THE FLAVONOIDS OF THE RHIZOMES OF *Rhodiola rosea*.
II. A FLAVONOLIGNAN AND GLYCOSIDES OF HERBACETIN

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Three new derivatives of herbacetin (3,4',5,7,8-pentahydroxyflavone) have been isolated from the rhizomes of roseroot sedum for the first time. Conclusions concerning their structures have been drawn on the basis of chemical transformations and UV, PMR, and mass spectra. The structure of herbacetin 7-O-α-rhamnopyranoside is proposed for rhodionin (I). Rhodiosin (II) has the structure of herbacetin 7-O-(3''-O-β-D-glucopyranosyl-α-L-rhamnopyranoside). The biose of which it contains a residue, which has been called rhodiose, is the first example of a 3-O-β-D-glucopyranosyl-L-rhamnopyranose residue to be found in natural flavonoid glycosides. A probable structure is put forward for the flavonolignan rhodiolin (III) — the product of the oxidative coupling of coniferyl alcohol with the 7,8-dihydroxy grouping of herbacetin.

The further study of the flavonoids of an ethanolic extract of the rhizomes of *Rhodiola rosea* L. (roseroot sedum) [1] has led to the isolation of minor amounts of three new derivatives of herbacetin, which we propose to call rhodionin (I), rhodiosin (II), and rhodiolin (III).

All three compounds give the color reactions of flavonoids and belong to the flavonols with a free 3-OH group according to the qualitative reaction with zirconyl chloride and citric acid [2], their UV spectra (maxima in methanol at 382 or 386 nm) and their bright yellow fluorescence in UV light; each of the three substances also contains a 5-OH group (PMR and UV spectra).

Rhodionin (I) and rhodiosin (II) were readily hydrolyzed by acid (2% HCl, 20 min), and gave the same aglycone, which was identified as herbacetin (3,4',5,7,8-pentahydroxyflavone) (IV); the carbohydrate moiety in (I) consists of rhamnose and in (II) of rhamnose and glucose. On the hydrolysis of (I) and (II), as on the hydrolysis of herbacetin 8-glycoside [3], no migration of the 8-OH group into position 6, such as is known for 5,7,8-hydroxy flavonoids [4], took place.

The qualitative reaction with p-benzoquinone (gossypetone test) indicated the presence of a 5,8-dihydroxy grouping in (I) and (II) and their aglycone. The UV spectra of (I) and (II) with all reagents were identical (Table 1), i.e., the natures of the substitution of herbacetin in (I) and (II) are the same and the position of glycosylation is the 7-OH group (absence of a bathochromic shift of band II with sodium acetate). Consequently, (I) is a herbacetin 7-rhamnoside and (I) is a herbacetin 7-glucorhamnoside. The PMR spectra (Figs. 1a and 2a) confirm the structure of herbacetin 7-O-α-L-rhamnopyranoside for rhodionin: The multiplicities, SSCCs, and CSs of the protons of the sugar residue in (I) and its acetate correspond to the $^1C_4$ conformer of α-L-rhamnopyranoside [5]. The specific rotation also corresponds to the α anomer.

Compound (II) is a bioside as was shown not only by the UV spectra but also by the NMR spectrum of the acetate in deuterochloroform, which contained the signals of four phenolic and six alcoholic CH_3COO groups.

In the bioside (II) the glycosidic bond was cleaved more readily than the bond between the sugars. Controlled mild acid hydrolysis (0.05% HCl) led to herbacetin (IV) and to a single carbohydrate component — a biose which we have called rhodiose, which was cleaved completely by 2% HCl and partially by 0.5% HCl into glucose and rhamnose. In no case was the formation of an intermediate flavonoid monoglycoside observed. It must be mentioned that

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TABLE 1. Maxima in the UV Spectra of Herbacetin Derivatives

Compound	MeOH	NaOMe	NaOAc	NaOAc+H_3BO_3	$AlCl_3$	$AlCl_3$+HCl
Rhodionin (I)	225, 248, 277, 333, 386	247, 277, 398*	277, 333, 388	277, 335, 385	267, 285, 370, 447	265, 285, 366, 447
Rhodiosin (II)	225, 248, 277, 333, 386	245, 275, 398*	277, 332, 390	277, 333, 386	266, 285, 370, 448	266, 285, 370, 448
Rhodiolin (III)	230, 260, 281, 333, 382	262, 275, 295, 434	280, 335, 393	280, 333, 382	287, 368, 444	287, 368, 444
Herbacetin (IV)	226, 278, 330, 385	280, 390, 670*	258, 280, 370, 580*	260, 315, 375, 470*	260, 288, 368, 460	270, 282, 364, 444
Rhodalgin (herbacetin 8-O-arabinoside) [3]	225, 272, 327, 376	282, 326, 418	280, 318, 396	278, 318, 384	225, 273, 312, 359, 436	225, 273, 312, 359, 436
3,7-Dimethylherbacetin [11]	225, 278, 307, 328, 372	240, 388*	276, 305, 326, 380	276, 306, 326, 367	240, 287, 320, 358, 443	238, 286, 319, 351, 434

*Substance unstable.

in the chromatographic system 1 that is frequently used (see the Experimental part) it is practically impossible to distinguish rhodiose from glucose.

Attempts to obtain a monoglycoside from the bioside (II) by enzymatic hydrolysis with β-glucosidase did not lead to the desired result — the substance did not hydrolyze. Partial hydrolysis was achieved by heating (II) with formic acid in cyclohexanol, and the resulting monoglycoside contained rhamnose and was identical with compound (I) according to UV spectra, the products of acid hydrolysis, chromatographic mobility, and qualitative reactions. Thus, the sequence of attachment of the fragments in the rhodiosin molecule has been established; herbacetin—rhamnose—glucose.

The method of attaching the glucose was determined with the aid of PMR spectroscopy. Datiscin (2',3,5,7-tetrahydroxyflavone 3-rutinoside) [6] was used as the comparison sample. When the spectra of glycosides (I), (II), and datiscin in acetone were compared, it was not difficult to observed that the CSs of the signals of the aromatic protons of the rhamnose coincided for (I) and (II) (5.7 ppm) and differed sharply from that for datiscin (4.7 ppm). In datiscin, the signal of the anomeric proton of the glucose is located at 5.17 ppm, and in rhodiosin at 4.7 ppm. These results may serve as a conformation of the fact that in the molecule of (II) it is the rhamnose, and not the glucose, residue that is attached directly to the flavonoid nucleus.

In the PMR spectra of the full acetates of these compounds (Fig. 2), the CSs of the 2 H-6 signals of the glucose residues [in (II) — 4.18 ppm] show that this group is acetylated in rhodiosin and not in datiscin. In the same region of the spectra of the acetate of (II), in addition to the H-5 signals of a rhamnose residue that is common for all three compounds, a signal appears (a doublet of doublets with SSCCs of 3 and 10 Hz), which belongs to the proton at C-3 of the rhamnose residue. The presence of this signal at 4.3 ppm is evidence that the hydroxyl at C-3 of the rhamnose residue is not acetylated and, consequently, it is to this position that the glucose residue is attached, and the SSCCs characterize it as a β-D-glucopyranose residue in the $^4C_1$ conformation.

Thus, rhodiose has the structure of 3-O-β-D-glucopyranosyl-L-rhamnopyranose, and rhodiosin (II) is herbacetin 7-O-α-rhodioside and has the structure shown in Fig. 1b.

An isomer of rhodiose, scillabiose (4-O-β-D-glucopyranosyl-L-rhamnopyranose) is present in the cardiac glycoside scillaren A [7]. No substances containing a glucosylrhamnose residue have been found among natural flavonoid glycosides, although three isomers of rhamnosylglucose are known: the widely distributed rutinose (6-O-α-L-rhamnosyl-D-glucose), neohesperidose (2-O-α-L-rhamnosyl-D-glucose), and rungiose (3-O-α-L-rhamnosyl-D-glucose).

It must be mentioned that herbacetin (IV) is an unusual flavonoid in many of its properties and the determination of the position of glycosylation of its glycosides solely on the basis of UV spectra (see Table 1) may lead to errors. Thus it is known [8] that the instability of the substance on the addition of sodium methanolate is diagnostic for a 3,4'-di-

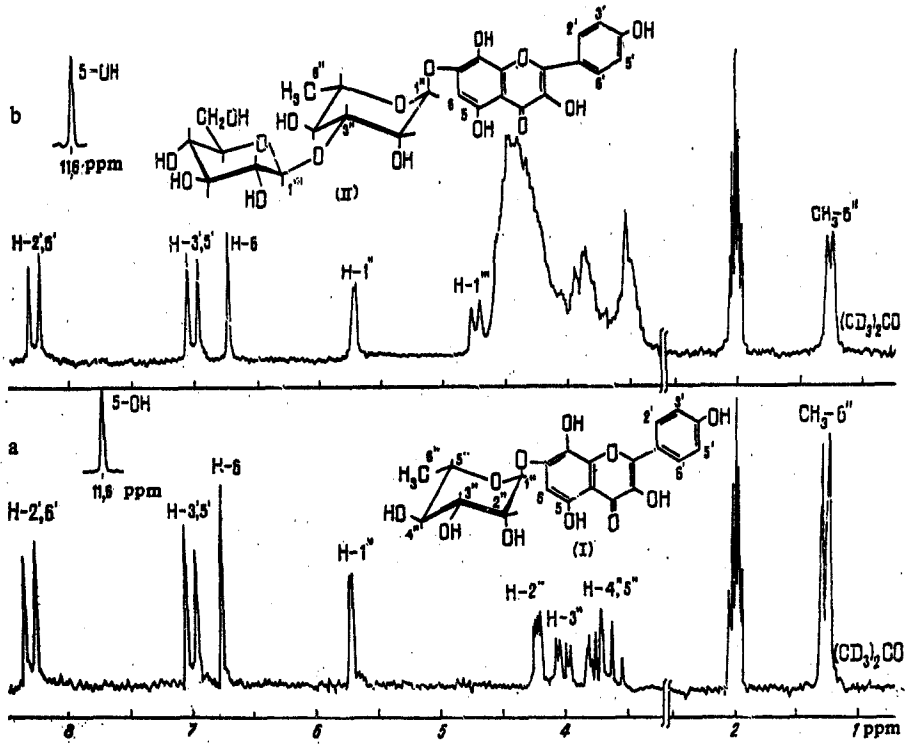


Fig. 1. PMR spectra of rhodionin (I) (a) and of rhodiosin (II) (b) in acetone-d_6.

hydroxy grouping. On this basis, a number of herbacetin glycosides that we have isolated previously from *Rhodiola algida* that were stable to NaOMe were originally determined as 4'-glycosides [9]. However, then, using chemical proofs we assigned them all to the 8-O-glycosides of herbacetin [3, 10]. Herbacetin 8-ether has UV spectra [8] similar to those of the 8-glycosides, and herbacetin 3,7-dimethyl ether [11] decomposes on the addition of NaOMe. Its UV spectrum methanol has a maximum at 372 nm that is usually characteristic for flavonols, but in this case it is possibly connected with the presence of a 5,8-dihydroxychromone grouping. Consequently, in our view, for herbacetin derivatives additional proofs of a free 3-OH group are required (other than UV spectra).

Herbacetin decomposes extremely readily in solutions, and on chromatograms its yellow spot becomes blue after only 30 min and then darkens. Herbacetin 7-glycosides (compounds (I) and (II)) behave similarly. In contrast to them, the 8-O-glycosides of herbacetin are stable and remain unchanged for several years. Thus, the instability of herbacetin and its 7-O-glycosides may be connected with the 5,8-dihydroxy grouping.

Attention is attracted by some other anomalies in the properties of compounds (I) and (II): the unusually large chemical shift of the signal of the proton at C-6 in the PMR spectra of (I) and (II) (Table 2), which is more characteristic for H-8. However, such an interpretation of this signal is excluded since we have shown that in compounds (I) and (II) there is an OH group in position 8. Furthermore, in the herbacetin obtained on their hydrolysis the CS of the singlet signal corresponds to the usual position of the H-6 signal, just as in 8-O-glycosides of herbacetin [3, 9, 10]. Thus, the observed anomalous properties of (I) and (II) may also be connected with the presence of a 5,8-dihydroxy grouping.

Flavonolignins — flavonoids containing an additional C_6-C_3 fragment (mainly coniferyl alcohol) — form a very small group of natural compounds. The complications which the research worker comes up against in determining their structures is vividly demonstrated by the history of silybin, which attracted the attention of scientists in connection with the detection of antihepatotoxic activity in it. It was isolated from *Silybum marianum* [12, 13] and its structure was established by cleavage [14] and synthesis [15]. Its absolute configuration was decided after a one-stage biomimetic synthesis of silybin and its isomer isosilybin [16, 17].

Rhodiolin (III) we assigned to the flavonolignins on the basis of the following facts. The molecular weight of 480 established by mass spectrometry corresponds to the formula

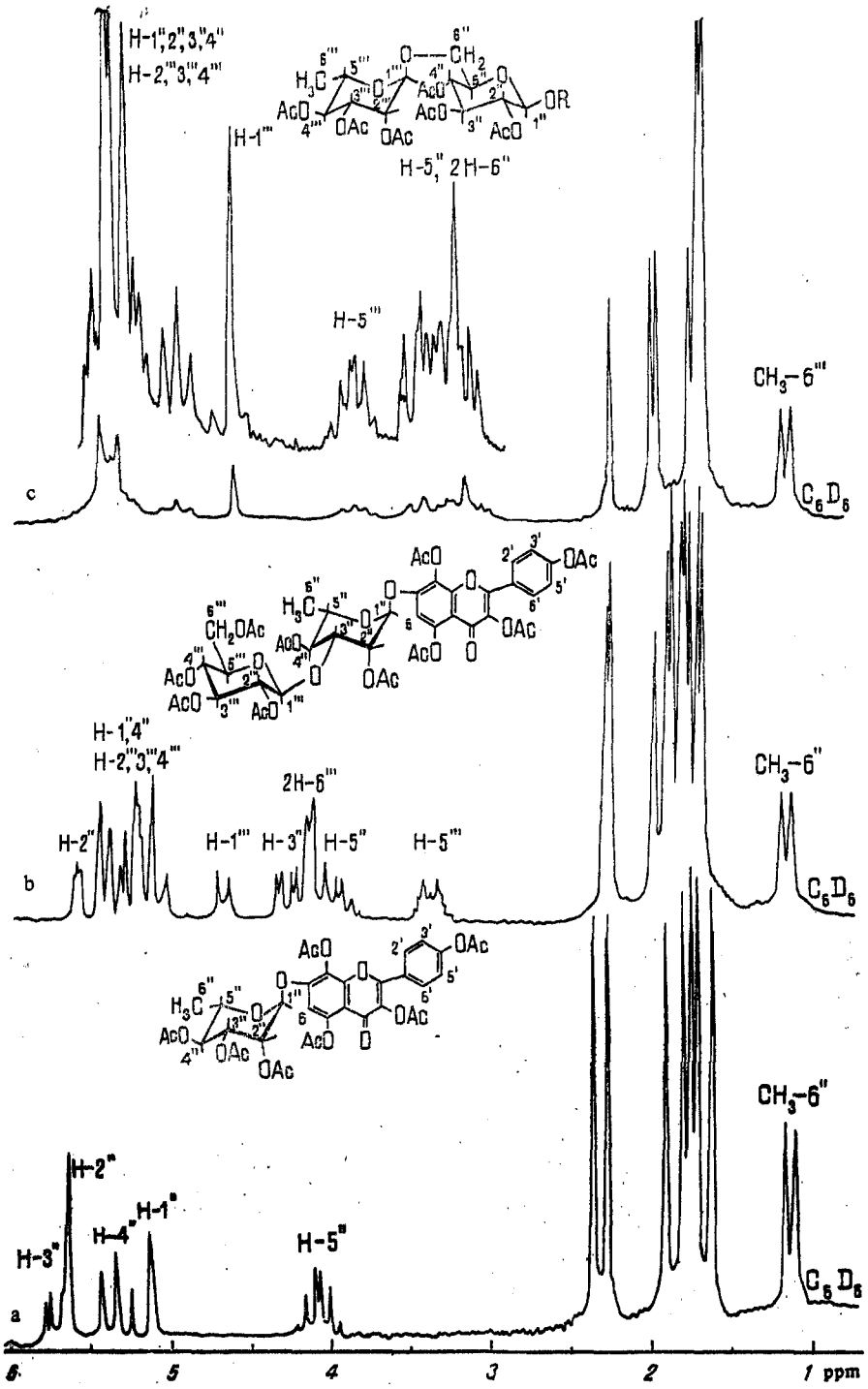


Fig. 2. Fragments of the PMR spectra of the acetates of rhodionin (a), of rhodiosin (b), and of datiscin (c) in benzene-d_6.

$C_{25}H_{20}O_{10}$. Rhodiolin contains, in addition to an aromatic CH_3O group, five acetylated hydroxyls, and the PMR spectrum of the pentaacetate (Fig. 3b) shows that four of them are of phenolic and one of alcoholic nature. The presence of a coniferyl alcohol residue in the molecule follows from the PMR spectrum and the features of the mass spectrum: Fragments with m/z 180, 138, and 124 correspond to similar fragments in the decomposition of cinnamyl alcohol [18, 19]: M^+, $M - C_2H_2O$, and $M - CH_4O$.

Substance (III) is far more stable in solution than (I) and (II); however, on fusion it partially decomposed and the melt was found by TLC to contain a product identical with herbacetin. On being heated with pyridine hydrochloride, rhodiolin gave a product likewise

TABLE 2. Details of the PMR Spectra of the Flavone Fragment of Herbacetin Derivatives

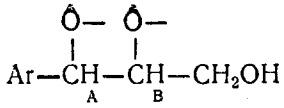
Compound	Solvent	Chemical shifts (δ, ppm)			
		5-OH s	H-2', '6 d, 9Hz	H-3', 5' d, 9Hz	H-6 s
1. Rhodionin (I)	$(CD_3)_2CO$	11,6	8,30	7,02	6,73
Acetate of (I)	C_6D_6		7,62	7,00	6,87
Acetate of (I)	$CDCl_3$		7,80	7,25	6,97
2. Rhodiosin (II)	$(CD_3)_2CO$	11,6	8,28	7,02	6,73
Rhodiosin (II)	C_5D_5N		8,56	7,20	7,20
Acetate of (II)	C_6D_6		7,64	7,06	6,92
Acetate of (II)	$CDCl_3$		7,80	7,24	6,97
3. Rhodiolin (II)	$(CD_3)_2CO$	11,6	8,24	7,00	6,32
Acetate of (III)	$CDCl_3$		7,96	7,26	6,72
4. Herbacetin (IV)	DMSO	12,3	8,30	7,00	6,30
Herbacetin (IV)	CD_3OD		8,20	6,90	6,28
TMS ether of (IV)	CCl_4		8,00	6,76	6,06
Acetate of (IV)	$CDCl_3$		7,70	7,18	6,90
5. Rhodalgin (herbacetin 8-arabinoside) [3]	DMSO	12,3	8,30	6,97	6,33
Rhodalgin	$(CD_3)_2CO$	12,2	8,38	7,04	6,33
TMS ether of rhodalgin	CCl_4		8,14	6,75	6,06
Acetate of rhodalgin	$CDCl_3$		7,85	7,20	6,80
6. 3,7-Dimethylherbacetin (TMS ether) [11]	CCl_4		8,13	6,90	6,27
Acetate [11].	$CDCl_3$		8,13	7,30	6,78

coinciding in chromatographic mobility with herbacetin and identical with it according to UV and mass spectra and color reactions.

Since all the proton signals corresponding to the herbacetin residue are clearly distinguishable in the PMR spectrum of rhodiolin (Fig. 3), its bond with the C_6-C_3 chain must be effected through oxygen.

It was shown above that compound (III) contains free hydroxy groups at C_3 and C_5. The absence of bathochromic shift of the 281 nm band in the UV spectrum with sodium acetate (see Table 1) shows that the 7-OH group of herbacetin is substituted, and the negative gossypetone test permits us to speak of substitution of the 8-OH group (which is also confirmed by the UV spectra with sodium methanolate, which are similar to those of herbacetin 8-glycoside; see Table 1).

Thus, the conferyl alcohol residue must be attached to the 7,8-dihydroxy grouping of the herbacetin. The binding of two hydroxy groups, i.e., the formation of a 1,4-benzodioxane ring, was also established from the fact that the aliphatic region of the PMR spectrum of (III) includes the signals of the following system



Here we had to decide on two positions: the configuration of the C_A-C_B substituents and the position of the ester bonds with respect to the herbacetin fragment.

It is known that in unsymmetrically substituted 1,4-benzodioxanes the value of $J_{2,3}$ for 2,3-cis substituents is 2 Hz [20], and for 2,3-trans substituents $J_{2,3}$ = 8 Hz [21]. Taking into account the doublet at δ 5.27 (J = 8 Hz) observed in the spectrum (Fig. 3) and assigned to the H_A benzyl proton, it can be stated that the relative configuration of the substituents in the dioxane fragment of rhodiolin in transoid.

The question remains open of the isomerism of the positions in the benzodioxane part of the molecule, i.e., whether the structure (IIIa) or (IIIb) corresponds to rhodiolin.

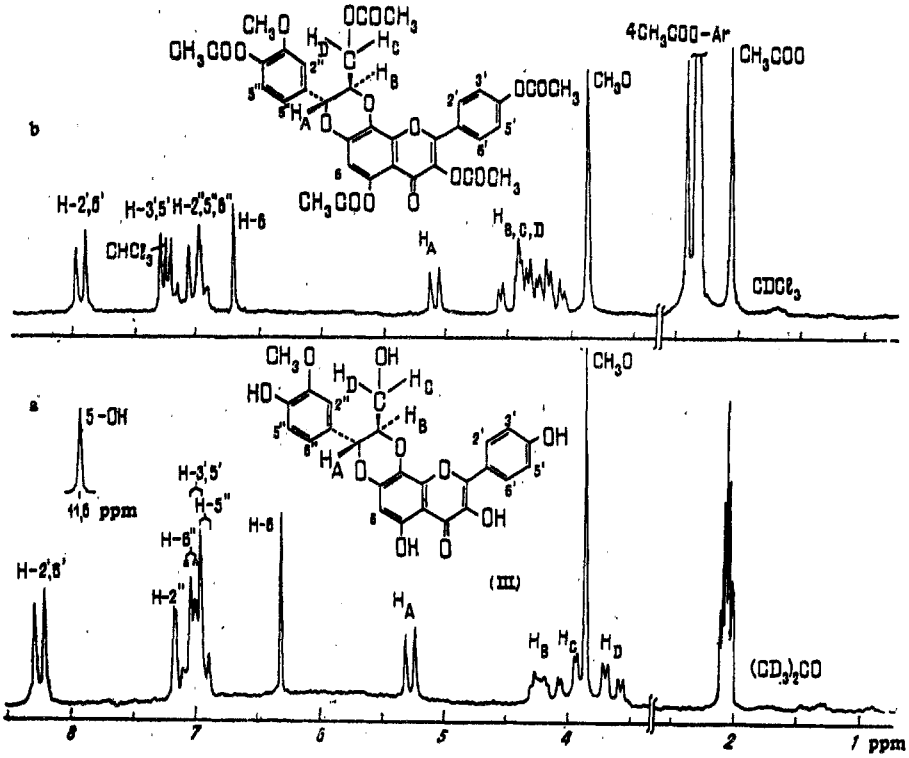
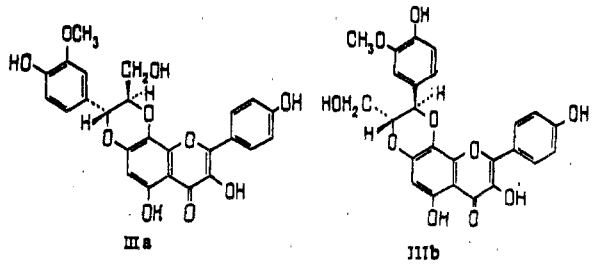


Fig. 3. PMR spectra of rhodiolin (III) in acetone-d_6 (a) and of its acetate in chloroform-d (b).



A structurally similar benzodioxane fragment with the trans configuration of the substituents is present in silybin and dehydrosilybin [21], isosilybin [17], hydnocarpin [22] and methoxyhydnocarpin [23]. In all the compounds mentioned, the coniferyl alcohol residue is attached to a 3',4'-dihydroxy grouping of the lateral phenyl ring of the flavonoid.

The results of a study of the absolute configuration led to the conclusion that natural silybin and isosilybin are diastereoisomeric mixtures, and their dehydro derivatives and the xanthonolignan kielcorin are mixtures of enantiomers [17, 24].

In view of the zero specific rotation of the flavonolignin that we have isolated, it may be assumed by analogy with the literature information given that rhodiolin is a natural mixture of enantiomers with the structures (IIIa) and (IIIb).

EXPERIMENTAL

For general information, see [1]. In the recording of the PMR spectra in deuteroacetone (100 MHz) to improve the solubility of the compounds (I-III) 1-3 drops of deuteropyridine was added.

Isolation. Substances (I-III) were obtained simultaneously with the isolation of tricin glycosides [1], but more polar mixtures of solvents (15% of methanol in chloroform) were required for their elution from the column. The further purification of the compound (III) was carried out by recrystallization from methanol (yield 10 mg). The fractions containing compounds (I) and (II) were passed through a column of polyamide or silica gel, (I)

being eluted with 7% methanol in chloroform and then being recrystallized from a mixture of water and acetone (4 : 1) (yield 20 mg); compound (II) was eluted with 10% methanol in chloroform and was recrystallized from a mixture of methanol and chloroform (3 : 1) or of water and acetone (1 : 2) (yield 30 mg).

Rhodiolin (I). Yellow acicular crystals with the composition $C_{21}H_{20}O_{11}$, mp 232-235°C (decomp.), $[\alpha]_D^{20} -150°$ (c 0.2; ethanol). For UV spectrum, see Table 1. For the PMR spectrum of the flavone fragment, see Table 2; that of the carbohydrate fragment in deuteroacetone, ppm: 5.72 (d, 2 Hz, H-1"); 4.2 (dd, 2 and 3 Hz, H-2"); 4.0 (dd, 3 and 10 Hz, H-3"); 3.74 (dd, 6 and 10 Hz, H-5"); 3.63 (t, 10 Hz, H-4"); 1.3 (d, 6 Hz, CH_3 of rhamnose).

The Heptaacetate of (I). mp 232-233°C, $[\alpha]_D^{20} -62°$ (c 0.4; chloroform). Fragment of the PMR spectrum in deuterobenzene, ppm: 5.7 (dd, 3 and 10 Hz, H-3"); 5.6 (br. s, H-2"); 5.34 (t, 10 Hz, H-4"); 5.1 (br. s, H-1"); 4.08 (dd, 6 and 10 Hz, H-5"); 2.35, 2.26, 1.90, 1.79, 1.75, 1.70, 1.61 (singlets, 7 CH_3COO groups); 1.12 (d, 6 Hz, CH_3); in deuterochloroform: 5.57 (H-1"), 5.46 (H-2"); 5.38 (H-3"); 5.18 (H-4"); 3.9 (H-5"); 2.44, 2.36, 2.28, 2.25 (four aromatic CH_3COO-); 2.13, 2.02, 1.97 (3 aliphatic CH_3COO-); 1.18 (CH_3 of rhamnose).

Rhodiosin (II). Yellow acicular crystals with the composition $C_{27}H_{30}O_{16}$, mp 192-196°C (decomp.), $[\alpha]_D^{20} -78.5°$ (c 0.5; methanol). For the UV spectra see Table 1. Fragment of the PMR spectrum in deuteroacetone, ppm: 5.72 (d, Hz, H-1"); 4.72 (d, 7 Hz, H-1"'); 4.6-3.4 (m, 10 H of the carbohydrate moiety); 1.27 (d, 6 Hz, CH_3 of rhamnose); in deuteropyridine, 6.24 (d, 2 Hz, H-1"); 5.06 (d, 7 Hz, H-1"'); 4.9-3.8 (10 H of sugar residues); 1.5 (CH_3).

Decaacetate of (II). mp 140-141°C, $[\alpha]_D^{20} -45.6°$ (c 0.5; chloroform). Fragment of the PMR spectrum in deuterobenzene, ppm: 5.7-5.1 (m, 6 H); 4.7 (d, 7 Hz, H-1";); 4.3 (dd, 3 and 10 Hz, H-3"); 4.18 (m, 2H-6" '); 4.0 (dd,6 and 10 Hz, H-5"); 3.4 (m, H-5"'); 2.30, 2.27, 2.00, 1.92, 1.89, 1.83, 1.81, 1.77, 1.73, 1.71 (singlets of 10 CH_3COO groups); 1.14 (d, 6 Hz, CH_3); in deuterochloroform: 5.57 (d, 2 Hz, H-1"); 5.40 (dd, 2 and 3 Hz, H-2"); 5.3-4.8 (m, 4 H); 4.7 (d, 7 Hz, H-1"'); 4.2 (m, 2 H-6"); 4.1 (dd, 3 and 10 Hz, H-3"); 3.9-3.6 (m, H-5", 5"'); 2.4, 2.38, 2.32, 2.28 (4 aromatic CH_3COO-); 2.16, 2.09, 2.06, 2.01, 1.99, 1.97 (6 aliphatic CH_3COO-; 1.18 (d, CH_3 of rhamnose).

Rhodiolin (III). Yellow acicular crystals with the composition $C_{25}H_{20}O_{10}$, mp 235-237°C $[\alpha]_D^{20} \pm 0°$ (c 0.33; acetone). For the UV spectra, see Table 1. Mass spectrum at 180°C, m/z (int. %); M^+ 480 (16), M − 18, 462 (0.8), 302 (100), 301 (25), 273 (9), 245 (10), 229 (7), 228 (7), 180 (56), 169 (9), 168 (12), 152 (12), 151 (17), 147 (16), 138 (16), 137 (88), 124 (48), 121 (32), 119 (24), 107 (22), 91 (36). PMR spectrum in deuteroacetone, ppm: 11.6 (s, 5-OH); 8.24 (d, 9 Hz, H-2',6'); 7.17 (d, 2 Hz, H-2'), 7.04 (dd, 2 and 8 Hz, H-6"); 7.00 (d, 9 Hz, H-3',5'); 6.94 (d, 8 Hz, H-5"); 6.32 (s, H-6); 5.27 (d, 8 Hz, H_A); 4.23 (m, H_B); 4.00 (d, 3 and 12 Hz, H_3); 3.88 (s, OCH_3); 3.66 (dd, 4 and 12 Hz, H_D).

The Pentaacetate of (III). mp 231-233°C, colorless needles, $[\alpha]_D^{20} \pm 0°$ (c 2.0; chloroform). PMR spectrum in deuterochloroform, ppm: 7.96 (H-2',6'); 7.26 (H-3',5); 7.12 (H-5); 7.00 (m, H-2",6"); 6.72 (H-6); 5.10 (d, 8 Hz, H_A); 4.60-4.07 (m, 3 H); 3.9 (s, CH_3O); 2.45 (s, 3 H); 2.37 (s, 9 H); 2.09 (s, 3 H).

Qualitative Reactions. 1. Gossypetone test: ethanolic solutions of samples were mixed with an ethanolic solution of freshly-sublimed p-benzoquinone. The solution of substance (III) remained unchanged while solutions of substances (I), (II), and (IV) acquired a red-brown color, which indicates the presence of a free phenolic p-dihydroxy grouping (i.e., free OH groups in positions 5 and 8 of the flavone nucleus).

2. The zirconyl-citric acid test (II): The yellow coloration of compound (I)-(III) with zirconyl chloride did not disappear on the addition of citric acid (free 3-OH group).

3. Coloration with the diazo reagent (diazotized sulfanilic acid in a saturated solution of Na_2CO_3) on Silufol plates: (I) and (II) — bright green; (III) — red; (IV) — blue-violet; rhodalgin — yellow. The products in melts (after melting-point determinations) can readily be distinguished by these colorations; in addition to the initial substances, (IV) was detected in (I) and (III), and (IV) and (I) in (II).

Acid Hydrolysis. 1. Hydrolysis with 2% HCl at 100°C took place completely in 20 min for compounds (I) and (II) (rhamnose was found in the hydrolysate of (I) and rhamnose and glucose in that of (II)) while compound (III) did not change. The aglycone in (I) and (II) was identified as herbacetin (IV) by comparison with an authentic sample, by UV and MR spectra (Tables 1 and 2), and by the mass spectrum: M^+ 302, and characteristic fragments with m/z 168, 121, and others.

2. The hydrolysis of (II) with 0.5% HCl (100°C, 30 min) led to the formation of (IV), glucose, rhamnose, and rhodiose. Under similar conditions, rutin yielded rutinose, rhamnose, and glucose.

3. The hydrolysis of (II) with 0.05% HCl (100°C for 30 min) led to the formation of (IV) and only one sugar — rhodiose.

R_f values on FN-15 paper in the butan-1-ol—acetic acid—water (4 : 1 : 2) (1), butan-1-ol—pyridine—water (6 : 4 : 3) (2), and water-saturated phenol (3) systems:

	1	2	3
Rhodiose	0.12	0.41	0.52
Rutinose	0.04	—	—
Glucose	0.10	0.32	0.37
Rhamnose	0.30	0.52	0.63

Partial Hydrolysis of Rhodiosin. 1. Under the usual conditions of enzymatic hydrolysis with the aid of β-glucosidase (38°C, 24 h), compound (II) did not change.

2. With heating, 20 mg of substance (II) was dissolved in 2 ml of cyclohexanol, and then 0.7 ml of 97% HCOOH was added and the solution was heated at 105°C for 5 h. The cooled reaction mixture was deposited on a column of polyamide (5 × 2 cm) which was then washed with 200 ml of water, and the flavonoids were eluted with 20 ml of ethanol and were rechromatographed on polyamide using mixtures of chloroform and methanol. At a concentration of 7% of methanol, 4 mg of a substance was isolated which was identical with (I) according to R_f, UV spectra, and the products of acid hydrolysis; 10% methanol eluted from the column 8 mg of the initial compound (II). A longer time of hydrolysis led to the decomposition of both substances — the starting material and the intermediate compound.

The acetylation of (I), (II), and (III) was performed with acetic anhydride in pyridine (20°C, 24 h), the reaction mixture being poured into ice water, and the precipitates being washed with water and recrystallized from ethanol.

Reaction of Rhodiolin with $C_5H_5N \cdot HCl$. A mixture of 5 mg of substance (III) and 15 mg of pyridine hydrochloride was heated at 175-185°C for 1.5 h. After cooling, the reaction mixture was acidified with 5% HCl and extracted with ether. The ethereal extract was evaporated and the residue was chromatographed on polyamide in chloroform—methanol. The initial substance (III) was isolated, together with herbacetin (IV), which was identified by qualitative reactions, TLC, and UV and mass spectra.

SUMMARY

Three new derivatives of herbacetin (3,4',5,7,8-pentahydroxyflavone) have been isolated from the rhizomes of *Rhodiola rosea* L. The structure of herbacetin 7-O-α-L-rhamnopyranoside is proposed for rhodionin (I); rhodiosin (II) has the structure of herbacetin 7-O-(3''-O-β-D-glucopyranosyl-α-L-rhamnopyranoside) the biose moiety that it includes, which has been called rhodiose, is the first example of 3-O-β-D-glucopyranosyl-L-rhamnopyranose found in natural flavonoid glycosides.

A probable structure has been put forward for the flavonolignan rhodiolin (III) — the product of the oxidative coupling of herbacetin and coniferyl alcohol.

LITERATURE CITED

1. V. A. Kurkin, G. G. Zapesochnaya, and V. G. Klyaznika, Khim. Prir. Soedin., 581 (1982).
2. L. Hörhammer, H. J. Gehrmann, and L. Endres, Arch. Pharm., 262/64, 113 (1959).
3. T. T. Pangarova and G. G. Zapesochnaya, Khim. Prir. Soedin., 712 (1975).
4. G. G. Zapesochnaya and T. T. Pangarova, Khim. Prir. Soedin., 554 (1973).
5. G. G. Zapesochnaya, Khim. Prir. Soedin., 695 (1982).
6. G. G. Zapesochnaya, N. A. Tyukavkina, and S. K. Eremin, Khim. Prir. Soedin., 180 (1982).
7. W. Karrer, Konstitution und Vorkommen der organischen Pflanzenstoffe, Birkhäuser Verlag, Basel (1958), p. 261.
8. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer Verlag, Berlin (1970).
9. T. T. Pangarova, G. G. Zapesochnaya, and E. L. Nukhimovskii, Khim. Prir. Soedinen., 667 (1974).

10. G. G. Zapesochnaya, Khim. Prir. Soedin., 519 (1978).
11. M. Sakakibara, B. N. Timmermann, N. Nakatani, H. Waldrum, and T. J. Mabry, Phytochemistry, 14, 849 (1975).
12. B. Janiak and R. Hänsel, Planta Med., 8, 71 (1960).
13. H. Wagner, in: Recent Flavonoid Research, Akademiai Kiado, Budapest, 51 (1973).
14. A. Pelter and R. Hänsel, Chem. Ber., 108, 790 (1975).
15. R. Hänsel, J. Schulz, and A. Pelter, Chem. Ber., 108, 1482 (1975).
16. L. Merlini, A. Zanarotti, A. Pelter, M. P. Rochefort, and R. Hänsel, J. Chem. Soc. Chem. Commun., 695 (1979).
17. A. Arnone, L. Merlini, and A. Zanarotti, J. Chem. Soc. Chem. Commun., 696 (1979).
18. C. Koppel and H. Schwartz, Org. Mass Spectrom., 11, 101 (1976).
19. G. G. Zapesochnaya and V. A. Kurkin, Khim. Prir. Soedin., 723 (1982).
20. A. R. Martin, S. K. Mallick, and J. F. Caputo, J. Org. Chem., 39, 1808 (1974).
21. R. Hansel, J. Schulz, A. Pelter, H. Rimpler, and A. F. Rizk, Tetrahedron Lett., 4417 (1969).
22. K. R. Ranganathan and T. R. Seshadri, Tetrahedron Lett., 3481 (1973).
23. K. R. Ranganathan and T. R. Seshadri, Indian J. Chem., 12, 993 (1974).
24. H. Nielsen and P. Arends, Phytochemistry, 17, 2040 (1978).

SYNTHESIS, SOME REACTIONS, AND BIOLOGICAL PROPERTIES OF IMIDAZO[1,2-f]THIOPURIN-7-ONE DERIVATIVES*

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The thionation of 1,8-dimethyl-6-H-2-phenolimidazole[1,2-f]xanthine with the aid of P_2S_5 in γ-picoline leads to 1,8-dimethyl-2-phenyl-5-thioxoimidazo[1,2-f]purin-7-one. On alkylation with methyl iodide, the latter is converted into 1,8-dimethyl-5-methylthio-2-phenylimidazo-[1,2-f]purin-7-one. The reductive desulfuration of 1,8-dimethyl-5-methylthio-2-phenylimidazo[1,2-f]purin-7-one with Raney nickel in aqueous ethanol forms 1,8-dimethyl-2-phenyl-7,8-dihydroimidazo[1,2-f]purin-7-one; the actions of colamine and benzylamine on the same compounds form 5-(β-hydroxyethylamino)- and 5-benzylamino-1,8-dimethyl-2-phenylimidazo[1,2-f]purin-7-one, respectively. The thionation of 1,8-dimethyl-2-phenylimidazo[1,2-f]xanthine in γ-picoline with an excess of phosphorus pentasulfide leads to 1,8-dimethyl-2-phenyl-5,7-dithioxoimidazo[1,2-f]purine. Some results of biological trials are given. The UV, IR, PMR, and mass spectra of the compounds obtained are discussed.

In the present paper, which represents a continuation of the investigations of the chemical properties of the 6-H-imidazo[1,2-f]xanthine system [2], the direct thionation reaction of this system is described and the possibility is shown of performing a number of transformations on the basis of the 5-thioxo derivative obtained.

The interest in thio derivatives of imidazo[1,2-f]purin-7-one is due to the fact that in its biochemical properties it can be assigned to nucleotide analogs and, like them, exhibits a high physiological activity, including antitumoral activity [3, 4].

According to the literature [5-7], the thionation of uncondensed xanthenes and their derivatives leads to 6-thioxo derivatives. The thionation of 1,8-dimethyl-6H-2-phenylimidazo[1,2-f]xanthine (I) with the aid of phosphorus pentasulfide in γ-picoline gives 1,8-dimethyl-2-phenyl-5-thioxoimidazo[1,2-f]purin-7-one (II) or the 5,7-dithioxo derivative (VIII). The alkylation of (II) with methyl iodide in aqueous alcoholic caustic soda leads to the

*See [1].

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