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# FLAVONOIDS FROM *Astragalus microcephalus*

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*Astragalus microcephalus* Willd., a representative of the genus *Astragalus* (Leguminosae L.), showed high antioxidant and leukopoietic activity in biological tests [1, 2]. The chemistry of this plant is little studied.

We previously reported [3] the isolation from stems of this plant of the aurone sulfuretin. Herein we communicate results for the isolation from leaves and the structure elucidation of compounds **1**–**5**. According to qualitative reactions and IR, UV, PMR, and $^{13}C$ NMR spectra, they are flavonoids [4–8].

The plant was collected near Lake Lisi (Georgia) during full flowering in 2008. Air-dried ground raw material (1.3 kg, leaves) was extracted by EtOH (80%, 3×, 1:10). The organic solvent was distilled off to produce an aqueous residue that was purified of lipophilic substances using $CHCl_3$. A white precipitate (4.2 g) consisting of cycloartanes formed [1]. The purified aqueous fraction was extracted exhaustively with EtOAc. The EtOAc extract was evaporated to a dry solid (1.5 g, total 1). The aqueous part left after EtOAc extraction was fractionated over a polyamide column (d, 5 cm; h, 50 cm) with elution successively by water and EtOH (45 and 90%). The 45% EtOH effluent contained total polar flavonoids (total 2, 8.2 g, 0.6% yield); 90%, total nonpolar flavonoids (total 3, 4 g, 0.3% yield).

Total 3 (2 g) showed 12 spots and was separated over a column of silica gel with elution by $CHCl_3$ and $CHCl_3$:MeOH with increasing concentration of the latter to afford **1**–**4**. Under analogous conditions, total 2 afforded compound **5**.

**Compound 1** crystallized from aqueous alcohol as pale yellow needle-like crystals, mp 157–159°C, MW 238.01; $C_{15}H_{10}O_3$, positive Bryant reaction [7]. IR spectrum (KBr, $\nu_{max}$, $cm^{-1}$): 3550 (OH), 1650, 1670 (γ-pyrone C=O). UV spectrum (MeOH, $\lambda_{max}$, nm): 250, 325; +$AlCl_3$: 250, 325; +$AlCl_3$ + HCl: 255, 320, 380sh; +$CH_3COONa$: 265, 359; +$CH_3COONa$ + $H_3BO_3$: 255, 270sh, 310; +$CH_3ONa$: 260, 325, 340.

PMR spectrum (400 MHz, $CD_3OD$, δ, ppm, J/Hz): 6.75 (1H, s, H-3), 6.95 (1H, d, J = 2.5, H-8), 7.00 (1H, d, J = 8.6, H-6), 7.78 (2H, d, J = 8.0, H-3′,5′), 7.79 (1H, d, J = 8.0, H-4′), 7.80 (2H, d, J = 7.20, H-2′,6′), 8.06 (1H, d, J = 8.0, H-5).

$^{13}C$ NMR spectrum (400 MHz, $CD_3OD$, δ, ppm): 162.6 (C-2), 106.5 (C-3), 176.1 (C-4), 126.3 (C-5), 114.9 (C-6), 161.7 (C-7), 102.4 (C-8), 157.3 (C-9), 116.0 (C-10), 131.0 (C-1′), 126.0 (C-2′,6′), 128.9 (C-3′,5′), 131.3 (C-4′).

Alkaline cleavage [2] of **1** produced benzoic acid and resorcinol. A comparison of the results with the literature [9–11] characterized the compound as 7-hydroxyflavone.

**Compound 2**, white needle-like crystals, mp 205–208°C, positive Bryant reaction [7], MW 256.25, $C_{15}H_{12}O_4$. IR spectrum (KBr, $\nu_{max}$, $cm^{-1}$): 3400–3300 (OH), 1667 (γ-pyrone C=O). UV spectrum (MeOH, $\lambda_{max}$, nm): 275, 315; +$AlCl_3$: 275, 310; +$AlCl_3$ + HCl: 275, 315; +$CH_3COONa$: 255sh, 288, 335; +$CH_3COONa$ + $H_3BO_3$: 275, 310; +$CH_3ONa$: 250, 295sh, 355.

PMR spectrum (400 MHz, $CD_3OD$, δ, ppm, J/Hz): 5.48 (1H, dd, J = 5.0, 11.0, H-2), 2.75 (1H, dd, J = 2.9, 12.9, quasiequatorial H-3), 3.10 (1H, dd, J = 2.9, 12.9, quasiaxial H-3), 7.80 (1H, d, J = 8.74, H-5), 6.52 (1H, d, J = 8.74, H-6), 6.45 (1H, s, H-8), 7.45 (2H, d, J = 8.25, H-2′,6′), 6.85 (2H, d, J = 8.0, H-3′,5′).

$^{13}C$ NMR spectrum (400 MHz, $CD_3OD$, δ, ppm): 80.41 (C-2), 44.52 (C-3), 190.80 (C-4), 129.43 (C-5), 111.2 (C-6), 165.3 (C-7), 103.5 (C-8), 164.52 (C-9), 115.1 (C-10), 131.11 (C-1′), 128.8 (C-2′,6′), 116.3 (C-3′,5′), 158.53 (C-4′).

Alkaline cleavage [2] formed *p*-hydroxycinnamic acid and resorcinol. Therefore, **2** was identified as 7,4′-dihydroxyflavone or liquiritigenin [12–15].

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**Compound 3**, white needle-like crystals, mp 230–232°C, negative cyanidine reaction, MW 284.26, $C_{16}H_{12}O_3$. Mass spectrum (70 eV, mz, $I_{rel}$, %): 284 (100), 148 (9), 137 (10), 131 (11). IR spectrum (KBr, $\nu_{max}$, $cm^{-1}$): 3430-3310 (OH), 1675 (γ-pyrone C=O). UV spectrum (MeOH, $\lambda_{max}$, nm): 247, 260sh, 290, 340sh; +$AlCl_3$: 248, 260sh, 290, 340sh; +$AlCl_3$ + HCl: 247, 285, 340sh; +$CH_3COONa$: 258, 300sh, 335; +$CH_3COONa$ + $H_3BO_3$: 252, 260sh, 284; +$CH_3ONa$: 255, 335.

PMR spectrum (400 MHz, $CD_3OD$, δ, ppm, J/Hz): 6.84 (1H, d, J = 2.2, H-8), 6.93 (1H, dd, J = 8.8, 2.2, H-6), 6.97 (2H, s, H-2′,6′), 7.04 (1H, dd, J = 1.5, 0.9, H-5′), 8.05 (1H, d, J = 8.8, H-5), 8.13 (1H, s, H-2).

$^{13}C$ NMR spectrum (100 MHz, $CD_3OD$, δ, ppm): 154.9 (C-2), 126.3 (C-3), 178.1 (C-4), 128.6 (C-5), 116.6 (C-6), 164.9 (C-7), 103.3 (C-8), 159.8 (C-9), 118.2 (C-10), 125.8 (C-1′), 112.6 (C-2′), 147.5 (C-3′), 149.2 (C-4′), 117.4 (C-5′), 121.6 (C-6′).

Compound **3** was characterized as 7,3′-dihydroxy-4′-methoxyisoflavone or calycosin [16–18].

**Compound 4**, orange crystals, mp 280–285°C, MW 270.24 (mass spectrometry), $C_{15}H_{10}O_5$, an aglycon, negative Shinoda reaction [4], base gives reddish-orange color. UV, PMR, and $^{13}C$ NMR spectral data correspond with 6,3′,4′-trihydroxyaurone or sulfuretin, which was previously isolated from *A. microcephalus* Willd. stems [3, 19].

**Compound 5**, pale white crystals, negative Bryant test [7] for flavonoid glycosides, MW [8] 564.0; $C_{26}H_{28}O_{14}$, a bioside. IR spectrum (KBr, $\nu_{max}$, $cm^{-1}$): 3460-3020 (OH), 1645 (γ-pyrone C=O), 1580, 1550, 1520 (>C=C<). UV spectrum (MeOH, $\lambda_{max}$, nm): 260, 350; +$AlCl_3$: 270, 350sh, 380sh, 400; +$AlCl_3$ + HCl: 270, 350sh, 390; +$CH_3COONa$: 270, 355; +$CH_3COONa$ + $H_3BO_3$: 260, 350; +$CH_3ONa$: 270, 320sh, 390. Analysis of UV spectra showed the presence of carbohydrate substituents on C-3 and no *ortho*-dihydroxy group in the side ring on C-3′ and C-4′. The acid hydrolysis products were kaempferol (mp 274–277°C), L-rhamnose, and D-xylose. Stepwise hydrolysis [2] of **5** gave the monoside afzelin (mp 183–185°C). UV spectrum (MeOH, $\lambda_{max}$, nm): 267, 355 [14] and D-xylose. The compound did not undergo alkaline hydrolysis. An analogous result was obtained upon treatment of the glycoside with rhamnodiastase enzyme. The production of afzelin and the negative test for enzymatic hydrolysis indicated that the L-rhamnose was bonded to C-3 of the aglycon. Therefore, D-xylose was the terminal sugar in the biose.

Chemical transformations and UV spectral data of **5** characterized it as kaempferol-3-*O*-α-L-rhamnoxyloside.

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