [MeOH containing HCl (0.01%), λ_{max}, nm): 534 nm; $+AlCl_3$, 545; E_{440}/E_{max} = 11%. Total acid hydrolysis gave the aglycon, which was identified as delphinidin, and D-glucose. The aglycon:sugar ratio was 1:2. Stepwise acid hydrolysis formed after 30 min an anthocyan corresponding to anthocyan A and D-glucose; after 60 min, the aglycon corresponding to delphinidin and D-glucose.

Thus, anthocyan **5** was identified as delphinidin-3-monoglucoside; **6**, cyanidin-3,5-diglucoside; **7**, delphinidin-3,5-diglucoside based on the results and a comparison with authentic samples and the literature [10, 11].

In both instances the qualitative composition of the anthocyans from flowers was almost the same with a difference in the ratio of the individual components. The content of **6** was elevated at a contaminated site. Photoelectrocalorimetry [12] established that flowers collected at a radioactive contaminated site had elevated amounts of **6** compared with samples collected at the Botanical Garden. The anthocyan content from Ramana was 1.58%; from the Botanical Garden, 1.23%.

REFERENCES

1. M. Yu. Goncharov, Author's Abstract of a Candidate Dissertation in Pharmaceutical Sciences, St. Petersburg (2002).
2. D. F. Alimova, L. A. Kuliev, and A. D. Udovin, *Chem. Nat. Comp.*, **43**, 326 (2007).
3. A. Sidibe, Author's Abstract of a Candidate Dissertation in Pharmaceutical Sciences, St. Petersburg (2003).
4. K. V. Sivak, *Rastit. Resur.*, **43**, 127 (2007).
5. I. A. Karaev, R. K. Aliev, and P. A. Yuzbashinskaya, *Dokl. Akad. Nauk AzSSR*, **11**, 359 (1955).
6. I. I. Karyagin, *Flora of Azerbaidzhan* [in Russian], Azerneshr, Baku, **5**, 1554.
7. M. V. Petrichenko, T. V. Sukhikina, and N. S. Fursa, *Rastit. Resur.*, **38**, 104 (2002).
8. E. N. Novruzov, *Izv. Nats. Akad. Nauk Az., Ser. Biol. Nauk*, 11 (2004).
9. L. Jurd, *Chemistry of Flavonoid Compounds*, Oxford (1962), 156.
10. J. B. Harborne, *J. Biochem.*, **1**-**2**, 22 (1958).
11. J. B. Harborne, *J. Chromatogr.* **1**, 473 (1958).
12. Yu. G. Skorikova and E. A. Shaftan, *Trudy BAV-3*, 451 (1968).