

FLAVONOIDS AND HYDROXYCINNAMIC ACIDS FROM *Astragalus asper*

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In continuation of research on the chemical composition of plants from the genus *Astragalus* [1], we studied polyphenolic compounds of *A. asper* Jacq growing in Caucasus foothills on Stavropol highlands near Stavropol [2]. Raw material was collected during flowering. Total polyphenolic compounds were worked up by known methods [1, 3]. For this, air-dried raw material (1 kg, aerial part) was ground to 3–5 mm and extracted with EtOH (70%, 1:10, 4×). The yield of biologically active compounds was about 94% [3].

The extracts were combined. EtOH was distilled off. The extracts were condensed in vacuo (pressure $4.0 \cdot 10^4$ N/m²). The condensed extract was purified of lipophilic substances using pentane and heptane. The principal groups of biologically active compounds were separated by selective extraction by CHCl₃, EtOAc, BuOAc, and BuOH [4]. Total polyphenolic compounds were obtained from the EtOAc and BuOAc extracts by distilling the solvent, combining them, and drying in a vacuum drying cabinet at 45–50°C and $2.6 \cdot 10^4$ N/m² [2]. The yield was 5.2% of air-dried mass of raw material.

Paper chromatography (PC) using systems I (BAW, BuOH:AcOH:H₂O, 4:1:2), II (AcOH, 15%), and III (AcOH, 2%) and specific detectors established that the polyphenols consisted of 10 compounds. Column chromatography over polyamide using water and a water:EtOH gradient (90:10→10:90) isolated eight pure compounds.

Compound 1. C₉H₈O₄, mp 196–198°C (MeOH); R_f 0.83 (system I), 0.28 (system III). UV spectrum (EtOH, $\lambda_{\max}$, nm): 217, 234, 300sh, 235. IR spectrum (KBr, $\nu_{\max}$, cm⁻¹): 3350 (OH), 1650–1630 (C=O), 1610–1575–1540 (Ar), 870. Alkaline fusion gave protocatechoic acid. The melting point of the product agreed with that of caffeic acid. Melting point depression was not observed. Compound 1 was identified as caffeic acid by comparison with the literature [4].

Compound 2. C₁₆H₁₈O₉, mp 203–206°C (MeOH); R_f 0.63 (system I), 0.66 (system III). UV spectrum (EtOH, $\lambda_{\max}$, nm): 245, 292, 328. IR spectrum (KBr, $\nu_{\max}$, cm⁻¹): 2900–2780 (OH), 1740–1715 (ester), 1660–1625 (C=O), 1605–1550 (Ar), 840. Alkaline hydrolysis formed caffeic acid and D-quinic acid. One spot was observed in a chromatogram of a mixed sample of 2 and chlorogenic acid. Melting point depression also was not observed for 2 and chlorogenic acid. This compound was identified as chlorogenic acid [1, 4].

Compound 3. C₁₅H₁₀O₇, mp 310–312°C (MeOH); R_f 0.64 (system I), 0.04 (system II). UV spectrum (EtOH, $\lambda_{\max}$, nm): 375, 265. IR spectrum (KBr, $\nu_{\max}$, cm⁻¹): 3380–3300 (OH), 1665 (C=O), 1615–1565–1515 (Ar). Fusion with crystalline KOH formed phloroglucinol and protocatechoic acid. Melting point depression of 3 and authentic quercetin was not observed. The compound was identified as quercetin [4].

Compound 4. C₂₁H₂₀O₁₁, mp 240–242°C (MeOH); $[\alpha]_D^{20}$ –59° (c 0.1, DMF). UV spectrum (EtOH, $\lambda_{\max}$, nm): 372, 257. Acid hydrolysis of the compound (1% H₂SO₄) formed an aglycon with mp 310–312°C that was identified by UV and IR spectroscopy as quercetin. The sugar component was identified by PC as D-glucose. The site of attachment of the sugar component to the aglycon was determined using UV spectroscopy with ionizing and complexing reagents. Comparison of molecular rotations in the studied compound established the β -configuration for the glycoside bond. Based on the results the compound was identified as quercimeritrin [4].

Compound 5. C₂₁H₃₀O₁₆, mp 190–192°C (MeOH); R_f 0.45 (system I), 0.51 (system II). UV spectrum (EtOH, $\lambda_{\max}$, nm): 256, 264sh, 354. IR spectrum (KBr, $\nu_{\max}$, cm⁻¹): 3595–3400 (OH), 1660–1658 (C=O), 1605–1575–1510 (Ar), 1085, 1062, 1025, 980, 900 (sugar). Acid hydrolysis (0.25% H₂SO₄, stepwise hydrolysis) formed quercetin-3-O-glycoside and L-rhamnose; then quercetin, D-glucose, and L-rhamnose. This was confirmed by PC with authentic samples. The compound was identified as rutin (quercetin-3-rutinoside) [4].

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Compound 6. $C_{21}H_{20}O_{12}$, mp 238–240°C (MeOH); $[\alpha]_D^{20} -69.2^\circ$ (c 0.1, MeOH); R_f 0.37 (system I), 0.58 (system II). UV spectrum (EtOH, λ_{max} , nm): 365, 355. IR spectrum (KBr, ν_{max} , cm^{-1}): 3300 (OH), 1637 (C=O), 1605, 1565, 1500, 1455 (Ar), 1080, 1057, 1028, 920, 890 (sugar). Acid hydrolysis (1% H_2SO_4) gave the aglycon quercetin and D-glucose that were identified by the aforementioned method. Comparison of the results with the literature identified **6** as quercetin-3-*O*- β -D-glucopyranoside [4].

Compound 7. $C_{15}H_{10}O_6$, mp 273–275°C (MeOH); R_f 0.82 (system I), 0.08 (system II). UV spectrum (EtOH, λ_{max} , nm): 370, 265. IR spectrum (KBr, ν_{max} , cm^{-1}): 3340 (OH), 1660 (C=O), 1615, 1570, 1510 (Ar). Fusion with crystalline KOH formed phloroglucinol and *p*-hydroxybenzoic acid. Based on the results and the literature compound **7** was identified as kaempferol [4].

Compound 8. $C_{21}H_{20}O_{11}$, mp 179–181°C (MeOH); $[\alpha]_D^{20} -6^\circ$ (c 0.1, DMF); R_f 0.2 (system I), 0.43 (system II). UV spectrum (EtOH, λ_{max} , nm): 357, 255, 326. IR spectrum (KBr, ν_{max} , cm^{-1}): 3425–3180 (OH), 1670 (γ -pyrone carbonyl), 1615–1610, 1575, 1510, 1455 (Ar), 1095, 1055, 1030, 902, 885 (sugar). Acid hydrolysis (1% H_2SO_4) formed kaempferol and D-glucose. This was proved by PC using authentic samples. Based on the results compound **8** was identified as kaempferol-3-glucoside (astragalin) [4].

Elemental analyses of all compounds agreed with those calculated [4]. Melting points were determined on a Kofler block. UV spectra were recorded on SF-46 and SF-56 spectrophotometers; IR spectra, on UR-20 and Specord-VIS (Germany) instruments.

These compounds were isolated from *A. asper* for the first time.

REFERENCES

1. A. A. Grossgeim, *Plant Resources of the Caucasus* [in Russian], Meditsina, Moscow, 1952.
2. N. N. Guzhva and A. M. Domunyan, in: *Materials of the Regional Conference on Pharmacy, Pharmacology, and Personnel Training* [in Russian], Pyatigorsk, 1999, p. 45.
3. N. N. Guzhva, S. F. Dzhumyrko, A. M. Kolpak, and V. P. Anisimova, *Khim. Prir. Soedin.*, 714 (1992).
4. N. N. Guzhva, Dissertation, Pyatigorsk Pharmaceutical Institute, Pyatigorsk, 1990.