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NEW FLAVONOID GLUCURONIDES FROM THE AERIAL PART OF *Scutellaria intermedia*

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The aerial part of the plant Scutellaria intermedia *Popov yielded five glucuronides, three of which were identified as chrysin 7-*O-β-*D-glucuronide, wogonin 7-*O-β-*D-glucuronide, and baicalein 7-*O-β-*D-glucuronide (baicalin). IR, UV, PMR, and* ^{13}*C NMR spectral data and acid hydrolysis of the two new glucuronides established their structures as 5,7,2′-trihydroxyflavone 2′-*O-β-*D-glucuronopyranoside (**4**) and scutevulin 2′-*O-β-*D-glucuronopyranoside (**5**).*

Keywords: *Scutellaria intermedia*, flavone glycosides, 5,7,2′-trihydroxyflavone 2′-*O*-*β*-D-glucuronopyranoside, scutevulin 2′-*O*-*β*-D-glucuronopyranoside.

Many species of the genus *Scutellaria* L. (skullcap) exhibit broad spectra of biological activity and are used in science-based and folk medicine [1–3]. Several of the 32 *Scutellaria* species indigenous to Uzbekistan are used in folk medicine to treat epilepsy, allergies, nervousness, hypertension, and other diseases [2, 4]. Plants of this genus have varied chemical compositions. Flavonoids, phenylpropanoids, phenolic acids, iridoids, clerodane-type diterpenoids, steroids, triterpenes, lignans, alkaloids, phytosterols, tanning agents, essential oils, and other classes of natural compounds have now been isolated from them [1–7].

Modern pharmacological research confirmed that extracts and individual flavonoids from plants of the genus *Scutellaria* possessed antitumor, hepatoprotective, antioxidant, anti-inflammatory, anticonvulsant, antibacterial, and antiviral activity [8–10]. The biological activity of *Scutellaria* flavonoids has prompted unwavering interest in them and an increasing number of scientific publications. Greater than 320 flavonoids have now been isolated from various species. The list is constantly being updated with new compounds [5–7].

Flavonoids from plants of the genus *Scutellaria* were studied systematically during our search for new biologically active compounds and their accessible sources. The present work focused on the aerial part of *S. intermedia* Popov growing on rocky and stony slopes, cliffs, and gravel beds of the western Tian-Shan and Pamir-Alay mountains [11]. The plant was collected during flowering (May 2015) in the foothills of Pap District, Republic of Uzbekistan. Information about flavonoids from this plant was missing in the literature available to us.

The EtOAc fraction of the EtOH extract of the aerial plant part was separated by column chromatography using various absorbents and was isolated eight flavonoids. Spectral data and direct comparisons with authentic samples identified them as chrysin, oroxylin A, 7-*O*-methylnorwogonin, 5,7,2′-trihydroxyflavone, scutevulin (5,7,2′-trihydroxy-8-methoxyflavone), 2(*S*)-5,7,2′-trihydroxyflavanone, baicalein, and apigenin [12].

Flavonoids **1**–**5** were isolated from the *n*-BuOH fraction. Three of them were identified based on spectral data and acid hydrolysis as the known flavonoids chrysin 7-*O*-*β*-D-glucuronide (**1**) [5, 7, 13], wogonin 7-*O*-*β*-D-glucuronide (**2**) [5, 7, 14], and baicalein 7-*O*-*β*-D-glucuronide (baicalin, **3**) [5–7, 15].

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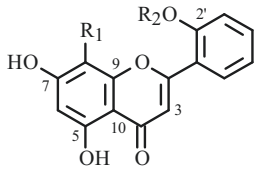
TABLE 1. ^{13}C NMR Spectra of Flavonoids **4**, **4a**, **5**, and **5a** (100 MHz, DMSO-d_6, δ, ppm)

C atom	**4**	**4a**	**5**	**5a**	C atom	**4**	**4a**	**5**	**5a**
2	161.2	161.8	160.4	161.5	3′	115.8	117.4	115.2	117.7
3	109.8	109.3	109.7	108.3	4′	132.3	133.0	132.2	132.5
4	182.3	182.5	181.7	181.9	5′	122.3	119.1	121.7	119.2
5	157.9	161.6	156.5	156.5	6′	128.9	128.8	128.4	128.6
6	98.5	99.6	99.1	99.2	1″	99.2		99.0	
7	164.1	166.3	157.0	157.3	2″	72.8		72.6	
8	94.1	94.5	127.3	127.2	3″	75.4		75.2	
9	161.1	158.3	149.5	150.2	4″	71.3		71.5	
10	103.5	103.6	103.2	103.8	5″	75.7		75.4	
1′	120.6	117.6	120.1	117.4	6″	170.1		170.3	
2′	155.7	158.0	154.5	156.6	OCH_3			61.1	60.8



4, 4a, 5, 5a

4: R_1 = H, R_2 = GlcA*p*; **4a:** $R_1 = R_2$ = H
5: R_1 = OCH_3, R_2 = GlcA*p*; **5a:** R_1 = OCH_3, R_2 = H

Flavonoid **4**, $C_{21}H_{18}O_{11}$, had a UV spectrum (λ_{max} 254, 260, 268, 326 nm) characteristic of flavone derivatives. Spectra taken with NaOAc and $AlCl_3$ indicated that free phenolic hydroxyls were present in the flavone 5- and 7-positions [15]. The IR spectrum of flavonoid **4** exhibited absorption bands for hydroxyls, carboxylic C=O, and γ-pyrone in addition to aromatic C=C bonds. The PMR spectrum of **4** in DMSO-d_6 showed resonances for protons of a 5,7,2′-trisubstituted flavone, a chelated 5-OH (12.84 ppm), an anomeric proton (δ 5.12 ppm, 1H, d, J = 7.4 Hz), and other carbohydrate protons. The TLC mobility and IR, PMR, and ^{13}C NMR spectral data (Table 1) were consistent with a flavonoid glycoside for **4**. The carbonyl absorption band at 1742 cm^{-1} in the IR spectrum suggested that the carbohydrate part was a uronic acid. In fact, acid hydrolysis of **4** produced 5,7,2′-trihydroxyflavone (**4a**) and D-glucuronic acid. Resonances of the D-glucuronic-acid C atoms appeared in the ^{13}C NMR spectrum at 99.2 (C-1″), 72.8 (C-2″), 75.4 (C-3″), 71.3 (C-4″), 75.7 (C-5″), and 170.1 ppm (C-6″) [15–17]. The bonding site of the carbohydrate was found by comparing ^{13}C NMR spectra of the compound and 5,7,2′-trihydroxyflavone (Table 1) and their UV spectra. Therefore, flavonoid **4** had the structure 5,7,2′-trihydroxyflavone 2′-*O*-β-D-glucuronopyranoside and was a new and previously unreported glycoside of 5,7,2′-trihydroxyflavone.

Flavonoid **5** was a yellow amorphous compound, $C_{22}H_{20}O_{12}$. Its IR spectrum had absorption bands for hydroxyls, two carbonyls, and aromatic C=C bonds. Its UV spectrum (λ_{max} 223, 275, 316, 345 nm) was characteristic of flavone derivatives. Bathochromic shifts of the absorptions upon adding NaOAc and $AlCl_3$ were consistent with free phenolic hydroxyls in the 5- and 7-positions. The PMR spectrum of **5** had resonances for 5,7,8,2′-tetrasubstituted flavone protons, a methoxyl, phenolic hydroxyls in the 5- and 7-positions, an anomeric proton, and other carbohydrate protons. Resonances in the ^{13}C NMR spectrum at 99.0 (C-1″), 72.6 (C-2″), 75.2 (C-3″), 71.5 (C-4″), 75.4 (C-5″), and 170.3 (C-6″) were indicative of a β-D-glucuronopyranosyl moiety in flavone glycoside **5** [15, 17]. This was confirmed by acid hydrolysis that produced scutevulin (5,7,2′-trihydroxy-8-methoxyflavone, **5a**) and D-glucuronic acid. The bonding site of the glucuronic acid to the phenolic 2′-OH was established by comparing ^{13}C NMR spectra of scutevulin and flavone glycoside **5**. Thus, **5** was a new natural glycoside of scutevulin and had the structure scutevulin 2′-*O*-β-D-glucuronopyranoside.

Flavone glycosides glycosylated at the C-2′ hydroxyl are rather common in plants of the genus *Scutellaria* [5, 7].

EXPERIMENTAL

UV spectra were recorded in EtOH on an SF-2000 spectrophotometer. IR spectra were taken on a PerkinElmer Spectrum FTIR spectrometer. PMR and ^{13}C NMR spectra were recorded on a Unity-400^{+}, Bruker AV-4 spectrometer at operating frequency 400 MHz (PMR) and 100 MHz (^{13}C NMR). Chemical shifts are given in ppm on the δ-scale. TLC was performed on Silufol UV-254 plates. Spots of flavonoids on TLC plates were viewed under UV light in a UFS-254/365 chromatographic irradiator and were also detected by treatment with NH_3 vapor, NaOH (0.5%) in EtOH, and vanillin (3%) in EtOH mixed with conc. HCl (4:1 ratio). Column chromatography used KSK SiO_2 100/160 μm, Woelm polyamide, and Sephadex LH-20 (Pharmacia). TLC used Sorbil PTSKh-P-A-UV plates and solvent systems 1) $CHCl_3$–EtOAc (9:1); 2) $CHCl_3$–EtOH (9:1); 3) $CHCl_3$–EtOAc–EtOH (6:1:3); 4) *n*-BuOH–EtOH–H_2O (5:3:2); and 5) *n*-BuOH–Py–H_2O (6:4:3).

Extraction and Isolation of Flavonoids. Air-dried ground aerial part of *S. intermedia* (1.13 kg) was extracted (5×) with EtOH (85%) at room temperature. The obtained extract was condensed *in vacuo* to 0.5 L, treated with an equal volume of distilled H_2O, shaken, and worked up sequentially with petroleum ether, EtOAc, and *n*-BuOH. The aqueous residue was acidified with H_2SO_4 solution (5%) and extracted (2×) with *n*-BuOH. The *n*-BuOH extracts from before and after acidification were combined. The extracts were evaporated *in vacuo* to afford petroleum-ether (7.2 g), EtOAc (21.8 g), and *n*-BuOH fractions (34.8 g).

A part of the EtOAc fraction (20.0 g) was chromatographed over a column of silica gel (600 g) using a petroleum ether–EtOAc gradient (80:20–0:100) to produce five subfractions (E1-E5). Rechromatography of subfraction E1 (2.38 g) over a column of silica gel using a hexane–EtOAc gradient (90:10–20:80) isolated chrysin (82.0 mg), oroxylin A (106.0 mg), and 7-*O*-methylnorwogonin (269.8 mg). Subfraction E2 (4.25 g) was chromatographed over a column of silica gel using a $CHCl_3$–PrOH gradient (98:2–70:30) to isolate from separate fractions 5,7,2′-trihydroxyflavone (49.6 mg) and scutevulin (135.2 mg). Subfraction E3 (3.82 g) was separated by preparative TLC using solvent system 2 to obtain 2(*S*)-5,7,2′-trihydroxyflavanone (21.4 mg). Subfraction E4 (2.89 g) was separated over a column of Sephadex LH-20 using an EtOH–H_2O gradient (95:5–20:80) to isolate baicalein (34.3 mg). Rechromatography of subfraction E5 (1.42 g) over an SiO_2 column with gradient elution by *n*-hexane–EtOAc (98:2–30:70) produced apigenin (25.3 mg).

The combined *n*-BuOH fraction (34.0 g) was also chromatographed over an SiO_2 column using an EtOAc–EtOH gradient (90:10–70:30) to produce four fractions. Subfraction B1 (4.37 g) was separated over a column of Sephadex LH-20 with elution by EtOH–H_2O (95:5–10:90) to isolate **1** (358.5 mg) and **2** (176.7 mg). Subfraction B2 (6.48 g) was rechromatographed over a polyamide column using $CHCl_3$–PrOH (98:2–0:100) to isolate **3** (214.1 mg). Subfraction B3 (5.85 g) was separated over a column of Sephadex LH-20 with elution by EtOH–H_2O (95:5–10:90) to isolate **4** (34.3 mg) and **5** (46.8 mg). Subfraction B4 (7.31 g) was chromatographed over a column of Sephadex LH-20 with elution by EtOH–H_2O (95:5–10:90) to isolate **4** (12.6 mg) and **5** (19.3 mg).

Acid Hydrolysis of Glucuronides. Glycosides **1**–**5** were hydrolyzed on a water bath using HCl solution (20%) for 5–6 h. The aglycon precipitated on cooling and was filtered off and recrystallized. The filtrate was evaporated *in vacuo* to a syrupy residue. Paper chromatography with an authentic sample detected D-glucuronic acid (system 5).

Chrysin 7-*O*-β-D-glucuronide (1), $C_{21}H_{18}O_{10}$, mp 219–221°C. UV spectrum (EtOH, λ_{max}, nm): 270, 305. ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 5.18 (1H, d, J = 6.8, H-1″), 6.48 (1H, br.s, H-6), 6.90 (1H, br.s, H-8), 7.06 (1H, s, H-3), 7.50–7.60 (3H, m, H-3′, 4′, 5′), 8.00–8.11 (2H, m, H-2′, 6′), 12.60 (1H, s, 5-OH). Acid hydrolysis of **1** produced chrysin and D-glucuronic acid.

Wogonin 7-*O*-β-D-glucuronide (2), $C_{22}H_{20}O_{11}$, mp 194–196°C. UV spectrum (EtOH, λ_{max}, nm): 275, 345. ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 3.78 (3H, s, 8-OCH_3), 5.32 (1H, d, J = 7.2, H-1″), 7.06 (1H, s, H-3), 7.12 (1H, s, H-6), 7.60 (3H, m, H-3′, 4′, 5′), 8.00 (2H, m, H-2′, 6′), 12.79 (1H, s, 5-OH). Acid hydrolysis of **2** produced wogonin and D-glucuronic acid.

Baicalin (baicalein 7-*O*-β-D-glucuronide) (3), $C_{21}H_{18}O_{11}$, mp 220–222°C. UV spectrum (EtOH, λ_{max}, nm): 245, 277, 313. ^1H NMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 5.24 (1H, d, J = 7.1, H-1″), 6.98 (1H, s, H-3), 7.09 (1H, s, H-8), 7.6 (3H, m, H-3′, 4′, 5′), 8.0 (2H, m, H-2′, 6′), 12.70 (1H, s, 5-OH). Acid hydrolysis of **3** produced baicalein and D-glucuronic acid.

5,7,2′-Trihydroxyflavone 2′-*O*-β-D-glucuronopyranoside (4), amorphous yellow compound, $C_{21}H_{18}O_{11}$, $[\alpha]_D$ –65.3° (*c* 0.13, Me_2CO). UV spectrum (EtOH, λ_{max}, nm): 254, 260, 268, 326; (+NaOMe): 246, 254, 262, 272, 373; (+NaOAc): 255, 263, 271, 357; (+$AlCl_3$): 246, 253, 264, 276, 340; (+$AlCl_3$/HCl): 242, 248, 257, 265, 280, 335. IR spectrum (KBr, ν_{max}, cm^{-1}): 3471 (OH), 1742 (carboxylic C=O), 1660 (γ-pyrone C=O), 1624, 1578 (aromatic C=C bonds). ^1H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.16–3.50 (m, H-2″–H-4″), 3.76 (1H, d, J = 9.0, H-5″), 5.12 (1H, d, J = 7.4,

H-1″), 6.16 (1H, d, J = 1.8, H-6), 6.43 (1H, d, J = 1.8, H-8), 6.97 (1H, s, H-3), 7.14 (1H, m, H-5′), 7.29 (1H, d, J = 8.0, 1.8, H-3′), 7.46 (1H, m, H-4′), 7.85 (1H, dd, J = 8.0, 1.8, H-6′), 10.68 (1H, s, 7-OH), 12.84 (1H, s, 5-OH). Table 1 presents the ^{13}C NMR spectrum. Acid hydrolysis of **4** produced 5,7,22-trihydroxyflavone (**4a**) and D-glucuronic acid.

Scutevulin 2′-*O*-β-D-glucuronopyranoside (5), yellow crystalline compound, $C_{22}H_{20}O_{12}$, mp 278–279°C (dec.), $[\alpha]_D$ –70.4° (*c* 0.09, Me_2CO). UV spectrum (EtOH, λ_{max}, nm): 223, 275, 316, 345; (+NaOMe): 230, 258, 282, 320, 344; (+NaOAc): 263, 281, 344, 377; (+$AlCl_3$): 223, 252, 284, 294, 336, 345, 397; (+$AlCl_3$/HCl): 225, 247, 282, 294, 332, 345, 398. IR spectrum (KBr, ν_{max}, cm^{-1}): 3476 (OH), 1742 (carboxylic C=O), 1665 (γ-pyrone C=O), 1615, 1574 (aromatic C=C bonds). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.24–3.43 (m, H-2″–H-4″), 3.84 (3H, s, OCH_3), 3.92 (1H, d, J = 9.0, H-5″), 5.23 (1H, d, J = 7.2, H-1″), 6.34 (1H, s, H-6), 7.06 (1H, s, H-3), 7.28 (1H, m, H-5′), 7.32 (1H, d, J = 8.0, 1.8, H-3′), 7.52 (1H, m, H-4′), 7.82 (1H, dd, J = 8.0, 1.8, H-6′), 10.78 (1H, s, 7-OH), 12.54 (1H, s, 5-OH). Table 1 presents the ^{13}C NMR spectrum. Acid hydrolysis of **5** produced scutevulin (**5a**) and D-glucuronic acid.

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