FLAVONOIDS OF *Scutellaria immaculata* ROOTS

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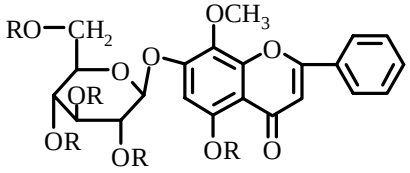
UDC 547.972

(±)-5,2′-Dihydroxy-6,7,6′-trimethoxyflavanone, (-)-5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavanone, chrysin, wogonin, apigenin, isoscutellarein, scutellarein, cosmosiin, and the new flavonoid wogonin-7-O-β-D-glucopyranoside, the structure of which was established using chemical transformations and spectral data, were isolated from Scutellaria immaculata *roots.*

Key words: *Scutellaria immaculata*, (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone, (-)-5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavanone, chrysin, wogonin, apigenin, isoscutellarein, scutellarein, cosmosiin, wogonin-7-O-*β*-D-glucopyranoside.

We have previously studied flavonoids from the aerial part of white skullcap and isolated several of them [1].

The present article contains results from an investigation of flavonoids from roots of *Scutellaria immaculata* Nevski. We used column chromatography to isolate from various fractions of the ethanol extract the new flavonoglycoside **1** in addition to the known flavonoids (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone [2], (-)-5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavanone [3], chrysin [4], wogonin [4], apigenin [5], isoscutellarein [6], scutellarein [7], and cosmosiin [7].



1 - 2

1: R = H; **2:** R = $COCH_3$

The UV spectrum of **1** (λ_{max}, nm, 276, 340) is characteristic of flavone derivatives [8, 9].

The IR spectrum of **1** contains absorption bands for hydroxyls, methoxyls, γ-pyrone carbonyl, aromatic C=C bonds, and glycoside C–O vibrations. The PMR spectrum of **1** exhibits signals for seven aromatic protons, an anomeric proton, a chelate hydroxyl proton, a methoxyl, and other protons of the carbohydrate part (see Experimental). Therefore, **1** is a glycoside.

Acetylation of **1** produced the pentaacetyl derivative **2**, the mass spectrum of which had a peak for the molecular ion with *m*/*z* 656 and strong peaks for fragment ions of the tetraacetylhexose with *m*/*z* 331, 271, and 169 [10]. Acid hydrolysis of **1** produced wogonin (5,7-dihydroxy-8-methoxyflavone) [4] and D-glucose.

The site of attachment of the carbohydrate unit to the 7-OH of the aglycon was established by studying UV spectra of **1** and its aglycon. Adding CH_3COONa gave no bathochromic shift of the absorption maxima, indicative of glycosylation of the flavone 7-OH [11].

The PMR spectrum of **1** gives a signal for the anomeric proton of D-glucose at 5.30 ppm as a doublet with SSCC 7.0 Hz, indicative of a *β*-glycoside bond between the carbohydrate and aglycon [11].

Therefore, **1** is wogonin-7-O-*β*-D-glucopyronoside (5-hydroxy-8-methoxy-7-O-*β*-D-glucopyranosylflavone).

Compound **1** is a new natural compound. (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone, (-)-5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavanone, chrysin, wogonin, apigenin, isoscutellarein, and scutellarein were isolated for the first time from *S. immaculata*.

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EXPERIMENTAL

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

TLC used Silufol UV-254 plates. Column chromatography was performed on KSK 100/160 μm silica gel; paper chromatography (PC), on Filtrak No. 12 chromatographic paper.

Spots of flavonoids in TLC were developed using ammonia vapor; sugars in PC, by spraying with anilinium acid phthalate and subsequent heating at 90-100°C.

PMR spectra were recorded on a Tesla BS-567A instrument (100 MHz, δ, ppm, 0 = HMDS); mass spectra, in an MX-1310 instrument at 50 eV ionizing potential; IR spectra, as KBr disks on a Perkin—Elmer System 2000 FT-IR Fourier-spectrometer; UV spectra, on a Perkin—Elmer Lambda 16 spectrometer. Melting points were determined on a Boetius instrument with a PHMK 0.5 visual device.

Extraction and Isolation of Flavonoids. Dried and ground roots (1.2 kg) of *S. immaculata* that were collected during fruting in August 1996 in Zhalilabad district in the foothills of the Kyzyl-Ungur ridge of Kyrgyzstan Republic were extracted eight times at room temperature with ethanol. The combined alcohol extract was condensed in vacuum to 0.8 L and diluted with water to 1.6 L. The aqueous alcohol extract was successively shaken with $CHCl_3$ (5 × 0.5 L), ethylacetate (8 × 0.5 L), and butanol (10 × 0.5 L). The solvents were removed to afford $CHCl_3$ (18.0 g), ethylacetate (14.0 g), and butanol (35.0 g) fractions.

The ethylacetate extract (14.0 g) was chromatographed over a column (2.8 × 160 cm) of silica gel (350 g) with elution successively by $CHCl_3$ and systems 1-3. Fractions of 400 mL were collected. Elution of the column by system 1 isolated (±)-5,2′-dihydroxy-6,7,6′-trimethoxyflavanone (0.22 g), (-)-5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavanone (0.25 g), chrysin (0.13 g); by system 2, wogonin (0.16 g), apigenin (0.14 g); by system 3, isoscutellarein (0.15 g), scutellareinn (0.12 g), and wogonin-7-O-*β*-D-glucopyranoside (**1**, 0.16 g).

(±)-5,2′-Dihydroxy-6,7,6′-trimethoxyflavanone, $C_{18}H_{18}O_7$, mp 217-218°C. UV spectrum (EtOH, λ_{max}, nm): 288, 345; +$AlCl_3$, 314, 375.

PMR spectrum (100 MHz, δ, ppm, J/Hz, $CDCl_3$): 2.75 (1H, dd, J = 3.1, J = 17.5, H-3 eq), 3.19 (1H, dd, J = 13.5, J = 17.5, H-3 ax), 3.70, 3.73, and 3.85 (3H, s, each 3 × OCH_3), 6.00 (1H, dd, J = 3.1, J = 13.5, H-2), 6.13 (1H, s, H-8), 6.55 (1H, br.d, J = 8.0, H-3′), 7.12 (1H, dd, J = 8.0, J = 8.5, H-4′), 6.41 (1H, br.d, J = 8.5, H-5′), 11.88 (1H, br.s, 5-OH). Mass spectrum *m*/*z* 346 $[M]^+$, 328 [M - H_2O], 313 [M - H_2O - CH_3] (100%), 285, 197, 196, 181, 168, 153, etc.

(-)-5,2′-Dihydroxy-6,7,8,6′-tetramethoxyflavanone, $C_{19}H_{20}O_8$, mp 148-149°C. UV spectrum (EtOH, λ_{max}, nm): 289, 358; +$AlCl_3$, 316, 372.

PMR spectrum (100 MHz, δ, ppm, J/Hz, $CDCl_3$): 2.73 (1H, dd, J = 3.1, J = 17.5, H-3 eq), 3.17 (1H, dd, J = 13.5, J = 17.5, H-3 ax), 3.71, 3,74, 3.87, and 4.03 (3H, s, each 4 × OCH_3), 6.01 (1H, dd, J = 3.1, J = 13.5, H-2), 6.36 (1H, br.d, J = 8.2, H-5′), 6.50 (1H, br.d, J = 7.8, H-3′), 7.10 (1H, dd, J = 7.8, J = 8.2, H-4′), 11.82 (1H, br.s, 5-OH). Mass spectrum *m*/*z* 376 $[M]^+$, 358 [M - H_2O], 343 [M - H_2O - CH_3], 315, 226 (a^+), 211, 183, 131, 119, 83, and 69 (100%).

Chrysin (5,7-dihydroxyflavone), $C_{15}H_{10}O_4$, mp 289-291°C. UV spectrum (MeOH, λ_{max}, nm): 247 sh, 268, 313; +CH_3COONa, 275, 359; +CH_3COONa/H_3BO_3, 269, 315; +$AlCl_3$, 252, 279, 330, 380; +$AlCl_3$/HCl, 251, 280, 326, 381.

PMR spectrum (100 MHz, δ, ppm, J/Hz, DMSO-d_6): 6.22 (1H, d, J = 2.0, H-6), 6.52 (1H, d, J = 2.5, H-8), 6.93 (1H, s, H-3), 7.58 (3H, m, H-3′,4′,5′), 8.30 (2H, m, H-2′,6′), 10.90 (1H, s, 7-OH), 12.83 (1H, s, 5-OH).

Wogonin (5,7-dihydroxy-8-methoxyflavone), $C_{16}H_{12}O_5$, mp 201-202°C. UV spectrum (MeOH, λ_{max}, nm): 247, 277, 319.

PMR spectrum (100 MHz, δ, ppm, J/Hz, C_5D_5N): 3.85 (3H, s, OCH_3), 6.70 (1H, s, H-3), 6.90 (1H, s, H-6), 7.41 (3H, m, H-3′,4′,5′), 7.97 (2H, m, H-2′,6′), 13.14 (1H, s, 5-OH).

Apigenin (5,7,4′-trihydroxyflavone), $C_{15}H_{10}O_5$, mp 246-247°C. UV spectrum (EtOH, λ_{max}, nm): 270, 298, 338.

PMR spectrum (100 MHz, δ, ppm, J/Hz, C_5D_5N): 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′,5′), 7.84 (2H, d, J = 9.0, H-2′,6′), 13.68 (1H, br.s, 5-OH).

Isoscutellarein (5,7,8,4′-tetrahydroxyflavone), $C_{15}H_{10}O_6$, mp 339-341°C. UV spectrum (EtOH, λ_{max}, nm): 282, 309, 327 sh, 365 sh.

PMR spectrum (100 MHz, δ, ppm, J/Hz, C_5D_5N): 6.52 (1H, s, H-6), 6.69 (1H, s, H-3), 6.95 (2H, d, J = 8.5, H-3′,5′), 7.70 (2H, d, J = 8.5, H-2′,6′), 12.70 (1H, s, 5-OH).

Scutellarein (5,6,7,4′-tetrahydroxyflavone), $C_{15}H_{10}O_6$, mp >340°C. UV spectrum (EtOH, λ_{max}, nm): 286, 339. PMR spectrum (100 MHz, δ, ppm, J/Hz, C_5D_5N): 6.70 (1H, s, H-3), 7.02 (2H, d, J = 9.0, H-3′,5′), 7.20 (1H, s, H-8), 8.29 (2H, d, J = 9.0, H-2′,6′), 12.90 (1H, br.s, 5-OH).

Wogonin-7-O-β-D-glucopyranoside (1), $C_{22}H_{22}O_{10}$, mp 147-149°C. UV spectrum (EtOH, λ_{max}, nm): 276, 340; $+CH_3COONa$, 277, 342. IR spectrum (KBr, ν_{max}, cm^{-1}): 3440 (OH), 2930 (OCH_3), 1660 (γ-pyrone C=O), 1620, 1575, 1514 (aromatic C=C), 1090, 1030, 1008 (glycoside C–O).

PMR spectrum (100 MHz, δ, ppm, J/Hz, DMSO-d_6): 3.76 (3H, s, OCH_3), 5.30 (1H, d, J = 7.0, H-1″), 4.00-4.50 (6H, sugar protons), 7.05 (1H, s, H-3), 7.12 (1H, d, J = 2.0, H-6), 7.57 (3H, m, H-3′,4′,5′), 8.02 (2H, m, H-2′,6′), 12.75 (1H, s, 5-OH).

Acid Hydrolysis of 1. Glycoside **1** (30 mg) was hydrolyzed by HCl (20 mL, 5%) for 4 h on a boiling-water bath. The resulting solid aglycon was filtered off and recrystallized from ethanol to afford wogonin (11 mg, mp 200-202°C). PC of the hydrolysate using system 4 detected D-glucose.

Pentaacetate of 1 (2). Glycoside **1** (25 mg) was dissolved in a mixture of pyridine (1.5 mL) and acetic anhydride (5 mL) and treated for 4 h as usual to afford the pentaacetate of composition $C_{32}H_{32}O_{15}$ (17 mg). Mass spectrum *m*/*z* 656 $[M]^+$, 331, 329, 284, 271, 169.

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