FLAVONOIDS OF *Scutellaria ocellata* AND *S. nepetoides**

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The known flavonoids oroxylin A, wogonin, apigenin, 3,7,4′-trihydroxyflavone, cinaroside, baicalin, and wogonoside were isolated from the aerial part of Scutellaria ocellata *Juz.; apigenin-7-O-*β*-D-glucuronide, norwogonoside, scutellarin, and the new flavoneglycoside nepetoside A, 5,8-dihydroxy-7-O-*β*-D-galacturonidopyranosylflavone, from the roots of* Scutellaria nepetoides *M. Pop. The structure of the last is established using chemical transformations and spectral data.*

Key words: *Scutellaria ocellata, Scutellaria nepetoides*, oroxylin A, wogonin, apigenin, 3,7,4′-trihydroxyflavone, cinaroside, baicalin, wogonoside, apigenin-7-O-*β*-D-glucuronide, norwogonoside, scutellarin, 5,8-dihydroxy-7-O-*β*-D-galacturonidopyranosylflavone.

Many plant species of the *Scutellaria* L. (Lamiaceae) genus are used in folk and traditional medicine [1]. Flavonoids are responsible for their high activity [2]. The genus *Scutellaria* is represented by ~360 species, 32 of which grow in Uzbekistan. Only 55 species have been investigated. The present article reports on a study of flavonoids from ocellar skullcap *Scutellaria ocellata* Juz. and catnip-like skullcap *S. nepetoides* M. Pop [3].

The chemical composition of ocellar skullcap has not been previously studied. Seven pure flavonoids were isolated by column chromatography from various fractions of the ethanol extract of the aerial part of *S. ocellata* collected at the end of flowering near Lake Kulikubon (Fergana district). Acid hydrolysis and UV, IR, mass, and NMR spectra identified them as the known flavonoids oroxylin A (**1**) [4, 5], wogonin (**2**) [4, 5], apigenin (**3**) [5-7], 3,7,4′-trihdyroxyflavone (**4**) [6,8], cinaroside (**5**) [5, 6, 9], baicalin (**6**) [5, 6, 10], and wogonoside (**7**) [5, 6, 10].

Roots of catnip-like skullcap (*S. nepetoides*) yielded apigenin-7-O-*β*-D-glucuronide, norwogonoside, and scutellarin [11]. Column chromatography of various fractions of the ethanol extract of roots of this species collected during fruiting on the right bank of Lake Kulikubon (Fergana district) yielded apigenin-7-O-*β*-D-glucuronide, norwogonoside, scutellarin, and the new flavonoid nepetoside A (**8**), $C_{21}H_{18}O_{11}$, mp 204-206°C. The UV spectrum (λ_{max}, nm, 277, 314) of **8** is characteristic of flavone derivatives [5, 12].

The IR spectrum contains absorption bands for hydroxyls, galacturonic acid carbonyl, γ-pyrone carbonyl, aromatic C=C, and glycoside C–O bonds. The PMR spectrum of **8** exhibits signals for seven aromatic protons, an anomeric proton, a chelated hydroxyl, and other carbohydrate protons (see Experimental). Therefore, nepetoside A is a glycoside.

Acid hydrolysis of **8** produced norwogonin and D-galacturonic acid. The site of attachment of the carbohydrate to the 7-OH of the aglycone was established from UV spectra of **8** and its aglycone. Adding CH_3COONa produced no bathochromic shift of the absorption maxima, indicating that the 7-OH of the flavone is glycosylated [6].

Furthermore, nepetoside A gives a positive gossypetin test, consistent with the presence of C-5 and C-8 hydroxyls [13]. The signal for the anomeric proton of D-galacturonic acid in the PMR spectrum of **8** appears at 5.95 ppm as a doublet with spin—spin coupling constant 7.0 Hz. This indicates that the carbohydrate is bound to the aglycone through a *β*-glycoside bond [6].

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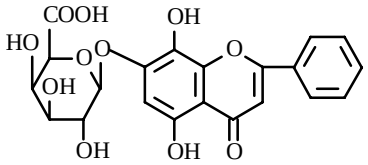
Thus, nepetoside A is a new natural compound, the structure of which is 5,8-dihydroxy-7-O-β-D-galacturonidopyranosylflavone or norwogonin-7-O-β-D-galacturonide. Flavonoids **1**-**7** were isolated from *S. ocellata* for the first time.



8

EXPERIMENTAL

The following solvent systems were used: $CHCl_3$—CH_3OH (19:1, 1; 9:1, 2; 85:15, 3; 4:1, 4) and butanol-pyridine-water (6:4:3, 5).

We used Silufol UV-254 plates for thin-layer chromatography (TLC). Column chromatography was performed over KSK 100/160 μ silica gel; paper chromatography (PC), on Filtrak No. 12 chromatographic paper. Spots of flavonoids on TLC were developed by ammonia vapors; sugar on PC, by spraying with acid anilinium phthalate with subsequent heating at 90-100°C.

PMR spectra were recorded on a Tesla BS-567A instrument at 100 MHz (δ, ppm, 0 = GMDS); mass spectra, in an MX-1310 instrument at 50 eV ionizing potential; IR spectra, on a Perkin—Elmer System 2000 FT-IR Fourier spectrometer in KBr; UV spectra, on a Perkin—Elmer Lambda 16 spectrometer.

Extraction and Isolation of Flavonoids from the Aerial Part of *S. ocellata*. The dried and ground aerial part (1.2 kg) of ocellar skullcap collected during flowering in July 1995 near Lake Kulikubon near Shakhimardan (Fergana district, Republic of Uzbekistan) was extracted at room temperature six times with ethanol. The combined alcoholic extract was condensed in vacuo to 0.7 L and diluted with water to 1.4 L. The aqueous alcoholic extract was shaken successively with $CHCl_3$ (5×0.5 L), ethylacetate (10×0.5 L), and butanol (8×0.4 L). Solvent was removed to give $CHCl_3$ (22.4 g), ethylacetate (13.0 g), and butanol (28.0 g) fractions.

The ethylacetate fraction (13.0 g) was chromatographed over a silica-gel (260 g) column (2.3×120 cm) with elution successively by $CHCl_3$ and systems 1-3. Fractions of 400 mL were collected. Elution of the column by system 1 isolated oroxylin A (0.06 g) and wogonin (0.09 g); by system 2, apigenin (0.1 g) and 3,7,4′-trihydroxyflavone (0.08 g); by system 3, cinaroside (0.84 g).

The butanol extract (28.0 g) was chromatographed over a silica-gel (560 g) column (3.0×140 cm) with elution successively by systems 3-4. Fractions of 500 mL were collected. Elution of the column by system 3 isolated cinaroside (0.6 g); by system 4, baicalin (0.14 g) and wogonoside (0.43 g).

Oroxylin A (5,7-dihydroxy-6-methoxyflavone) (1). $C_{16}H_{12}O_5$ ([M]$^+$ 284), mp 218-219°C. UV spectrum (EtOH, λ_{max}, nm): 249, 272, 321. Mass spectrum *m*/*z* 284 [M]$^+$, 269, 254, 241, 226, 202, 167, 142, 124, 113, 103, 77, 69.

Wogonin (5,7-dihydroxy-8-methoxyflavone) (2). $C_{16}H_{12}O_5$, mp 201-202°C. UV spectrum (EtOH, λ_{max}, nm): 247, 277, 319. Mass spectrum *m*/*z* 284 [M]$^+$, 269 [M - CH_3]$^+$ (100%), 241 [M - CH_3 - CO], 167, 139, 105, 102.

Apigenin (5,7,4′-trihydroxyflavone) (3). $C_{15}H_{10}O_5$ ([M]$^+$ 270), mp 346-347°C. UV spectrum (EtOH, λ_{max}, nm): 270, 298, 338. PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 6.62 (1H, d, J = 2.0, H-6), 6.71 (1H, d, J = 2.0, H-8), 6.80 (1H, s, H-3), 7.09 (2H, d, J = 9.0, H-3′, H-5′), 7.84 (2H, d, J = 9.0, H-2′, H-6′), 13.68 (1H, br.s, 5-OH).

3,7,4′-Trihydroxyflavone (4). $C_{15}H_{10}O_5$ ([M]$^+$ 270), mp 203-205°C. UV spectrum (EtOH, λ_{max}, nm): 267, 364.

Cinaroside (luteolin-7-O-β-D-glucoside) (5). $C_{21}H_{20}O_{11}$, mp 240-242°C. UV spectrum (EtOH, λ_{max}, nm): 256, 268, 350. PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 3.90-4.05 (sugar protons), 5.66 (1H, d, J = 7.0, H-1′), 6.67 (1H, d, J = 2.5, H-6), 6.77 (1H, s, H-3), 6.84 (1H, d, J = 2.5, H-8), 7.13 (1H, d, J = 8.0, H-5′), 7.38 (1H, dd, J = 8.0 and J = 2.5, H-6′), 7.74 (1H, d, J = 2.5, H-2′).

Acid hydrolysis of **5** produced luteolin and D-glucose (PC, system 5). Acetylation of **5** by acetic anhydride in pyridine gave the heptaacetate derivative $C_{35}H_{34}O_{18}$ ([M]$^+$ 742), mp 121-123°C.

Baicalin (baicalein-7-O-β-D-glucuronide) (6). $C_{21}H_{18}O_{11}$, mp 219-221°C. UV spectrum (EtOH, λ_{max}, nm): 245, 277, 313. Acid hydrolysis of **6** produced baicalein ($C_{15}H_{10}O_5$, mp 259-261°C, $[M]^+$ 270) and D-glucuronic acid (PC, system 5).

Wogonoside (wogonin-7-O-β-D-glucuronide) (7). $C_{22}H_{20}O_{11}$, mp 194-196°C. UV spectrum (EtOH, λ_{max}, nm): 275, 345. Acid hydrolysis of **7** produced wogonin and D-glucuronic acid (PC, system 5).

Extraction and Isolation of Flavonoids from Roots of *S. nepetoides*. The dried and ground roots (0.8 kg) of catnip-like skullcap collected during flowering in July 1996 on the right bank of Lake Kulikubon near Shakhimardan (Fergana district, Republic of Uzbekistan) was extracted at room temperature eight times with ethanol. The combined alcoholic extract was condensed in vacuo to 0.6 L and diluted with water to 1.2 L. The aqueous alcoholic extract was shaken successively with $CHCl_3$ (4×0.5 L), ethylacetate (9×0.4 L), and butanol (8×0.4 L). The solvents were removed to give $CHCl_3$ (17.0 g), ethylacetate (14.0 g), and butanol (25.0 g) fractions.

The ethylacetate extract (14.0 g) was chromatographed over a silica-gel (280 g) column (2.3×140 cm) with elution successively by $CHCl_3$ and systems 3-4. Fractions of 400 mL were collected. Elution of the column by system 3 yielded apigenin-7-O-β-D-glucuronide (0.09 g) and norwogonoside (0.18 g); by system 4, scutellarin (0.12 g).

Nepetoside A was isolated from the butanol extract by crystallization from methanol to afford **8** (0.21 g).

Nepetoside A (norwogonin-7-O-β-D-galacturonide) (8). $C_{21}H_{18}O_{11}$, mp 204-206°C. UV spectrum (EtOH, λ_{max}, nm): 277, 314; +CH_3COONa 275, 315. IR spectrum (KBr, ν_{max}, cm^{-1}): 3358 (OH), 1729 (COOH), 1664 (γ-pyrone C=O), 1612, 1575, 1492 (aromatic C=C), 1070, 1038 (glycoside C–O).

PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 4.00-4.70 (3H, m, H-2″, H-3″, H-4″), 4.87 (1H, d, J = 8.5, H-5″), 5.95 (1H, d, J = 7.0, H-1″), 6.77 (1H, s, H-6), 6.85 (1H, s, H-3), 7.39 (3H, m, H-3′, H-4′, H-5′), 7.70 (2H, m, H-2′, H-6′), 12.85 (1H, br.s, 5-OH).

Acid Hydrolysis of 8. Glycoside **8** (20 mg) was hydrolyzed in aqueous methanolic HCl (25 mL, 15%) for 6 h on a boiling-water bath. The methanol was removed in vacuo. The resulting precipitate was filtered off and recrystallized from ethanol to afford norwogonin (8 mg), $C_{15}H_{10}O_5$, mp 250-252°C, $[M]^+$ 270. The evaporated hydrolysate yielded D-galacturonic acid (PC, system 5).

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