anhydrous sodium sulfate and evaporated. The residue was treated with 0.5 N hydrogen chloride in absolute methanol at 100°C for 7 h. The fatty acid methyl esters obtained were analyzed by GLC.

SUMMARY

1. Lipopolysaccharides the carbohydrate components of which consists of heteropolysaccharides have been isolated from the marine blue-green algae Oscillatoria hildebrandtii and Nostoc sp.

2. Rhamnose residues are included in the carbohydrate chain of the LPS from O. hildebrandtii by 1→3-bonds with substituents in the second position.

3. The rhamnose residues in the carbohydrate chain of the LPS from Nostoc sp. are linked by both 1→2-and by 1→3-glycosidic bonds.

4. The lipid components from O. hildebrandtii and Nostoc sp. are constructed of residues of palmatic and stearic acids and glucosamine and glucose.

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FLAVONOIDS OF THE EPIGEAL PART OF Rhodiola rosea.

II. STRUCTURES OF NEW GLYCOSIDES OF HERBACETIN AND OF GOSSYPETIN

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The structure of four new flavonol glycosides isolated from the epigeal part of Rhodiola rosea have been established: 7-O-α-L-rhamnopyranosylgossypetin (rhodiolgin), 8-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranosylgossypetin (rhodiolgidin), 8-O-β-D-glucopyranosol-7-O-α-L-rhamnopyranosylherbacetin (rhodionidin), and 3-O-β-D-glucopyranosyl-8-O-β-D-xylopyranosylherbacetin (rhodalidin). The properties of the previously undescribed incomplete methyl ethers of herbacetin and gossypetin obtained during the structural analysis of the glycosides have been studied. It has been found that diazomethane methylates the 5-OH groups in the diglycosides investigated.

We have previously [1] reported the isolation from the epigeal part of Rhodiola rosea L. (Sedum rosea, roseroot stonecrop), family Crassulaceae of rhodionin (V), rhodalin (VI), and four new flavonoid glycosides – rhodiolgin (I), rhodiolgidin (II), rhodionidin (III), and rhodalidin (IV). In the present paper we have given information on the determination of the structures of the new compounds (I-IV).

On the basis of their PMR spectra and the results of acid hydrolysis, compound (I) was assigned to the monoglycosides (rhamnoside) and compounds (II-IV) to the diglycosides, containing, respectively, rhamnose and glucose (II and III) and xylose and glucose (IV). The

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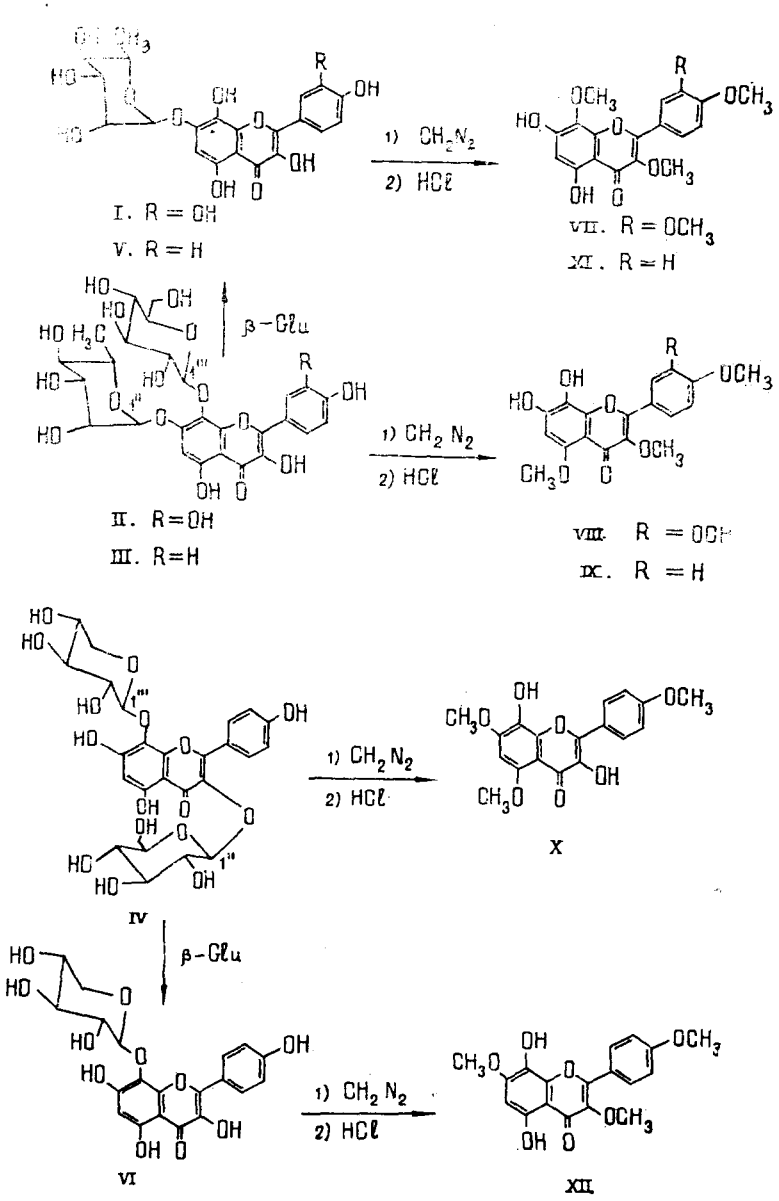
aglycone moiety of the molecules of (I) and (II) consisted of gossypetin (3,3',4',5,7,8-hexahydroxyflavone), while compounds (III) and (IV) were derivatives of herbacetin (3,4',5,7,8-pentahydroxyflavone)



Some reactions of the glycosides (I-VI)

A considerable complication in the structural analysis of these glycosides is the determination of the positions of the carbohydrate residues, since UV spectroscopy does not permit unambiguous conclusions to be drawn for herbacetin and gossypetin derivatives (Table 1) [2]. In view of this, we performed the methylation with diazomethane of all six glycosides (I-VI) and on subsequent acid hydrolysis obtained the corresponding incomplete methyl ethers (VII-XII, scheme). In the case of the diglycosides we could have expected that this would form dimethyl ethers of herbacetin and trimethyl ethers of gossypetin, respectively, but in all cases, even when methylation was repeated several times, compounds were obtained in which the phenolic hydroxyls, including the 5-OH group were completely methylated.

Structures of the Methyl Ethers of Herbacetin and Gossypetin

To establish the structures of the incomplete methyl ethers of herbacetin and gossypetin we used their PMR, UV, and mass spectra.

The PMR spectra confirmed the presence of the herbacetin or gossypetin skeleton and showed the presence of methoxy groups and their numbers, and also that of free 5-OH groups

TABLE 1. Maxima in the UV Spectra of the Herbacetin and Gossypetin Derivatives

Compound	MeOH	NaOMe	NaOAc	NaOAc+H_3BO_3	$AlCl_3$	$AlCl_3$+HCl
Rhodiolgin (I)	261, (278), 347, 388	275, 384*	262, (278), (347), 376	266, (354), 410	(264), 278, 476	274, 376, 450
Rhodiolgidin (II)	(262), 277, (305), 370	258, 368	275, (336), 390	268, (305), 388	284, (316), 456	284, (316), 360, (430)
Rhodionidin (III)	277, 308, 332, (370)	255, 370	278, 300, 332, (388)	277, 308, 332, (370)	243, 286, 321, 360, (436)	241, 286, 319, 352, (436)
Rhodalidin (IV)	273, (330), 360	281, (330), 410	281, (310), 388	280, (310), 374	281, 310, 355, 410	281, 310, 355, 410
Rhodionin (V)	277, 332, 386	(282), 308*	277, 332, 386	277, 332, 386	267, 285, 368, 448	267, 285, 368, 448
Rhodalin (VI)	(255), 273, (328), 374	280 (325) 420	280, (318), 396	278, (318), 382	274, (311), 357, 436	273, (311), 357, 436
5,7-Dihydroxy-3,3',4,4',8-tetramethoxyflavone (VII)	(256), 275, 340, (360)	285, (321), 392	285, (320) 380	(257), 277, 340, (360)	268, 283, 262, (416)	261, 285, 360, (415)
7,8-Dihydroxy-3,3',4',5-tetramethoxyflavone (VIII)	256, (271), 370	281, (321), 412	281, 324, 390	257, 275 287, (336), 436	267, (358), 433	265, (355), 432
7,8-Dihydroxy-3,4',5-trimethoxyflavone (IX)	(257), 270, (310) 368	280, (317), 408	279, (315), 385	271, 285, (335), 434	266, (348), 430	266, (348), 430
3,8-Dihydroxy-4',5,7-trimethoxyflavone (X)	220, 275, 325, 380	278, 325, 422*	275, 325, 380	275, 325, 380	226, 265, 285, 362, 440	226, 265, 285, 362, 440
5,8-Dihydroxy-3,4',7-trimethoxyflavone (XII)	(224), 275, (312), 360	284, (304), 394	284, (306), 385	276, (315), 360	232, 283, 313, 354, 419	231, 283, 312, 348, 418
	225, 279, 307, (330), 370	330*	279, 307, 370	279, 307, 370	225, 288, 324, 360, (445)	225, 288 324, 354, (430)

*Substance unstable. Inflections or shoulders are given in parentheses.

in compounds (VII), (XI), and (XII) and their alkylation in (VIII-X) (the spectra of these compounds lacked a signal at 12.4 ppm). Because of their poor solubility in benzene it was impossible to use PMR spectroscopy for determining the positions of the methoxy groups in compounds (VII-XII).

We then studied the features of the fragmentation of the molecular ions of substances (VII-XII) on electron impact (Table 2). In each of these compounds, M^+ was the main ion in the mass spectrum, with the exception of (VII) and (XI) for which the strongest peaks were those of the (M − 15) ions, which is characteristic for flavonols with an 8-OCH_3 group [3. 4]. The lateral-ring fragment (B_2) showed that in each of compounds (VII-XII) this ring contained only one or two methoxy groups and no free hydroxyls. The fragments of ring A had a low intensity and were uninformative.

It is interesting to note in the spectrum of each of these three compounds (VIII-X) having 5-OCH_3 groups the presence of the (M − 18) ion which produces the strong ions (M − 46) and (M − 61). It has been shown previously [5] that $(M - H_2O)^+$ and $(M - OH)^+$ peaks are formed by flavones with 5-OMe groups. The (M − 46) ions, with intensities of 30-40%, have been reported previously for the methyl and deuteromethyl ethers of chrysin, apigenin, and luteolin [4-7], and it has been shown [7] that it is mainly the CH_3O or CD_3O group at C-5 that participates in the formation of these ions. However, in not one of the cited papers [4-7] were (M − 46) peaks reported for flavonols, although a large series of compounds containing a CH_3O group at C-5 was studied: the complete and partial methyl or deuteromethyl ethers of galangin, of kaempferol, of quercetin, and of myricetin, 3,5,6,7,8-pentahydroxyflavone, and 3,4',5,6,7,8-hexahydroxyflavone. Only for 4',5,7-trimethylkaempferol, containing a single OH group at C-3, was the $(M - H_2O - CO)$ ion reported, and this was the main peak in the spectrum [5]. In our work we considered its 8-hydroxylated analog (X), in the spectrum of which this peak is the second in intensity (70%). It must be mentioned that the elimination from the (M − 46) ion of a CH_3 radical (and the formation of strong (M − 61 peaks) took place readily in two compounds (VIII and IX, Table 2) which contained CH_3O groups at C-3 and in which this process also took place to a considerable extent in the molecular ion. Thus, our results confirmed that the formation of the intense ions $(M - H_2O)$ $(M - CO - H)$, and $(M - H_2O - CO)$ is connected with the presence of 5-OMe group but in flavonols the presence of a hydroxy group in the molecule in position 3 or 8 is necessary for the formation of these ions.

The recording and analysis of the UV spectra was performed in accordance with [8]. The test with NaOAc readily characterized the presence of a 7-OMe groups in (X) and (XII) and of free 7-OH groups in the other compounds (see Table 1). The test with H_3BO_3 revealed the presence of o-7,8-dihydroxy groupings in two compounds (VIII and IX), the shift of the long-wave band (+66 nm) being considerably greater than when an o-dihydroxy grouping is present in the lateral ring (see, for example, (I) and (II), Table 1). Aluminum chloride does not react with a 7,8-dihydroxy grouping, and the spectra in its presence were identical with those obtained with $AlCl_3$ + HCl. The size of the shifts with $AlCl_3$ + HCl in comparison with the spectra in methanol were approximately the same for all the compounds, which did not permit a conclusion to be drawn concerning the presence of free 3- and 5-OH groups. The long-wave maximum in methanol likewise did not permit compounds with 3-OH groups to be singled out. With sodium methanolate, two compounds (X) and (XII) proved to be unstable, i.e., in addition to the 5,8-dihydroxy groupings reported previously [2] and additional factor for the instability of herbacetin derivatives has been found − a 3,8-dihydroxy groupings.

In sum, it was established by a combination of spectral characteristics that in the incomplete methyl ethers free hydroxy groups were located at C-5,7 (VII and XI), C-7,8 (VIII and IX), C-3,8 (X), and C-5,8 (XII), i.e., to them correspond the structures given in the scheme. The reaction with p-benzoquinone showed the presence of a free p-5,8-dihydroxy grouping only in (XII) and thereby confirmed its structure.

The methyl ethers (VII), (VIII), (IX), and (X) are new compounds; 3,4',8-trimethylherbacetin (XI) has been isolated from plants previously [9, 10]; and compound (XII) has been obtained in a study of other 8-glycosides of herbacetin [11].

Thus, the methylation of 7- and 8-monoglycosides of herbacetin and of gossypetin with diazomethane leaves a 5-hydroxy group unaffected. However, in the 7,8-diglycosides (II and III), as in the 3,8-diglycoside (IV), full methyl ethers are formed and the products obtained after hydrolysis contain 5-methoxy groups (compounds VIII-X). This confirms the

TABLE 2. Mass Numbers (m/z) of Characteristic Ions in the Mass Spectra of Incomplete Methyl Ethers of Herbacetin and Gossypetin (VII-XII) (in parentheses – relative intensities, %)

Ion	VII	VIII	IX	X	XI	XII
M^+	374 (73)	374 (100)	344 (100)	344 (100)	344 (70)	344 (100)
$(M-H)^+$	373 (11)	373 (7)	343 (6)	343 (11)	343 (12)	343 (50)
$(M-CH_3)^+$	359 (100)	359 (37)	329 (47)	329 (4)	329 (100)	—
$(M-OH)^+$	—	357 (4)	327 (6)	327 (3)		—
$(M-H_2O)^+$	—	356 (10)	326 (21)	326 (10)	—	—
$(M-CO-H)^+$	345 (4)	345 (9)	315 (9)	315 (8)	315 (4)	315 (4)
$(M-CH_3O)^+$	343 (4)	343 (5		313 (3)	—	—
$(M-CH_3-CO)^+$	331 (13)	331 (14)	301 (27)	301 (11)	301 (10)	301 (55)
$(M-H_2O-CO)^+$	—	328 (15)	298 (6)	298 (70)	—	—
$(M-H_2O-CO-CH_3)^+$	313 (3)	313 (38)	283 (50)	283 (3)	—	—
$(M-CH_3-2CO)^+$	303 (-)	303 (3)	273 (6)	273 (1)	273 (4)	—
$(A+H)^+$	—	—		197 (2)	183 (1)	183 (1)
A^+	—	182 (3)	182 (2)	—	—	—
$(A-CH_3)^+$	167 (4)	167 (3)	167 (3)	181 (3)	167 (4)	—
$(A-CH_3-CO)^+$	139 (8)	139 (4)	139 (3)	153 (6)	139 (7)	—
B^{2+}	165 (9)	165 (14)	135 (36)	135 (21)	135 (14)	135 (15)
M^{2+}	187 (5)	187 (13)	172 (14)	172 (10)	172 (4)	172 (3)

mutual influence of oxygen substituents at C-3,5,8 on the process of selective demethylation observed previously [12, p. 157] and also explains the fact that in plant sources flavonols with 5,7,8-oxysubstituents are present mainly in the form of 8- or 7-glycosides, while 3-glycosides are rarely found, 3-glycosylation being accompanied by the methylation or glycosylation of an 8-OH groups [13-17].

Structure of the Glycosides

Since a free 5-OH group was detected in the PMR spectra of the glycosides (I-VI) in all cases, the results of methylation permit the following conclusions to be drawn: in the first place, compounds (I) and (V) were, respectively, gossypetin 7-rhamnoside and herbacetin 7-rhamnoside, and (VI) was herbacetin 8-xyloside; in the second place, the diglycosides contained carbohydrate residues in positions 7 and 8 (II and III) or 3 and 8 (IV).

The pyranoid form of the carbohydrate rings, their conformations, and the types of glycosidic bonds were determined on the basis of the PMR spectra of the glycosides and their full acetates [18, 19] with the aid of polarimetric analysis. According to this, to rhodolgin corresponded the structure of gossypetin 7-O-α-L-rhamnopyranoside (I), and to rhodionin the analogous herbacetin structure (V) [2], while rhodalin was herbacetin 8-O-β-D-xylopyranoside [20] (see scheme).

The fact that substances (II-IV) were diglycosides was confirmed by the results of their

acetylation, since in the acetates it was possible with the aid of PMR to differentiate aromatic acetoxy groups, and the presence of seven signals of aliphatic CH_3COO groups excluded bioside structures. For the concrete assignment of the carbohydrate residues, the diglycosides were subjected to partial enzymatic hydrolysis with β-glucosidase. In this way, rhodiolgin (I) was obtained from rhodiolgidin (II), rhodionin (V) from rhodionidin (III), and rhodalin (VI) from rhodalidin (IV). These results made the assignment of the second carbohydrate residue (glucose) to position 8 in (II) and (III) and to position 3 in (IV) unambiguous. According to the results of enzymatic hydrolysis, and also on the basis of the PMR spectra of the native glycosides and their acetates (the CSs and SSCCs of the carbohydrate protons), the glucose residues in the diglycosides were represented by β-D-glucopyranose. All the facts given above permit the conclusion that rhodiolgidin, rhodionidin, and rhodaligin are represented by structures (II), (III), and (IV) shown in the scheme.

Analysis of the UV spectra (Table 1) of the glycosides (I-VI) permitted only a few clear conclusions to be drawn: the presence of free 7-OH-group in (IV) and (VI) and its glycosylation in the other compounds (test with NaOAc), and also the presence of a 3',4'-dihydroxy grouping in (I) and (II) (spectrum with H_3BO_3 and $AlCl_3$ + HCl). The long-wave maxima in methanol for compounds (I-VI) are located in a wide range of wavelengths (from 332 to 388 nm), whicm makes the singling out of compounds with a free 3-OH group ambiguous. For example, the simultaneous glycosylation of 7- and 8-OH groups (compounds II and III) has a very pronounced effect on the overall electronic state of the molecules and, although they each contain a 3-OH group, their long-wave maxima are located at 350 and 332 nm, respectively, while (III) has only an inflection of low intensity at 370 nm. In connection with this, the test with $AlCl_3$ + HCl, in comparison with methanol, also gave different shifts: +62 nm (I, V, VI), +50 nm (IV), and +20 nm (II and III), although, with the exception of (IV), they all contained free OH groups at C-3 and C-5.

With sodium methanolate, only the 7-glycosides (I and V) decomposed. Thus, the instability of this group of compounds (test with NaOMe) is connected not with the 3,4'-dihydroxy grouping, which is present in the majority of the glycosides (I-III, V, and VI), but is due to the presence of hydroxy groups in positions 5 and 8 (compounds I, V, and XII) or with a free 3,8-dihydroxy grouping (compound X). It must be stated that substances unstable with sodium methanolate (I, V, X, and XII) also decompose rapidly on Silufol plates, and spraying with aluminum chloride accelerates this process.

The Wessely–Moser rearrangement [21] for 5,7,8-trihydroxyflavonoids, leading under the conditions of acid hydrolysis to the 5,6,7-trihydroxy isomers, has been mentioned in the literature repeatedly. This spontaneous cyclic isomerization has been observed for the 7- and 8-glycosides of such flavones as hypolaetin, isoscutellarein, etc. [22-26]. In the flavonol series this isomerization requires more severe conditions [27], but, in view of the many anomalous properties of herbacetin, we considered it desirable to check their stability. In the present work, as also for ten 7- and 8-glycosides of herbacetin and gossypetin isolated previously [2, 11, 20, 28], the isomerization mentioned was not observed, although each of the compounds investigated contained a 5,7,8-trihydroxy grouping and in our work we subjected them to acid hydrolysis and to methylation with subsequent acid hydrolysis.

In the course of the study of the chemical composition of the epigeal part and rhizomes of roseroot stonecrop we have isolated 15 flavonoids, but only one glycoside – rhodionin – is common to all parts of the plant.

The glycosides (I-VI) considered in the present work were present in approximately equal amounts in wild-growing and cultivated specimens of the plant raw material.

In the chemotaxonomic aspect, the simultaneous presence in roseroot stonecrop of herbacetin and gossypetin derivatives is interesting, these aglycones (in the free form) having been found previously in only one representative of the family Crassulaceae – Sedum album [29]. It must be mentioned that in the recent literature information has been appearing ever more frequently on the simultaneous presence of these flavonoids in plants [16, 17], and in a number of cases structures previously assumed to be of the quercetagetin type have proved to be gossypetin derivatives [29]. The preferential nature of 8-hydroxylation and the considerable wider distribution in nature of such compounds as compared with the 6-hydroxyflavonoids [12, p. 350] may, in Harborne's opinion [29], reflect the greater tendency of position 8 of a flavonol to undergo anionic attack in the living cell.

The difficulty of differentiating them in the native form, the Wessely–Moser isomerization accompanying the acid hydrolysis of flavone glycosides, the instability of flavonol 7-glycosides and the experimental difficulties in isolation connected with this, the high reactivity of the 8-OH group of the 3,5,7,8-tetrahydroxy grouping, and a number of anomalous spectral properties reported previously [2] and given in the present paper – this is a far from complete list of the reasons why 8-hydroxyflavonoids could be taken erroneously for 6-hydroxyflavonoids.

EXPERIMENTAL

Spectral characteristics were obtained on the following instruments: Varian HA-100D at 100 MHz with tetramethylsilane as internal standard (PMR); Varian CH-8 at 70 eV (mass spectra); and Hitachi EPS-3T (UV spectra). Melting points were determined on a Kofler block. Angles of rotation were measured on a A-I EPL polarimeter. Chromatographic monitoring was performed by TLC (Silufol UV 254) in the chloroform–methanol (6:1 and 9:1) and chloroform–methanol–water (26:14:3) systems, and PC (for the identification of sugars) in the butanol–acetic acid–water (4:1:2), butanol–pyridine–water (6:4:3), and water saturated phenol systems.

Isolation. The fresh herbage of roseroot stonecrop in the flowering phase, the flowers, and the air-dry inflorescences in the fruit-bearing phase collected in the Kurchum region, East Kazakhstan province in August, 1981, and also the analogous organs of roseroot stonecrop grown under the conditions of Moscow province (time of gathering, May-August, 1981 and 1983) were extracted with methanol at room-temerature. The methanolic extracts (separately from each plant specimen) were evaporated in vacuum and chromatographed on polyamide using mixtures of water and ethanol. Compounds (II) and (III) were isolated from the evaporated aqueous eluates of all the extracts after repeated chromatographic purification on polyamide and silica gel using mixtures of chloroform and methanol (Table 3). Under similar conditions, compound (IV) was obtained from the 15-20% ethanolic eluates (extract of the herbage) (Table 3). The 50% ethanolic eluates of the flower and inflorescences contained the monoglycosides (I) and (V), which is was possible to purify by recrystallization (Table 3) without using chromatography on silica gel where they very rapidly decomposed. Glycoside (VI) was stable under these conditions and was isolated from the herbage and the inflorescences.

Rhodiolgin (I). Yellowish green crystals from aqueous acetone with the composition $C_{21}H_{20}O_{12}$, mp 176-178°C; $[\alpha]_D^{20}$ —11,3° (*c* 0,19) ; ethanol). UV spectra – see Table 1. PMR spectrum in $(CD_3)_2CO$, ppm: 11.58 (s, 5-OH); 7.91 (d, 2.5 Hz, H-2'); 7.80 (dd, 2.5 and 9 Hz, H-6'); 7.01 (d, 9 Hz, H-5'); 6.67 (s, H-6); 5.63 (br.s, H-1"); 4.15 (dd, 2 and 3 Hz, H-2"); 3.98 (dd, 3 and 10 Hz, H-3"); 3.8-3.5 (m, H-4",5"); 1.26 (d, 6 Hz, CH_3 of rhamnose).

Octaacetate of (I). mp 169-173°C; fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.60 (br. s, H-1"); 5.46 (br.s, H-2"); 5.36 (dd, 3 and 10 Hz, H-3"); 5.19 (t, 10 Hz, H-4"); 3.94 (dd, 6 and 10 Hz, H-5"); 2.50, 2.42, 2.36 (9 H) (the singlets of five aromatic CH_3COO groups); 2.19, 2.08, 2.04 (the singlets of three aliphatic CH_3COO groups); 1.19 (d, 6 Hz, CH_3 of rhamnose); in C_6D_6: 5.72 (H-3"); 5.66 (br.s, H-2"); 5.49 (H-4"); 5.19 (br.s, H-1"); 4.08 (H-5"); 2.46, 2.26, 2.04, 1.80 (9 H); 1.72, 1.63 (singlets of eight CH_3COO groups); 1.12 (CH_3 of rhamnose).

5,7-Dihydroxy-3,3',4',8-tetramethoxyflavone (VII). This was obtained by the methylation of (I) followed by acid hydrolysis. Light yellow crystals with the composition $C_{19}H_{18}O_8$, mp 227-229°C. UV spectra – Table 1. Mass spectrum at 160°C – Table 2. PMR spectrum in $CDCl_3$ (60°C), ppm: 12.4 (s, 5-OH); 7.82 (d, 2.5 Hz, H-2'); 7.80 (dd, 2.5 and 9 Hz, H-6'); 7.00 (d, 9 Hz, H-5'); 6.39 (s, H-6); 4.00, 3.97 (6 H), 3.90 (the singlets of four aromatic CH_3O groups).

Rhodiolgidin (II). Yellow crystals from ethanol with the composition $C_{27}H_{30}O_{17}$, mp 194-197°C (decomp), $[\alpha]_D^{20}$—30° (*c* 0,09 ; ethanol). UV spectra – see Table 1. PMR spectrum in C_5D_5N (ppm): 8.49 (d, 2.5 Hz, H-2'); 8.07 (dd, 2.5 and 9 Hz, H-6'); 7.18 (d, H-5'); 7.18 (s, H-6); 6.30 (br.s, H-1"); 6.11 (d, 7 Hz, H-1"'); 4.6-3.8 (m, 10 H of the carbohydrate moiety); 1.52 (d, 6 Hz, CH_3 of rhamnose).

Undecaacetate of (II). mp 145-148°C; fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.6-4.9 (m, 8 H); 4.1-3.5 (m, 4 H); 2.52, 2.45, 2.36, 2.34 (singlets of four aromatic CH_3COO groups); 2.21, 2.14, 2.09, 2.04 (6 H), 2.00, 1.93 (seven aliphatic CH_3COO groups); 1.23 (d, 6 Hz, CH_3 of rhamnose); in C_6D_6: 5.9-5.1 (m, 8 H), 4.1-2.9 (m, 4 H); 2.46, 2.33, 1.95 (6 H),

TABLE 3. Scheme for the Isolation of the Glycosides from the Plant Samples

Plant organ	Polyamide, water–ethanol	Polyamide, chl–MeOH	Silica gel, chl–MeOH	Solvent for recrystallization	Glycoside isolated (yield, %)
Flowers	100:0 →	80:20 →	80:20 →	— — — — — —	→ III (0.05)
		75:25, 70:30 →	75:25, 70:30 →	— — — — — —	→ II (0,05)
	50:50 →	91:9 →	— — — →	water–acetone (4:1)	→ V (0,1)
		88:12 →	— — — →	water–acetone (3:1)	→ I (0.1)
Herbage	100:0 →	80:20 →	80:20 →	— — — — —	→ III (0,001)
		75:25, 70:30 →	75:25, 70:30 →	— — — — — —	→ II (0,001)
	85:15, 80:20 →	85:15, 80:20 →	85:15, 80:20 →	— — — — — —	→ IV (0.01)
	50:50 →	93:7 →	93:7 →	— — — — — —	→ VI (0.01)
Inflorescence	100:0 →	80:20 →	80:20 →	— — — — — —	→ III (0.01)
		75:25, 70:30 →	75:25, 70:30 →	— — — — — —	→ II (0.01)
	50:50 →	93:7 →	93:7 →	— — — — — —	→ VI (0,05)
		91:9 →	— — — →	water–acetone (4:1)	→ V (0,1)
		88:12 →	— — — →	water–acetone (3:1)	→ I (0,1)

1.81 (6 H), 1.74 (9 H), 1.66, 1.64 (singlets of eleven CH_3COO groups); 1.13 (d, 6 Hz, CH_3 of rhamnose).

7,8-Dihydroxy-3,3',4',5-tetramethyoxyflavone (VIII). This was obtained from (II) by methylation followed by hydrolysis. Light yellow cyrstals with the composition $C_{19}H_{18}O_8$, mp 251-253°C. UV spectra – see Table 1. Mass spectrum at 160°C – see Table 2. PMR spectrum in $CDCl_3$ (ppm): 7.89 (d, 2.5 Hz, H-2'); 7.84 (dd, 2.5 and 9 Hz, H-6'); 7.32 (d, 9 Hz, H-5'); 6.67 (s, H-6); 4.05, 3.95 (9 H) (singlets of four aromatic CH_3O groups); no 5-OH signal.

Rhodionidin (III). Yellow crystals with the composition $C_{27}H_{30}O_{16}$, mp 209-211°C (water), $[\alpha]_D^{20}$ −32° (*c* 0,1; ethanol). UV spectra – see Table 1. PMR spectrum in $(CD_3)_2CO$ + 5 drops of C_5D_5N (ppm): 11.6 (s, 5-OH); 8.20 (d, 9 Hz, H-2',6'); 6.94 (d, 9 Hz, H-3',5'); 6.76 (s, H-6); 5.73 (br.s, H-1''); 5.36 (d, 7 Hz, H-1'''); 4.6-3.3 (m, 10 H of the carbohydrate moiety); 1.26 (d, 6 Hz, CH_3 of rhamnose).

The Decaacetate of (III). mp 202-204°C. Fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.56-4.94 (8 H); 4.0-3.48 (4 H); 2.50, 2.44, 2.32 (3 aromatic CH_3COO groups); 2.20, 2.12, 2.08, 2.03, 2.01, 1.99, 1.82 (seven aliphatic CH_3COO groups); 1.12 (d, 6 Hz, CH_3 of rhamnose); in C_6D_6: 5.9-5.1 (m, 8 H); 4.2-2.9 (m, 4 H); 2.32 (6 H); 1.94, 1.78, 1.75, 1.72, 1.69 (6 H), 1.64, 1.60 (singlets of 10 CH_3COO groups); 1.09 (d, 6 Hz, CH_3 of rhamnose).

7-8-Dihydroxy-3,4'-5-trimethyoxyflavone (IX). This was obtained from (III) by methylation followed by hydrolysis. Light yellow crystals with the composition $C_{18}H_{16}O_7$, mp 298-301°C. UV spectra – see Table 1. Mass spectrum at 142°C – see Table 2. PMR spectrum in C_5D_5N (ppm): 8.58 (d, 9 Hz, H-2,6'); 7.14 (d, 9 Hz, H-3',5'); 6.70 (s, H-6); 3.95, 3.84, 3.76 (singlets of three aromatic CH_3CO groups); fragment of the PMR spectrum in DMSO: 8.13 (d, 9 Hz, H-2',6'); 7.16 (d, 9 Hz, H-3',5'); 6.46 (s, H-6); no 5-OH signal.

Rhodalidin (IV). Yellow crystals with the composition $C_{26}H_{28}O_{16}$, mp 242-245°C (aqueous acetone), $[\alpha]_D^{20}$ +48° (*c* 0,09; ethanol). UV spectra – see Table 1. PMR spectrum in C_5D_5N (ppm): 8.95 (d, 9 Hz, H-2',6'); 7.26 (d, 9 Hz, H-3',5'); 6.72 s, H-6); 6.32 (d, 8 Hz, H-1''); 5.22 (d, 7 Hz, H-1'''); 4.8-3.7 (m, 10 H of the carbohydrate moiety).

The Decaacetate of (IV). mp 114-117°C; fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.6-4.9 (m, 8 H of sugar residues) 4.24 (dd, 5 and 12 Hz, H-$5_e'''$); 4.0-3.5 (m, 3 H); 3.39 (dd, 8 and 12 Hz, H-$5_a'''$) 2.43, 2.37, 2.33, (three aromatic CH_3COO groups); 2.12, 2.06, 2.05, 2.02, 2.00, 1.92, 1.89 (seven aliphatic CH_3COO groups); in C_6D_6: 5.8-4.8 (m, 8 H of sugar residues); 4.2-3.0 (m, 5 H of sugar residues); 2.30. 2.02, 1.94, 1.80, 1.77, 1.67 (15 H) (ten CH_3COO groups).

3,8-Dihydroxy-4',5,7-trimethoxyflavone (X). This was obtained by the methylation of (IV) followed by acid hydrolysis. Greenish yellow crystals with the composition $C_{18}H_{16}O_7$,

mp 184-186°C – see Table 2. PMR spectrum in $CDCl_3$ (ppm): 8.25 (d, 9 Hz, H-2',6'); 7.02 (d, 9 Hz, H-3',5'); 6.44 (s, H-6); 4.04, 3.99, 389 (singlets of three aromatic CH_3O groups); no signal of 5-OH.

Rhodionin (V). Yellow needles from aqueous acetone with mp 232-235°C (decomp.), $[\alpha]_D^{20}$ — 150° (*c* 0,2; ethanol). PMR spectrum in $(CD_3)_2CO$ (ppm); 11.6 (s, 5-OH); 8.2 (d, 9 Hz, H-2',6'); 7.0 (d, 9 Hz, H-3',5'); 6.7 (s, H-6); 5.63 (d, 2 Hz, H-1"); 4.3 (dd, 2 and 3 Hz, H-2"); 4.00 (dd, 3 and 10 Hz, H-3"); 3.8-3.5 (m, H-4",5"); 1.3 (d, 6 Hz, CH_3 of rhamnose).

Pentaacetate of (V). mp 232-233°C. PMR spectrum in $CDCl_3$ (ppm): 7.80 (d, 9 Hz, H-2',6'); 7.25 (d, 9 Hz, H-3',5'); 6.97 (s, H-6); 5.57 (br.s, H-1"); 5.46 (br.s, H-2"); 5.38 (dd, 3 and 10 Hz, H-3"); 5.18 (t, 10 Hz, H-4"); 3.90 (dd, 6 and 10 Hz, H-5"); 2.44, 2.36, 2.28, 2.25 (four aromatic CH_3COO groups); 2.13, 2.02, 1.97 (three aliphatic CH_3COO groups); 1.18 (d, 6 Hz, CH_3 of rhamnose).

5,7-Dihydroxy-3'4',8-trimethoxyflavone (XI). This was obtained from rhodionin (V). Light yellow crystals with the composition $C_{18}H_{16}O_7$, mp 173-174°C. UV spectra – see Table 1. Mass spectrum at 110°C – see Table 2. PMR spectrum in $CDCl_3$ (ppm): 12.4 (s, 5-OH); 8.12 (d, 9 Hz, H-2',6'); 7.04 (d, 9 Hz, H-3',5'); 6.42 (s, H-6); 4.00, 3.91, 3.88 (singlets of three aromatic CH_3O groups).

Rhodalin (VI). Yellow cyrstals from methanol, mp 261-264°C, PMR spectrum in C_5D_5N (ppm): 9.03 (d, 9 Hz, H-2',6'); 7.43 (d, Hz, H-3',5'); 6.77 (s, H-6); 5.3 (d, 7 Hz, H-1"); 4.6-3.6 (m, 5 H of a xylose residue).

Heptaacetate of (VI). mp 114-115°C. PMR spectrum in $CDCl_3$ (ppm): 7.85 (d, 9 Hz, H-2', 6'); 7.25 (d, 9 Hz, H-3',5'); 6.80 (s, H-6); 5.3-4.8 (4 H of a xylose residue); 4.18 (dd, 5 and 13 Hz, H-$5''_a$); 3.30 (dd, 7 and 13 Hz, H-$5''_e$); 2.40, 2.37, 2.32, 2.30 (four aromatic CH_3COO groups); 1.99 (6 H), 1.86 (three aliphatic CH_3COO groups).

5,8-Dihydroxy-3,4',7-trimethoxyflavone (XII). This was obtained from (VI). Light yellow crystals from ethanol, mp 202-204°C. UV spectra and mass spectrum – see Tables 1 and 2. PMR spectrum in $CDCl_3$ (ppm): 12.3 (s, 5-OH); 8.10 (d, 9 Hz, H-2'.6'); 6.90 (d, 9 Hz, H-3',5') 6.35 (s, H-6); 3.90, 3.82, 3.80 (singlets of three aromatic CH_3O groups).

Acid-Hydrolysis. The hydrolysis of 2-3 mg of each of compounds (I–VI) was carried out in 2 ml of 2% HCl at 100°C for 20-30 min. By PC and TLC, rhamnose was detected in the hydrolysates of compounds (I) and (V), rhamnose and glucose in those of (II) and (III), xylose and glucose in that of (IV), and xylose in that of (VI).

The aglycone of compounds (I) and (II) was identified as gossypetin (M^+ 318 and characteristic fragments with m/z 168 and 137), and (III-VI) yielded herbacetin (M^+ 302, and fragments with m/z 168 and 121), as was confirmed by UV and PMR spectroscopy and comparison with authentic samples.

Enzymatic Hydrolysis. A solution of 5 mg of compound (II) (or (III) or (IV)) in 2 ml of water was treated with 1 mg of β-glucosidase, and the mixture was left at 38°C for 24 h. Then the hydrolysate was passed through a column of polyamide (8 × 20 mm), which was washed with 5 ml of water, after which 10 ml of ethanol eluted the intermediate monoglycoside (I) (III) and (IV) gave (V) and (VI), respectively). The monoglycosides obtained were identified with the aid of acid hydrolysis, UV spectroscopy, and direct comparison with the native glycosides (I), (V), and (VI).

Methylation. A solution of 20 mg of (I) (or (II-VI)) in 2 ml of methanol (or acetone) was mixed with an excess of an ethereal solution of diazomethane and the mixture was left for 48 h. After the solvent had been driven off, the residue was heated with 2 ml of 5% HCl for 30 min. The precipitate tnat separated after cooling was separated off and chromatographed on silica gel with petroleum ether – chloroform (0→100%). The final purification of the substance was carried out by recrystallization from ethanol. This gave the methyl ethers (VII-XII), respectively.

The acetylation of glycosides (I-VI) was performed with acetic anhydride in the presence of pyridine (20°C for 24 h), the reaction mixture was poured into ice water, and the precipitate was washed with water and recrystallized from ethanol.

Qualitative Reactions. 1) Gossypetone test: an ethanolic solution of one of compounds (I-XII) was mixed with an ethanolic solution of freshly sublimed p-benzoquinone. The solutions of substances (I), (V), and (XII) acquired a red-brown coloration, and the other

substances did not react (the solution remained yellow).

2) Coloration with a 1% ethanolic solution of $AlCl_3$ on Silufol plates with heating. At the moment of visualization all the substances acquired a bright yellow color; after 2-3 h the spot of rhodionin (V) had become blue, those of rhodiolgin (I) and compound (XII) green, and that of compound (X) violet. The spots of the other substances retained their yellow color.

SUMMARY

Four new flavonol glycosides have been isolated from the epigeal part of Rhodiola rosea, and the following structures are proposed for them: 3,3',4'5,8-pentahydroxy-7-α-L-rhamnopyranosyloxyflavone (rhodiolgin), 8-β-D-glucopyranosyloxy-3,3',4',5-tetrahydroxy-7-α-L-rhamnopyranosyloxyflavone (rhodiolgidin); 8-β-D-glucopyranosyloxy-3,4',5-trihydroxy-7-α-L-rhamnopyranosyloxyflavone (rhodionidin), and 3-β-D-glucopyranosyloxy-4',5,7-trihydroxy-8-β-D-xylopyranosyloxyflavone (rhodalidin).

The properties of the previously undescribed incomplete methyl ethers of herbacetin and gossypetin obtained during the structural analysis of the glycosides have been studied. Using the compounds studied as examples, several laws of the methylation with diazomethane of the 5-OH group in the flavone series have been revealed.

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