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STRUCTURES OF TWO NEW FLAVONOIDS FROM *Scutellaria ramosissima*

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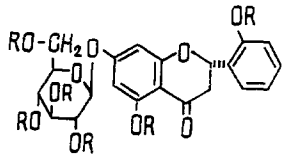
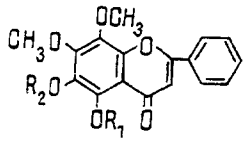
The new 2(S)-2',5-dihydroxy-7-β-D-glucopyranosyloxyflavanone has been isolated from the epigeal part of Scutellaria ramosissima *M. Pop. Oroxylin A, woganin, 2',6'-trihydroxy-6,7,8-trimethoxyflavone, and vanillic and syringic acids, and also the new substance 5,6-dihydroxy-7,8-dimethoxyflavone, have been isolated from the roots of this plant for the first time. The structures of the flavonoids isolated have been established on the basis of chemical transformations and spectral characteristics.*

We have previously isolated new flavanones from the epigaeal part of *Scutellaria ramosissima* M. Pop. [1]. Continuing this investigation, from an ethyl acetate fraction of an alcoholic extract we have now isolated the new compound (**1**). The UV spectrum of substance (**1**) was characteristic for flavanone derivatives [2]. This was confirmed by its PMR spectrum, which contained the signals of the H-2 proton in the form of a doublet of doublets with SSCC of 7.0 and 10.0 Hz and the signals of the 2H-3 protons in the form of a two-proton multiplet [2]. In addition, the spectrum showed the signals of six aromatic protons, an anomeric proton, and two protons of the carbohydrate moiety, and also those of a chelate hydroxy group (see the Experimental part). Consequently, the substance under consideration was a glycoside.

When glycoside (**1**) was acetylated, a hexaacetyl derivative (**2**) was obtained the mass spectrum of which contained, in addition to a low-intensity peak of the molecular ion with *m/z* 686, intense peaks of fragmentary ions of the tetraacetylhexose residue with *m/z* 331, 271, and 169 [3]. The acid hydrolysis of glycoside (**1**) gave 2(S)-2',5,7-trihydroxyflavanone [1, 4] and D-glucose. The site of attachment of the carbohydrate residue was established by a comparative study of the UV spectra of glycoside (**1**) and its aglycon. In contrast to the spectrum of the aglycon, in the spectrum of glycoside (**1**) the addition of CH_3COONa did not lead to a bathochromic shift of the absorption maxima, which showed glycosylation of the 7-OH group of the flavanone [2].



1, R=H
2. R=COCH$_3$



3. R$_1$=R$_2$=H
4. R$_1$=R$_2$=COCH$_3$
5. R$_1$=H, R$_2$=CH$_3$

In the PMR spectrum of glycoside (**1**), the signal of the anomeric proton of the D-glucose residue appeared at 5.37 ppm in the form of a doublet with the SSCC 7.0 Hz. This showed the presence of a β-glycosidic bond of the carbohydrate residue with the aglycon [2].

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TABLE 1. Chemical Shifts of the Carbon Atoms of 5,6-Dihydroxy-7,8-dimethoxy-flavone in a Mixture of $CDCl_3 + CD_3OD$ (δ, ppm; 0 – TMS)

C Atom	δ	C Atom	δ
2	164.2 s	10	106.6 s
3	104.7 d	1′	131.2 s
4	183.0 s	2′	126.1 d
5	142.9 s	3′	129.0 d
6	133.8 s	4′	131.8 d
7	147.4 s	5′	129.0 d
		6′	126.1 d
8	133.2 s		
		—OCH_3	61.2 q
9	142.2 s		61.9 q

We have also studied the flavonoids of the roots of this plant gathered on the slopes of the Kurama range in the flowering period. By chromatography on a column of silica gel we isolated from an alcoholic extract of the roots the new flavone (**3**) and also the known flavonoids oroxylin A [5], wogonin [5, 6], norwoginin [7], and 2′,5,6′-trihydroxy-6,7,8-trimethoxyflavone [7], and vanillic and syringic acids.

The UV spectrum of compound (**3**) showed that it was a flavone derivative [2], and its IR spectrum contained the absorption bands of hydroxy and methoxy groups, of a γ-pyrone carbonyl, and of aromatic nuclei.

The PMR spectrum of the flavone under consideration showed the signals of the protons of two methoxy groups, of H-3, of an unsubstituted ring B, and of a chelate hydroxy group (5-OH) (see the Experimental part). The acetylation of compound (**3**) with acetic anhydride in pyridine gave the diacetyl derivative (**4**) while methylation with an ethereal solution of diazomethane gave the monomethyl ether (**5**). Thus, in ring A of the flavone under consideration there were two methoxy groups and two phenolic hydroxy groups. The mass spectrum of substance (**3**) contained the 100% peak of an ion with *m/z* 299 formed as the result of the splitting out of OCH_3 from the molecular ions with *m/z* 314. This showed the presence of an OCH_3 group in the C-8 position of the flavone nucleus [8]. Flavone (**3**) gave a positive qualitative reaction with $SrCl_2$, showing the presence of an *ortho*-dihydroxy grouping in the C-5 and C-6 positions [5]. Consequently, the substance under consideration had the structure of 5,6-dihydroxy-7,8-dimethoxyflavone.

The proposed structure of (**3**) was confirmed by the results of a study of its ^{13}C NMR spectrum (Table 1).

The assignment of the signals of the ^{13}C nuclei of flavone (**3**) was made on the basis of a comparative study of its ^{13}C NMR spectrum and that of 2′,5,6,6′-tetrahydroxy-7,8-dimethoxyflavone [5] and of flavonoids unsubstituted in ring B [9].

The study of the flavonoids of *S. ramosissima* is continuing.

EXPERIMENTAL

General Observations. We used the following solvent systems: chloroform–methanol: 1) (97:3), 2) (19:1); 3) chloroform–hexane (9:1); 4) *n*-butanol–pyridine–water (6:4:3). PC was conducted on type FN-11 paper. For the TLC and CC conditions, see [1].

PMR spectra were recorded on a Tesla BS-567A 100 MHz instrument (δ, ppm; 0 – HMDS), and ^{13}C spectra on a Varian CFT-20 20 MHz instrument (δ, ppm, 0 – TMS). Mass spectra were obtained on a MKh-1310 instrument at an ionizing voltage of 50 V; UV spectra were recorded on a UR-20 instrument in KBr, and UV spectra on a SPECORD UV-Vis spectrometer in ethanol.

Isolation of 2(S)-7-β-D-Glucopyranosyloxy-2′,5-dihydroxyflavanone (1). On further elution of a column containing the ethyl acetate fraction [1] with system 2, fraction 124 yielded 0.06 g of the flavanone (**1**) with the composition $C_{21}H_{22}O_{10}$, mp 209-210°C. λ_{max}, mm, 289, 330; $+CH_3COONa$ 288, 332. PMR spectrum (Py-d_5), ppm: 2.78-3.13 (m, 2H-3), 3.75-4.56 (glucose protons 5.37 (d, 7.0 Hz, H-1"), 6.15 (dd, 7.0 Hz and 10.0 Hz, H-2), 6.25 (d, 2.0 Hz, H-6), 6.33 (d, 2.0 Hz, H-8), 6.98 (dd, 7.5 Hz and 7.5 Hz, H-5′), 7.08 (dd, 7.5 and 2.0 Hz, H-3′), 7.19 (ddd, 7.5; 7.5 and 2.0 Hz, H-4′), 7.62 (dd, 7.5 and 2.0 Hz, H-6′), 12.75 (br.s, 5-OH).

Hexaacetate (2) of (1). Glycoside (1) (14 mg) was dissolved in a mixture of 1 ml of pyridine and 2 ml of acetic anhydride. After 3 h, working up by the method generally adopted yielded 12 mg of a hexaacetate with mp 92-94°C. Mass spectrum, *m/z* (%): M^+ 686, 644, 331, 284, 271, 169, and others.

Acid Hydrolysis. Glycoside (1) (10 mg) was hydrolyzed in 15 ml of 5% aqueous methanolic hydrochloric acid in the boiling water bath for 4 h. The precipitate of the aglycon that had deposited was filtered off and was recrystallized from aqueous methanol. This gave 6 mg of 2(S)-2′,5,7-trihydroxyflavanone with mp 198-200°C, composition $C_{15}H_{12}O_5$ (M^+ 272) [1, 4]. D-Glucose was detected in the hydrolysate by PC in system 4.

Isolation of Flavonoids from the Roots. The dried and comminuted roots (1.8 g) of *S. ramosissima* gathered in the flowering period in August, 1991 (Tamchik Pass, Pap region, Namangana province) was extracted at room temperature with ethanol eight times. The combined alcoholic extract was concentrated in vacuum to 0.8 liter and was diluted with water to 1.6 liters. The aqueous alcoholic extract was shaken successively with petroleum ether (8 × 0.5 liter), chloroform (8 × 0.5 liter), ethyl acetate (10 × 0.5 liter), and butanol (10 × 0.5 liter). The solvents were distilled off, to give 11.0 g of petroleum-ether fraction, 15.0 g of chloroform fraction, 13.0 g of ethyl acetate fraction, and 35.0 g of butanol fraction.

The chloroform extract (15.0 g) was chromatographed on a column (3 × 120 cm) of silica gel (300 g), with elution successively by mixtures of chloroform and hexane (4:6)-(7:3), 500 ml fractions being collected. On the elution of the column with the above-described system in a ratio of (1:1), fraction 37-45 yielded 0.2 g of oroxylin A, and fractions 46-47 gave 0.23 g of wogonin. On elution of the column with the chloroform–hexane (7:3) system, fractions 52-82 yielded 0.3 g of 5,6-dihydroxy-7,8-dimethoxyflavone, fractions 97-101 – 0.4 g of 2′,5,6′-trihydroxy-6,7,8-trimethoxyflavone, and fraction 102 – 0.1 g of norwogonin.

The ethyl acetate extract (13.0 g) was chromatographed on a column (3 × 100 cm) of silica gel (260 g) in a chloroform–methanol gradient system. On elution of the column with the above-mentioned system in a ratio of (97:3), fraction 2 yielded 0.2 g of 5,6-dihydroxy-7,8-dimethoxyflavone, fractions 11-17 – 0.3 g of 2′,5,6′-trihydroxy-6,7,8-trimethoxyflavone, fractions 24-25 – 0.1 g of syringic acid, and fractions 26-29 – 0.1 g of vanillic acid.

Oroxylin A (5,7-dihydroxy-6-methoxyflavone). $C_{16}H_{12}O_5$ (M^+ 284) with mp, 218-219°C (benzene–hexane), λ_{max}, nm: 249, 272, 321. PMR spectrum (Py-d_5), ppm: 3.85 (s, $-OCH_3$), 6.78 (s, H-3), 6.85 (s, H-8), 7.36 (m, H-3′, 4′, 5′), 7.83 (m, H-2′, 6′), 13.56 (br. s, 5-OH).

Wogonin (5,7-dihydroxy-8-methoxyflavone). $C_{16}H_{12}O_5$ (M^+ 284), mp, 202°C (benzene–hexane), λ_{max}, nm: 247, 277, 319 [6].

Norwogonin (5,7,8-trihydroxyflavone) $C_{15}H_{10}O_5$, mp, 250-252°C, (decom.), λ_{max}, nm: 280, 362; mass spectrum, *m/z* (%): M^+ 270(M^+), 178, 168, 119, 101, 86(100).

5,6-Dihydroxy-7,8-dimethoxyflavone (3). Orange-yellow crystals with the composition $C_{17}H_{14}O_6$, mp 182-184°C (from chloroform), λ_{max}, nm: 282, 319; ν_{max}, cm^{-1}: 3540-3280(OH), 1654 (γ-pyrone C=O), 1635, 1575 (C=C bonds PMR spectrum (Py-d_5), ppm: 3.92; 4.08 (s, each 2 × OCH_3), 6.88 (s, H-3), 7.39 (m, H-3′, 4′, 5′), 7.91 (m, H-2′, 6′), 13.08 (br.s, 5-OH). Mass spectrum, *m/z* (%): M^+ 314(56), 299 ($M-CH_3$)(100), 284(47), 269(78), 241(28), 202(25), 197(28), 164(44), 149(56), 105(56).

Diacetate (4) of (3). A solution of 20 mg of flavone (3) in 1 ml of pyridine was treated with 2 ml of acetic anhydride. After 3 h, the reaction mixture was worked up by the usual method, giving 16 mg of the diacetate, with mp 155-156°C. PMR spectrum ($CDCl_3$), ppm: 2.28 and 2.35(s, each, 2 × Ar–$OCOCH_3$), 3.98 and 4.00 (s, each, 2 × $-OCH_3$), 6.58 (s, H-3), 7.47 (m, H-3′, 4′, 5′), 7.83 (m, H-2′, 6′).

Monomethyl Ether (5) of (3). A solution of 5 mg of flavone (3) in 3 ml of anhydrous methanol was treated with an ethereal solution of diazomethane, and the mixture was left in the refrigerator. After a day, the solvent was distilled off, and more of the ethereal solution of diazomethane was added to the residue (3 × 3 ml). This led to a substance with the composition $C_{18}H_{16}O_6$ with mp 86-87°, λ_{max}, nm: 280, 320; mass spectrum *m/z* (%): M^+ 328(75), 313 ($M-CH_3$) (100), 299(8), 298(12), 283(18), 244(83), 135(92), 119(83), 109(96).

2′,5,6′-Trihydroxy-6,7,8-trimethoxyflavone. $C_{18}H_{16}O_8$, mp 242–244°C, λ_{max}, nm, 269, 315; ν_{max}, cm^{-1}: 3545-3275(OH), 1650 (γ-pyrone C=O), 1630, 1570, 1510 (C=C bond), Mass spectrum, *m/z* (%): 360 (M^+), 345 ($M-CH_3$) (100), 330, 315 and others. PMR spectrum (Py-d_5), ppm: 3.84; 3.86 and 3.92 (s, each, 3 × OCH_3), 6.69 (d, 8.0 Hz, H-3′, 5′), 6.83 (s, H-3), 7.20 (dd, 8.0 Hz, H-4′), 13.20 (br.s, 5-OH).

Vanillic Acid. $C_8H_8O_4$ (M^+ 168) mp, 212-213°, λ_{max}, nm: 261, 292. PMR spectrum (Py-d_5), ppm: 3.63 (s, $-OCH_3$), 7.19 (d 8.5 Hz, H-5), 7.97 (d, 2.0 Hz, H-2), 8.07 (dd, 2.0 and 8.5 Hz, H-6).

Syringic Acid. $C_9H_{10}O_5$ (M^+ 198), mp 202-203°C.

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