FLAVONOIDS OF THE EPIGEAL PART OF Scutellaria ramosissima

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Chrysin 7-O-β-D-glucuronide and two new flavanones have been isolated from the epigeal part of Scutellaria ramosissima. It has been established on the basis of spectral characteristics and the results of chemical transformations that they have the structures of 2(S)-2',5,7-trihydroxyflavanone 7-O-(methyl β-D-glucopyranosiduronate) and 2(S)-2',5,7-trihydroxyflavanone 7-O-(ethyl β-D-glucopyranosiduronate).

Plants of the genus Scutellaria (Libiatae) are rich sources of flavanoids [1] and are widely used in scientific and folk medicines [3].

Scutellaria ramosissima M. Pop is a perennial semishrub growing in rocky areas in the subalpine zone of the mountains of Central Asia. We have investigated the flavonoids of the epigeal part of the plant gathered at the beginning of fruit bearing on the slopes of the Chatkal range. Three flavonoids (I-III) were isolated from an alcoholic extract of the epigeal part, and the results of a study of their chemical structures are given in the present paper.

The UV spectrum of compound (I) (λ_{max}, nm: 211.5, 224 sh., 283, 330 sh.; log ε 4.39, 4.25, 4.22, 3.59) was characteristic for flavanone derivatives, and its IR spectrum contained absorption bands due to hydroxy groups (3560-3220 cm^{-1}), an ester carbonyl group (1740 cm^{-1}), a γ-pyrone (1650 cm^{-1}), aromatic nuclei (1635, 1587, 1510 cm^{-1}), and the C–O vibrations of glycosides (1100, 1078, 1050 cm^{-1}).

The flavanone nature of (I) was confirmed by its PMR spectrum, which contained the signals of the H-2 proton in the form of a double doublet with SSCCs of 5.5 and 10 Hz and the signals of the two H-3 protons in the form of a two-proton multiplet [5]. In addition, the spectrum showed the signals of six aromatic protons, of a chelate hydroxy group (5-OH), of an ethoxycarbonyl (-$COOCH_2CH_3$) group, of an anomeric proton, and of the other protons of the carbohydrate moiety (Table 1). The chemical shifts of the C-2 and C-3 carbon atoms in the ^{13}C NMR spectrum were also characteristic for flavanones, and the chemical shift of the signal of the C-2 carbon (74.6 ppm) showed the presence of a free or a substituted phenolic hydroxy group in the C-2' position [6].

An absorption band at 1740 cm^{-1} in the IR spectrum permitted the assumption of the presence of a uronic acid residue in the flavanone (I) molecule. A one-proton doublet at 4.61 ppm (J = 8.7 Hz), which is characteristic for the H-5" proton of a D-glucuronide, and the signals of the protons of an ethoxycarbonyl group in the PMR spectrum of compound (I) showed that the carbohydrate residue of the substance under consideration probably consisted of the ethyl ester of a glucuronic acid [7].

The mass spectrum of flavanone (I), contained, in addition to the peak of the molecular ion with m/z 476, intense peaks of ions from the aglycon with m/z 272, 254 (272 – H_2O), 153 (a + H), and 120 (c). Consequently, the aglycon of glycoside (I) was a trihydroxyflavanone containing two hydroxy groups in ring A and one hydroxy group in ring B.

The acid hydrolysis of glycoside (I) gave an aglycon with the composition $C_{15}H_{12}O_5$ (M^+ 272) and D-glucuronic acid. By a study of UV, IR, mass, CD, and PMR spectra and a comparison of physicochemical properties with literature information, the aglycon obtained was identified as 2(S)-2',5,7-trihydroxyflavanone (see the Experimental) [8].

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TABLE 1. PMR Spectra of Flavanones (I) and (II) in C_5D_5N, δ, ppm (J, Hz)

Proton	I	II
H-3	5,90 dd (5,5; 10,0)	5,92 dd (5,3; 10,0)
2H-3	2,75–3,27 m	2,82–3,26 m
H-6	6,42 s	6,43 s
H-8	6,42 s	6,43 s
H-3′	6,92 d (7,4)	6,93 d (7,3)
H-4′	7,10 dd (7,5; 7,4)	7,12 dd (7,5; 7,3)
H-5′	6,82 dd (7,5; 7,5)	6,83 dd (7,5; 7,3)
H-6′	7,49 d (7,5)	7,51 d (7,3)
H-1″	5,69 d (6,5)	5,72 d (6,5)
H-5″	4,61 d (8,7)	4,65 d (8,5)
H-2″–4″	4,10–4,50	4,05–4,50
$-OCH_2-CH_3$	3,97 q (7,0)	—
	0,92 t (7,0)	—
OCH_3	—	3,48 s
5-OH	12,30	12,32

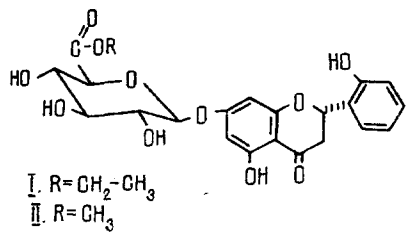
TABLE 2. Chemical Shifts of the Carbon Atoms of 2',5,7-Trihydroxyflavanone (IV) and of Flavanone (I) in DMSO-d_6 (δ, ppm, O – TMS)

C atom	IV [8]	I	C atom	IV	I
2	74,0	74,6 d	4′	129,5	130,2 d
3	41,1	41,3 t	5′	119,2	119,9 d
4	196,5	197,8 s	6′	127,1	127,6 d
5	163,7	163,6 s	1″	—	99,5 d
6	95,9	97,0 d	2″	—	73,1 d
7	166,8	165,3 s	3″	—	75,6 d
8	95,1	95,7 d	4″	—	71,7 d
9	163,4	163,6 s	5″	—	75,6 d
10	101,8	103,9 s	6″	—	169,3 s
1′	124,9	124,9 s	$-OCH_2-CH_3$	—	61,4 t
2′	154,4	154,9 s		—	14,5 q
3′	115,6	116,0 d			

In the PMR spectrum of compound (I), the signal of the anomeric proton of D-glucuronic acid appeared at 5.69 ppm in the form of a doublet with an SSCC of 6.5 Hz, which corresponds to a β-bound D-glucopyranosiduronate.

The position of attachment of the carbohydrate residue was established as the result of a comparison of the ^{13}C NMR spectra of 2(S)-2',5,7-trihydroxyflavonone (IV) and glycoside (I) (Table 2). On passing from the aglycon (IV) to the glycoside (I) the signal of the C-7 carbon shifted upfield by –1.5 ppm, while the signals of the C-6, C-8, and C-10 carbons underwent paramagnetic shifts by +1.1, +0.6, and +2.1 ppm, respectively.

Consequently, the carbohydrate residue in glycoside (I) was attached to the hydroxyl in position C-7 of the aglycon, and the flavonoid isolated had the structure of 2(S)-2',5,7-trihydroxyflavanone 7-O-(ethyl β-D-glucopyranosiduronate) (I):



I. R=CH_2-CH_3
II. R=CH_3

The UV and IR spectra of flavanone (II) were close to those of compound (I). As compared with the NMR spectrum of flavanone (I), that of substance (II) lacked the signals of the protons of an ethoxycarbonyl group but a three-proton singlet due to a methoxycarbonyl ($-COOCH_3$) group appeared at 3.48 ppm. Otherwise, the spectra of these substances were very close (see Table 1).

The mass spectrum of compound (II) had the peak of the molecular ion with m/z 462, which is 14 mass units smaller than that for substance (I). The acid hydrolysis of flavonone (II) led to 2(S)-2',5,7-trihydroxyflavanone (IV) and D-glucuronic acid.

The facts given above, and also a comparative analysis of the PMR and mass spectra of the flavanones (I) and (II) showed that the latter differed from the former by the presence of a methyl group instead of the ethyl group and had the structure of 2(S)-2',5,7-trihydroxyflavanone 7-O-(methyl β-D-glucopyranosiduronate) (II).

On the basis of a study of spectral characteristics and the results of acid hydrolysis, flavonoid (III) was identified as chrysin 7-O-β-D-glucopyranuronoside [9].

It must be mentioned that glucuronides are widely distributed among the flavonoids of plants of the genus Scutellaria [3]. The isolation of methyl glucuronides from representatives of this genus has been reported repeatedly [10]. Apigenin 7-(ethyl glucosiduronate) has been detected in the plant Achillea cartilaginea [7].

EXPERIMENTAL

General Remarks. For thin-layer chromatography (TLC) we used Silufol UV-254 plates. Column chromatography was conducted on type KSK silica gel with a grain size of 100-160 μm. In TLC, the substances were revealed in UV light and by treatment with ammonia vapors. The following solvent systems were used: chloroform–methanol: (9:1) (1), (97:3) (2), and (95:5) (3); and n-butanol–pyridine–water (6:4:3) (4).

PMR and ^{13}C NMR spectra were taken on a Tesla BS-567 A spectrometer (δ, ppm, PMR, 0 – TMS) at frequencies of 100 MHz for protons and 25.142 MHz for carbon in Py-d_5 and DMSO-d_6, respectively. Mass spectra were obtained on an MKh-1310 instrument at an ionizing voltage of 50 V. IR spectra were recorded on a UR-20 instrument in KBr, and UV spectra on a Specord UV-Vis spectrometer.

Isolation of the Flavonoids. The dried and comminuted epigeal part (1 kg) of Scutellaria ramosissima gathered at the beginning of fruit-bearing in September, 1989 (village of Aksak-ata, Tashkent province) was extracted at room temperature with ethanol eight times. The combined alcoholic fraction was concentrated in vacuum to 0.5 liter and was diluted with water to 1 liter. The aqueous alcoholic extract was treated successively with petroleum ether (5 × 0.45 liter), chloroform (5 × 0.5 liter), ethyl acetate (12 × 0.45 liter), and butanol (10 × 0.45 liter). After the solvents had been distilled off, 16.0 g of petroleum ether fraction, 8 g of chloroform fraction, 20 g of ethyl acetate fraction, and 36 g of butanol fraction were obtained. The ethyl acetate extract (20 g) was chromatographed on a column (3 × 120 cm) of silica gel (330 g) with elution successively by chloroform and systems 2 and 3, 500-ml fractions being collected. When the column was eluted with system 2, fractions 59-70 yielded 0.2 g of flavanone (I), and then fractions 110-117 gave 0.21 g of flavanone (II). Elution of the column in system 3 led to 0.13 g of flavanone (III).

2(S)-2',5,7-Trihydroxyflavonone 7-O-(Ethyl β-D-Glucopyranosiduronate) (I). $C_{23}H_{24}O_{11}$, mp 130-132°C (methanol), $[\alpha]_D$ – 115.8° (c 0.48; methanol). Mass spectrum, m/z (%): M^+ 476(3), 458 (M – H_2O; 3.5), 273(24), 272(64), 255(58), 254(272 – H_2O; 100), 253(69), 226(8), 153(92), 152(28), 120(17). For the PMR and ^{13}C NMR spectra, see Tables 1 and 2.

2(S)-2',5,7-Trihydroxyflavonone 7-O-(Methyl β-D-Glucopyranosiduronate) (II). $C_{22}H_{22}O_{11}$, mp 141-142°C (methanol), $[\alpha]_D$ –98.0° (c 0.62; dimethylformamide), $\lambda_{max}^{ethanol}$, nm: 213, 225.5, 284, 331 (log ε 3.99; 3.86; 3.81; 3.19); ν_{max}^{KBr} cm^{-1}: 3480-3330 (OH), 1724 (ester C=O), 1643 (γ-pyrone C=O), 1620, 1580 (C=C bond), 1097, 1057, 1045, 1026 (C–O of glycosides).

Mass spectrum, m/z (%): M^+ 462(8), 444 (M – H_2O; 4) 273(33), 272(76), 255(43), 254 (272 – H_2O; 100), 253(79), 153(83), 152(16), 120(12). For the PMR spectrum, see Table 1.

Acid Hydrolysis. Glycosides (I) and (II) (25-30 mg in each case) were hydrolyzed in 25 ml of a 20% solution of hydrochloric acid in the water bath for 5-6 h. The precipitate

of aglycon that had deposited was filtered off, was recrystallized from aqueous methanol. This gave a white crystalline substance with mp 199-201°C, composition $C_{15}H_{12}O_5$ (IV) (M^+ 272 and fragments with m/z 254, 153, 120); $\lambda_{max}^{ethanol}$, nm: 287, 329; $+CH_3COONa$ 255, 280*, 327; $+AlCl_3$ 240*, 276*, 315, 383; $+CH_3ONa$ 243*, 326; ν_{max}^{KBr}, cm^{-1}: 3434 (OH), 1642 (C=O), 1600, (C=C bond); CD (c 0.006; methanol): $\Delta\varepsilon$ +2.80 (307 nm), $\Delta\varepsilon$ −18.6 (284 nm). By paper chromatography in system 4, hydrolysates of compounds (I) and (II) were found to contain glucuronic acid.

Chrysin 7-O-β-D-Glucopyranuronoside (III). $C_{21}H_{18}O_{10}$, mp 219-221°C (methanol); $\lambda_{max}^{ethanol}$ nm: 270, 305 (log ε 3.58; 3.27); $+CH_3COONa$, 268, 308; $+AlCl_3$, 250, 282, 329, 378.

PMR spectrum (Py-d_5: 4.00-4.67 (m, H-2', 3', 4'), 4.84 (d, 8.5 Hz, H-5"), 5.88 (d, 6.5 Hz, H-1"), 6.70 (d, 2 Hz, H-6), 6.76 (s, H-3), 6.97 (d, 2 Hz, H-8), 7.15-7.33 (H-3', 4', 5'), 7.51-7.73 (H-2', 6').

The acid hydrolysis of glycoside (III) (15% hydrochloride acid, 5 h) formed D-glucuronic acid and chrysin with mp 286-288°C (M^+ 254, and characteristic fragments with m/z 152 and 102), $\lambda_{max}^{ethanol}$ 269, 311 nm.

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