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FLAVONOIDS OF THE AERIAL PART OF *Scutellaria immaculata**

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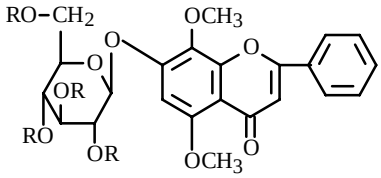
*Chrysin-7-O-glucuronide, scutellarein-7-O-glucoside, apigenin-7-O-glucoside, baicalein-7-O-glucoside, norwogonin-7-O-glucoside, oroxyloside, wogonoside, and the new glycoside immaculoside-5,8-dimethoxy-7-O-β-D-glucopyranosylflavone were isolated for the first time from the aerial part of white skullcap (*Scutellaria immaculata *Nevski). The structure of the last was established using chemical transformations and spectral data.*

Key words: *Scutellaria immaculata*, chrysin-7-O-glucuronide, scutellarein-7-O-glucoside, apigenin-7-O-glucoside, baicalein-7-O-glucoside, norwogonin-7-O-glucoside, oroxyloside, wogonoside 5,8-dimethoxy-7-O-β-D-glucopyranosylflavone.

We continue our study of flavonoids from *Scutellaria* L. (Lamiaceae) species growing in Central Asia.

White skullcap (*Scutellaria immaculata* Nevski) is a perennial shrub that grows in cliff crevices mainly in steep rocky mountain gorges of Central Asia [1].

We investigated flavonoids from the aerial part of the plant collected during fruiting (August 1996) in the Kyzyl-Ungur foothills. Column chromatography of the alcohol extract over a silica-gel column afforded the new flavone glycoside immaculoside (**1**) and the known flavonoids chrysin-7-O-glucuronide [2], scutellarein-7-O-glucoside [3], apigenin-7-O-glucoside [4], baicalein-7-O-glucoside [5], norwogonin-7-O-glucoside [6], oroxyloside [7], and wogonoside [8,9].



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

The UV spectrum of **1** (λ_{max}, nm, 270, 331) is characteristic of flavone derivatives [10]. The IR spectrum contains absoption bands of hydroxyl, methoxyl, carbonyl of γ-pyrone, and C–O of glycosides and aromatic rings. The PMR spectrum of **1** exhibits signals for seven aromatic protons, an anomeric proton, two methoxyls, and other protons of the carbohydrate (see Experimental). Therefore, the examined compound is a glycoside.

Acetylation of **1** produced the tetraacetyl derivative **2**, the mass spectrum of which gives a peak for the molecular ion with *m*/*z* 628 and strong peaks for fragments from the tetraacetylhexose with *m*/*z* 331, 271, and 169 [11]. Acid hydrolysis of **1** produced 7-hydroxy-5,8-dimethoxyflavone [6] and D-glucose.

The site of attachment of the carbohydrate to the 7-OH of the aglycone was established by studying the UV spectra of the glycoside and its aglycone. Addition of CH_3COONa did not produce a bathochromic shift of the absorption maxima. This is consistent with glycosylation of the flavone 7-OH [10].

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The PMR spectrum of immaculoside contains a doublet for the anomeric proton of D-glucose at 5.44 ppm with spin—spin coupling constant 6.5 Hz. This is indicative of a β-glycosidic bond of the carbohydrate to the aglycone [10]. Therefore, immaculoside has the structure 5,8-dimethoxy-7-O-β-D-glucopyranosylflavone.

Immaculoside (**1**) is a new natural compound. Chrysin-7-O-glucuronide, scutellarein-7-O-glucoside, apigenin-7-O-glucoside, oroxyloside, and wogonoside are isolated from *Scutellaria immaculata* for the first time.

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).

We used Silufol UV-254 plates for thin-layer chromatography (TLC). Column chromatography was carried out on KSK 100/160 μ silica gel; paper chromatography (PC), on Filtrak No. 12 chromatographic paper. Spots of flavonoids on TLC were visualized by ammonia vapor; spots of sugar on PC, by spraying with acidic 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 = HMDS); mass spectra, in an MX-1310 instrument at ionizing potential 50 eV; IR spectra, on a Perkin—Elmer System 2000 FT-IR Fourier spectrometer in KBr; UV spectra, on a Perkin—Elmer Lambda 16 spectrometer. Melting points were determined on a Boetius instrument with an RNMK 0.5 optical fitting.

Extraction and Isolation of Flavonoids. The dried and ground aerial part (2.0 kg) of white skullcap collected during fruiting in August 1996 in Jalalabad district in the Kyzyl-Ungur foothills of the Republic of Kyrgyzstan was extracted at room temperature eight times with ethanol. The combined extract was concentrated in vacuo to 0.9 L and diluted with water to 1.8 L. The aqueous alcoholic extract was successively shaken with $CHCl_3$ (5×0.5 L), ethylacetate (8×0.5 L), and butanol (8×0.5 L). The solvents were removed to afford $CHCl_3$ (42.0 g), ethylacetate (20.0 g), and butanol (51.0 g) fractions.

The ethylacetate extract (20.0 g) was chromatographed over a silica-gel (400 g) column (2.6×130 cm) with elution successively by $CHCl_3$ and systems 1-3. Fractions of 500 mL were collected. Elution of the column by system 1 isolated chrysin-7-O-glucuronide (0.18 g); system 2, baicalein-7-O-glucoside (0.12 g); system 3, oroxyloside (0.23 g).

The butanol extract (51.0 g) was chromatographed over a silica-gel (1020 g) column (5×110 cm) with elution successively by systems 1-3. Fractions of 500 mL were collected. Elution of the column by system 1 isolated chrysin-7-O-glucuronide (0.12 g); system 2, baicalein-7-O-glucoside (0.14 g), scutellarein-7-O-glucoside (0.20 g), apigenin-7-O-glucoside (0.24 g), and norwogonin-7-O-glucoside (0.18 g); system 3, wogonoside (0.24 g) and immaculoside (0.19 g).

Chrysin-7-O-glucuronide, $C_{21}H_{18}O_{10}$, mp 219-221°C. UV spectrum (EtOH, λ_{max}, nm): 270, 305; +CH_3COONa 268, 308; +$AlCl_3$ 250, 282, 329, 378.

PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 4.00-4.67 (3H, m, H-2″, H-3″, H-4″), 4.84 (1H, d, J = 8.5, H-5″), 5.88 (1H, d, J = 6.5, H-1″), 6.70 (1H, d, J = 2.0, H-6), 6.76 (1H, s, H-5), 6.97 (1H, d, J = 2.0, H-8), 7.15-7.38 (3H, m, H-3′, H-4′, H-5′), 7.51-7.73 (2H, m, H-2′, H-6′). Acid hydrolysis of the glucoside produced chrysin with mp 290-292°C, $C_{15}H_{10}O_4$. $[M]^+$ 254 and D-glucuronic acid (PC, system 4).

Oroxyloside (oroxylin-7-O-glucuronide), $C_{22}H_{20}O_{11}$, mp 199-201°C. UV spectrum (EtOH, λ_{max}, nm): 280, 315; +CH_3COONa 281, 316. Acid hydrolysis of oroxyloside isolated oroxylin A with mp 217-218°C, $C_{16}H_{12}O_5$, $[M]^+$ 284 and D-glucuronic acid (PC, system 4).

Scutellarein-7-O-glucoside, $C_{21}H_{20}O_{11}$, mp 193-195°C. UV spectrum (MeOH, λ_{max}, nm): 288, 337; +CH_3COONa 290, 340; +$AlCl_3$ 292 sh, 306, 371. Acid hydrolysis of the glucoside produced scutellarein with mp >340°C, $C_{15}H_{10}O_6$, $[M]^+$ 286 and D-glucose (PC, system 4).

Apigenin-7-O-glucoside (cosmosiin), $C_{21}H_{20}O_{10}$, mp 227-229°C. UV spectrum (EtOH, λ_{max}, nm): 268, 339; +CH_3COONa 267, 339; +$AlCl_3$ 279, 301, 343; +CH_3ONa 267, 397.

PMR spectrum (100 MHz, C_5D_5N, δ, ppm, J/Hz): 3.59-4.64 (sugar protons), 5.72 (1H, d, J = 7.0, H-1″), 6.64 (1H, d, J = 2.5, H-6), 6.78 (1H, s, H-3), 6.96 (1H, d, J = 2.5, H-8), 7.12 (2H, d, J = 8.0, H-3′, H-5′), 7.81 (2H, d, J = 8.0, H-2′, H-6′), 13.62 (1H, br.s, 5-OH). Acid hydrolysis of the glycoside produced apigenin with mp 345-346°C, $C_{15}H_{10}O_5$, $[M]^+$ 270 and D-glucose (PC, system 4).

Norwogonin-7-O-glucoside, $C_{21}H_{20}O_{10}$, mp 278-280°C. UV spectrum (EtOH, λ_{max}, nm): 279, 350. IR spectrum (KBr,

ν_{max}, cm^{-1}): 3450 (OH), 1660 (C=O of γ-pyrone), 1618, 1575, 1517 (aromatic C=C), 1075, 1024, 1005 (C–O of glycosides). Acid hydrolysis of the glycoside produced norwogonin with mp 250-252°C, $C_{15}H_{10}O_5$, $[M]^+$ 270 and D-glucose (PC, system 4).

Wogonoside (wogonin-7-O-glucuronide), $C_{22}H_{20}O_{11}$, mp 194-196°C. UV spectrum (MeOH, λ_{max}, nm): 276, 345. Acid hydrolysis of wogonoside produced wogonin with mp 200-202°C, $C_{16}H_{12}O_6$. Mass spectrum *m*/*z* 284 $[M]^+$, 269 $[M - CH_3]^+$ (100%), 241 [M - CH_3 - CO], 167, 139, 105, 102, and D-glucuronic acid (PC, system 4).

Immaculoside (1), $C_{23}H_{24}O_{10}$, mp 197-199°C. UV spectrum (EtOH, λ_{max}, nm): 270, 331; +CH_3COONa 271, 332. IR spectrum (KBr, ν_{max}, cm^{-1}): 3445 (OH), 2930 (OCH_3), 1664 (C=O of γ-pyrone), 1619, 1578, 1516 (aromatic C=C), 1072, 1022, 1009 (C–O of glycosides).

PMR spectrum (100 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.85, 3.92 (3H, s, 2×OCH_3 each), 3.38-4.00 (sugar protons), 5.44 (1H, d, J = 6.5, H-1″), 6.72 (1H, s, H-6), 6.81 (1H, s, H-3), 7.52-7.74 (3H, m, H-3′, H-4′, H-5′), 7.92-8.15 (2H, m, H-2′, H-6′).

Acid Hydrolysis of 1. Glycoside **1** (25 mg) was hydrolyzed in HCl (15 mL, 5%) for 4 h on a boiling-water bath. The resulting precipitate of aglycone was filtered off and recrystallized from ethanol to produce 7-hydroxy-5,8-dimethoxyflavone (8 mg) with mp 259-262°C (dec.), $C_{17}H_{14}O_5$. Mass spectrum *m*/*z* (%): 298 $[M]^+$, 283 $[M - CH_3]^+$ (100%), 255, 105, 102, etc., and D-glucose (PC, system 4).

Tetraacetate of 1 (2). Glycoside **1** (20 mg) was dissolved in the mixture of pyridine (1.5 mL) and acetic anhydride (4 mL). Treatment for 4 h by the usual method gave the tetraacetate of formula $C_{31}H_{32}O_{14}$ (15 mg). Mass spectrum *m*/*z* $[M]^+$ 628, 331, 329, 298, 271, 169.

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