FLAVONE GLYCOSIDES OF *Rhodiola krylovii*

E. A. Krasnov and L. A. Demidenko

UDC 547.972

We have studied the chemical composition of a new species endemic to the Altai-Sayan mountain region — *Rhodiola krylovii* Polozh. et Revjak (Krylov's stonedrop), family Crassulaceae [1], collected in the Kosh-Agachsk region in the flowering phase. By two-dimensional PC and by TLC on silica gel, not less than five flavone glycosides, localized mainly in the epigeal organs of the plant, have been found in ethanolic extracts. To isolate them, the ground rhizomes were treated with boiling chloroform, and the flavonoids were extracted with 70% ethanol. The extracts were evaporated to an aqueous residue, which was treated with ether and with ethyl acetate. The dry ethereal and ethyl acetate extracts were separated on columns of polyamide. On elution with chloroform containing 10-15% of ethanol and rechromatography under similar conditions, four flavonoid compounds (1-4) were isolated:

Substance 1, composition $C_{22}H_{20}O_{12}$, mp 244-246°C, $[\alpha]_D$ +84.2° (c 0.25; CH_3OH), R_f 0.49 (PC on FN-4; here and below in the 60% CH_3COOH system).

Substance 2, composition $C_{25}H_{26}O_{15}$, mp 262-264°C, $[\alpha]_D$ +34.6° (c 0.13; CH_3OH), R_f 0.45.

Substance 3, composition $C_{21}H_{20}O_{11}$, mp 253-254°C, $[\alpha]_D$ −96° (c 0.2; CH_3OH), R_f 0.50.

Substance 4, composition $C_{25}H_{26}O_{15}$, mp 274-277°C, $[\alpha]_D$ −29° (c 0.36; CH_3OH), R_f 0.54.

On chromatograms, all the substances had a yellow-green fluorescence in UV light, and after treatment with a 1% ethanolic solution of $AlCl_3$ they acquired a green coloration, which shows the presence of a free hydroxy group at C_3 in each case. When these compounds were hydrolyzed with 2% HCl, they gave the same aglycone, with mp 288-289°C, which was identified as herbacetin (3,4',5,7,8-pentahydroxyflavone). When the hydrolysate was neutralized with $Ba(OH)_2$ and chromatographed on paper and in a thin layer of silica gel, L-arabinose (from substance 1), L-arabinose and D-xylose (from substance 2), L-rhamnose (from substance 3), and D-xylose (from substance 4) were identified. The gossypetin reaction with p-benzoquinone was negative for all the glycosides, which shows the substitution of the hydroxy group at C_8 of herbacetin.

The IR spectra of substances 2, 3, and 4 were similar and contained the following absorption bands (cm^{-1}): 3400-3200 (OH), 1662-1666 (C=O of a γ-pyrone), 1620, 1575, 1560, 1523 (aromatic nuclei), and 1007-1009 (O—C—O of a glycosidic unit), while substance 1 had additional bands at 1695 and 1725 cm^{-1} (C=O of an ester). The UV spectra of the compounds were characteristic for herbacetin glycosides.

On the basis of electronic spectra with ionizing and complex-forming reagents, in all the compounds we detected free hydroxy groups in positions 3, 5, and 7. In substances 1 and 3 the carbohydrate components were present at C_8, and in glycosides 2 and 4 at C_4' and C_8.

When substance 2 was subjected to stepwise hydrolysis with 0.5% HCl, herbacetin 8-arabinoside and herbacetin 4'-xyloside, separated by preparative paper chromatography in 15% CH_3COOH, were obtained.

On the basis of their physicochemical properties and direct comparisons with authentic samples, substance 1 was identified as acetylrhodalgin [4, 5] and substance 2 as rhodolide (herbacetin 8-α-L-arabinopyranoside 4'-O-β-D-xylopyranoside [5].

Substance 3 did not change under the action of β-glucosidase, and under mild conditions of acid hydrolysis [6] there was not appreciable cleavage. Thus, substance 3 has the structure of herbacetin 8-O-α-L-rhamnopyranoside.

The results of quantitative acid hydrolysis (yield of herbacetin 52.5%) and UV spectroscopy showed that substance 4 was a diglycoside in which the carbohydrate substituents were located at C_4' and C_8. The products of enzymatic hydrolysis with β-glucosidase and of

Tomsk Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 106-107, January-February, 1984. Original article submitted July 12, 1983.

0009-3130/84/2001-0103$08.50

acid hydrolysis contained two monosides, one of which was identified from its melting point and chromatographic characteristics as the intermediate product of the cleavage of substance 2 — herbacetin 4'-xyloside — while the other glycoside gave a negative gossypetin test, which indicated that the second xylose molecule was attached at position 8 of herbacetin.

The investigations performed permitted us to suggest for substance 4 the structure herbacetin 8-O-β-D-xylopyranoside 4'-O-β-D-xylopyranoside.

LITERATURE CITED

1. A. V. Polozhii and N. V. Revyakina, Nov. Sistem. Vyssh. Rast., 16, 125 (1979).
2. T. J. Mabry et al., The Systematic Identification of Flavonoids, Springer, New York (1970), p. 125.
3. J. B. Harborne et al., The Flavonoids, Springer, New York, (1975), p. 376.
4. T. T. Pangarova and G. G. Zapesochnaya, Khim. Prir. Soedin., 712 (1975).
5. E. A. Krasnov and L. A. Demidenko, Khim. Prir. Soedin., 404 (1979).
6. A. Hunger and T. Reichstein, Helv. Chim. Acta, 2 (1952).

CEREBROSIDES AND CEREBROSIDE SULFATES OF THE BRAIN OF THE HARP SEAL

S. G. Batrakov, V. B. Muratov, O. G. Sakandelidze, A. V. Sulima, and B. V. Rozynov

UDC 547.952:599.745.3

Galactocerebrosides (GCs) and galactocerebroside sulfates (GCSs) are among the main lipids of the plasmatic membranes of the nerve tissues of mammals, and the bulk of the GCs and GCSs are concentrated in the brain and spinal cord [1]. These lipids are of interest in many aspects and, in particular, from the point of view of their participation in the functioning of biological membranes [1]. Below we describe the results of an analysis of the GCs and GCSs of the brain of the harp seal *Phoca groenlandica* at the age of one month.

The comminuted brain was extracted repeatedly first with acetone and then with a mixture of chloroform and methanol (2:1). The extracts, which, according to TLC, contained GCs and GCSs, were subjected to alkaline methanolysis under mild conditions [2]. The lipophilic substances from the methanolysate were chromatographed on a column of DEAE-cellulose (AcO^- form). The $CHCl_3$—MeOH (9:1) system eluted neutral lipids, and then the column was washed with $CHCl_3$—MeOH (2:1), with acetic acid, and with methanol, after which the $CHCl_3$—MeOH (2:1) + 5% of concentrated aqueous ammonia system eluted a chromatographically homogeneous fraction of GCSs. The above-mentioned neutral lipids were chromatographed on a column of silica gel by a procedure described previously [2], which yielded two homographically homogeneous fractions of GCs — with residues of 2-hydroxy fatty acids (h-GC fraction) and with residues of unsubstituted fatty acids (u-GC fraction). The amount of GCSs and the total amount of GCs in the natural brain were 3.2 and 14.2 mg/g, respectively, which are fairly close to the amounts of the same lipids in the brain of many terrestrial mammals and the dolphin [1]. To determine carbohydrates, fatty acids, and bases in the structures of the h-GCs, the u-GCs, and the GCSs, these lipids were subjected to alkaline methanolysis under severe conditions [3]. The methanolysis products — methyl glycosides, sphingosine bases, and fatty acid methyl esters — were separated by the usual methods [3]. Only galactose was found in the carbohydrate fractions of all three methanolysates. The results of the periodate oxidation of the native glycolipids, the mass spectrometry of their trimethylsilyl derivative (see [4]), and the oxidation of the corresponding per-O-acetates by chromium trioxide [5] showed that the h-GCs, the u-GCs, and the GCSs consisted of β-galactopyranosides and that the sulfate groups in the GCSs were located at $C_{(3)}$ of the carbohydrate residue. The sphingosine bases were converted into N-2,4-dinitrophenyl derivatives, and these were analyzed in

Institute of Immunology, Ministry of Health of the USSR, Moscow. M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 107-108, January-February, 1984. Original article submitted August 27, 1983.

0009-3130/84/2001-0104$08.50