PROANTHOCYANIDINS OF Rhodiola semenovii

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The structures of two compounds previously isolated from Rhodiola semenovii, rhodokhinoside (I) and rhodikhim (II), have been established.

Continuing a study of the physicochemical properties of compounds isolated previously from Rodiola semenovii [1] we have established the structures of two proanthocyanidins – rhodikhinoside (I) and rhodikhim (II).

The UV spectrum of rhodokhinoside had characteristic maxima for galloylated proanthocyanidins at 220 and 278 nm with shoulders at 245 and 310 and a minimum at 258 nm. The IR spectrum of the substance had characteristic absorption bands of hydroxy groups (3500-3200 cm^{-1}), of the α-carbonyl of an aromatic acid (1695), and of condensed aromatic rings (1620, 1545, 1515, 1450), of a =C–CH group (1320), and of a =C–O–C group (1250 and 1045 cm^{-1}).

The interpretation of the series of resonance lines in the ^{13}C NMR spectrum of (I) obtained under the conditions of complete decoupling from protons was effected by the mutual comparison of the ^{13}C NMR spectra of catechins and of model proanthocyanidins. In the ^{13}C NMR spectrum of rhodokhinoside signals appeared of (–)-epigallocatechins and of residues of β-glucose and of gallic acid. Resonance signals in the 155.0-157.7 ppm region related to the C-5, C-7, and C-9 carbon atoms of the phloroglucinol nucleus – ring A. An intense resonance signal at 146.0 ppm related to C-3' and C-5' of ring B. The C-4' carbon atom of the epigallocatechin residue was screened and, as a result of a diamagnetic shift, resonated at 133.6 ppm. Resonance signals from C-1' appeared in the 129.1-130.7 ppm region. Relatively intense signals at 106.7 ppm related to the C-2' and C-6' carbons of ring B. The chemical shift of the signal of the C-10 carbon (with the exception of that of the "lower" catechin block) amounted to 99.7 ppm, which showed the C-4 → C-6 type of interflavan bond [2]. The signals of the C-6 carbon atoms through which the interflaven bond was made were observed in the 106.7 ppm region and those of the C-8 and C-6 carbon atoms free from the interflavan bond at 97.6 ppm.

The chemical shifts of the resonance signals of the carbon atoms of ring B were close to the corresponding values of the CSs of the epigallocatechins [3] (Table 1). The epigallocatechin occupying the "lower" position in the proanthocyanidin chain was esterified in the C-3 position as was shown by the values of the chemical shifts of the C-2 and C-3 atoms of ring C (78.8 and 69.1 ppm) [4, 5].

It is interesting to note that the signals of a glucose residue with a galloylated hydroxy group appeared in the spectrum. The C-6 chemical shift of the sugar residue in rhodikhinoside (63.0 ppm) was close to the chemical shift of the sixth carbon atom of glucose with an acylated hydroxy group [6].

The results of the mild acid and thiolytic decomposition of (I) showed the absence of a sugar residue as a component of the "lower" epigallocatechin block, and, consequently, if the stereochemical hidnrance in the proanthocyanidin molecule is taken into account it may be assumed that it was the "upper' block of epigallocatechin that was glycosylated.

The molecular mass of rhodikhim (II) was ~1700, $[\alpha]_D^{22}$ –26° (c 0.32; ethanol). Alkaline fusion of the substance led to phloroglucinol and gallic and para-hydroxybenzoic acids.

According to its UV and IR spectra, rhodikhim belonged to the polymeric proanthocyanidins. The IR spectrum of the compound contained the absorption bands of an aromatic acid

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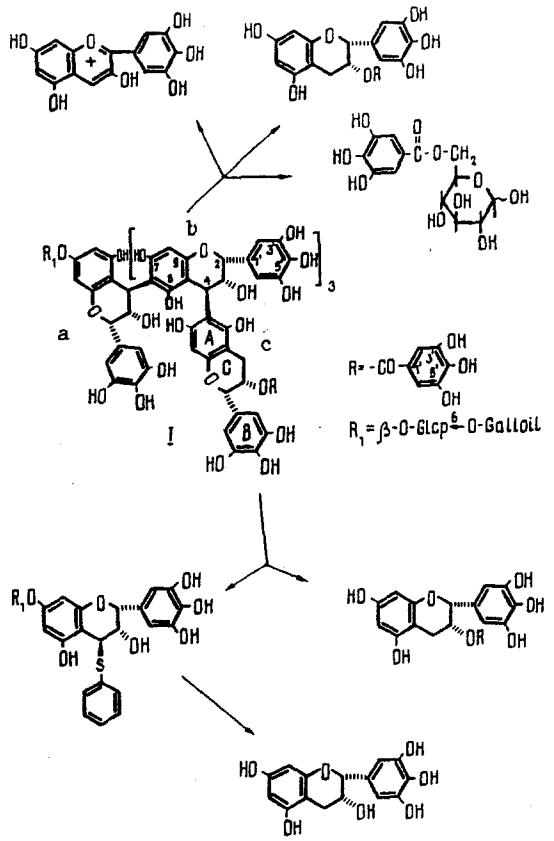
TABLE 1. Chemical Shifts in the ^1H NMR Spectrum of Rhodikhinoside (ppm, 0 – TMS, acetone-d_6–water (1:1))

Carbon atom	Fragments of rhodikhinoside				
	a	b	c	glucose	galloyl
2	79,2	78,0**	78,8*		
3	73,0	71,9	69,1		
4	37,8	37,8	—*		
6	97,6	106,7	106,7**		
8	97,6	97,6	97,6		
10	101,0**	99,7	99,7		
5,7,9	155,0	156,6	157,7		
1′	131,7	129,1	130,7	101,0**	120,7
2′	106,7	106,7	106,7**	73,0	110,0
3′	146,0	146,0	146,0	77,5	146,0
4′	133,6	133,6	133,6	71,3	138,9
5′	146,0	146,0	146,0	78,0**	146,0
6′	106,7	106,7	106,7	63,0	110,0
—COO—					166,4; 168,6

*The signal was not observed because of masking by a signal from the solvent.
**The signals may be inverted.

(1690) and intense absorption bands of hydroxy groups (3500-3200) and of –CH–, =C–O, and =C–O–C groups (2930, 1330, 1250, and 1045 cm^{-1}, respectively).



In the ^{13}C NMR spectrum of rhodikhim signals appeared of (+)-afzelichin, (–)-epiafzelichin, and (±)-gallocatechin, and of gallic acid residues (Table 2) [7, 8].

In the proanthocyanidin chain, the "lower" position was occupied by (±)-gallocatechin gallate, as followed from the chemical shifts of the C-2, C-3, and C-4 carbon atoms – 80.3, 69.7, and 26.3 ppm, respectively. Presumably the "upper" position was occupied by galloylated (+)-afzelichin.

The chemical shifts of the signals of the C-2, C-3, and C-4 carbon atoms of ring C (81.5, 73.1, and 34.5 ppm) were close to the corresponding values for galloylated proanthocyanidins with the 2,3-trans-3,4-trans configuration [9, 10].

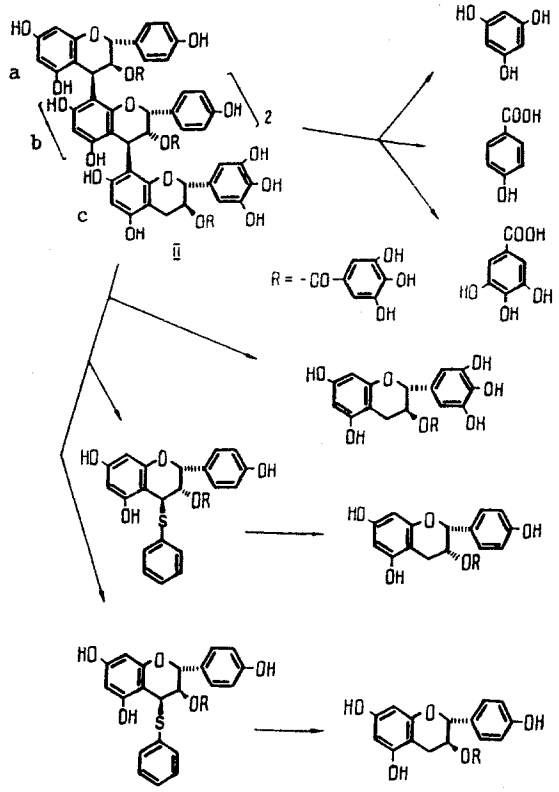
TABLE 2. Chemical Shifts in the ^{13}C NMR Spectrum of Rhodikhim, ppm, 0 – TMS (solvent layer – acetone-d.)

Carbon atom	Fragments of rhodikhim			
	a	b	c	galloyl
2	81,5	77,2	80,3	
3	73,2	73,1	69,7	
4	34,5	34,2	26,3	
6	98,6	98,6	98,6	
8	98,6	106,8	106,8	
10	103,8	103,8	103,8	
5,7,9	156,7	156,7	156,7	
1′	131,6	131,6	131,6	121,2
2′	130,3	130,3	106,8	109,7
3′	114,6	114,6	146,8	146,8
4′	156,7	156,7	134,4	140,0
5′	114,6	114,6	146,8	146,8
6′	130,3	130,3	106,8	109,7
—COO—				164,1 166,5

Consequently, the "middle" position in the rhodikhim molecule was occupied by galloylated (–)-epiafzelichin blocks, which were characterized by the chemical shifts of the C-2, C-3 and C-4 atoms of ring C (77.2, 73.1, and 34.2 ppm, respectively).

The chemical shift of the C-10 carbons of the "upper" proanthocyanidin blocks, amounting to 103.8 ppm, showed that the interflavan bond in the proanthocyanidin was of the C4 → C8 type [11].

The degradation products obtained under the conditions of mild and severe acid and thiolytic cleavages of rhodikhim confirmed the hypotheses given above.



EXPERIMENTAL

General Information. The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Hitachi EPS-3T instrument. IR spectra were taken on a Carl Zeiss (Jena), UR-20 instrument using tablets molded with potassium bromide. The ^{13}C NMR spectra were recorded on a Tesla BS 567 A/25 MHz instrument in Me_2CO-d_6 solution and in a mixture of Me_2CO-d_6 and D_2O, with TMS as the internal standard (δ scale). The concentrations of the substances ranged between 15 and 20%. Molecular masses were determined on a MOM 3170 ultracentrifuge and by gel filtration on a calibrated column of Sephadex

LH-20. A JASCO J-20 instrument was used to determine optical activities. PC and TLC on Silufol UV-254 plates were used for the identification of the substances and the determination of their homogeneity. Solvent systems: 1) chloroform–methanol–ethyl acetate (10:1.5:1) and (5.5:1.5:1); 2) chloroform–methanol–formic acid–water (5:10:8:3); and 3) BAW (4:1:5).

Isolation of the Proanthocyanidins and Their Glycosides. The roots of Rhodiola semenovii (4 kg) were extracted with ethanol six times. The extracts were combined and evaporated in vacuum at 40°C to 2 liters. The concentrated extract was treated successively with diethyl ether, ethyl acetate, and n-butanol, giving 105, 35, and 148 g of extracts, respectively. The ethyl acetate extract contained the total amount of proanthocyanidins, consisting of nine components, while the butanolic extract consisted of five components.

Separation of the Total Proanthocyanidins. The ethyl acetate extract (20 g) was mixed with 20 g of cellulose and was transferred to a column containing cellulose. On elution, 50 ml fractions were collected: with hexane, fractions 1-40; with hexane–ethyl acetate (1:1), fractions 41-60; (1:3), fractions 61-100; (1:4), fractions 101-190; (1:5), fractions 191-258. Monitoring was carried out by TLC in systems 1 and 3.

Fractions 64-212 contained a mixture of three proanthocyanidins (2.1 g). The mixture was rechromatographed on cellulose with elution by chloroform and by chloroform–ethyl acetate ((1:1)–(1:20)).

Rhodisin [1]. The residue from fractions 60-67 (0.196 g) was chromatographed on a column of Sephadex LH-20 with elution by 80% ethanol. This gave 0.177 g of an amorphous powder with MM ~920, decomposing at 290-300°C, $[\alpha]_D^{22}$ + 2° (c 0.35; ethanol), R_f 0.62 (system 2). UV spectrum: λ_{max} 220, 245, 278, 310 nm, λ_{min} 258 nm). IR spectrum: ν_{max} 3500, 2935, 1675, 1625, 1545, 1510, 1435, 1320, 1270, 1200, 1040, 830, 770, 730 cm^{-1}.

Rhodikhinoside (I). The residue from fractions 92-103 (0.281 g) was chromatographed on Sephadex LH-20 with 60% ethanol as eluent. This gave 0.23 g of an amorphous substance with MM ~1900, decomposing at 290-300°C, $[\alpha]_D^{22}$ −26° (c 0.17; ethanol), R_f 0.37 (system 2). UV spectrum; λ_{max} 220, 245, 278, 310 nm. IR spectrum; ν_{max} 3500, 2935, 1695, 1620, 1545, 1515, 1450, 1320, 1250, 1200, 1045, 830, 805, 774, 730 cm^{-1}.

Rhodikhim (II). The residue from fractions 121-133 (0.301 g) was transferred to a column of Sephadex LH-20 and was eluted with 80% ethanol. This gave 0.246 g of an amorphous substance with MM ~1700, decomposing at 290-300°C, $[\alpha]_D^{22}$ −26° (c 0.32; ethanol), R_f 0.48 (system 2). UV spectrum: λ_{max} 220, 245, 278, 305 nm. IR spectrum: ν_{max} 3500, 2930, 1690, 1617, 1540, 1445, 1330, 1250, 1045, 830, 777, 735 cm^{-1}.

Alkaline Cleavage of Rhodikhinoside. A 20-ml flask was charged with 30 mg of the substance and, with the passage of a slow current of nitrogen, 5 ml of 50% KOH was added. The flask was immersed in a bath at a temperature of 155-160°C, and then the temperature was raised over 5 min to 230°C. The reaction mixture was rapidly cooled, acidified, diluted with water, and extracted with ethyl acetate. The extract was dried, the solvent was disilled off, and the residue was chromatographed on polyamide. This gave two compounds: (I) – $C_6H_6O_3$, M^+ 126, mp 218-219°C – and (II) – $C_7H_6O_5$, M^+ 170, mp 220°C (decomp) –, which were identified (IR) as phloroglucinol and gallic acid, respectively.

The alkaline cleavage of rhodikhim was carried out by the method described above. Three compounds were obtained: (I) – $C_6H_6O_3$, M^+ 126; (II) –$C_7H_6O_5$, M^+ 170; and (III) – $C_7H_6O_3$, M^+ 138. The substances obtained were identified as phloroglucinol and gallic and p-hydroxybenzoic acids, respectively.

Acid Cleavage of Rhodikhinoside. A solution of the substance (66 mg) in 2 ml of ethanol was treated with 1 ml of a 2N solution of hydrochloric acid, and the mixture was heated in the water bath with a reflux condenser in a current of nitrogen for 2 h. The reaction mixture (colored crimson) was diluted with water and extracted with ethyl acetate (3 × 2 ml). The extract was washed and dried, and the solvent was distilled off. The residue was chromatographed on a column of Sephadex LH-20, with elution by 60% ethanol. This gave 8 mg of (−)-epigallocatechin gallate, mp 210-211°C, $[\alpha]_D^{22}$ −132 (c 0.38; methanol–water) and 4 mg of monogalloylglucose, M^+ 332, mp 135-137°C, $[\alpha]_D^{24}$ +26.5° (c 0.21; acetone); PMR (acetone-d_6): 3.0-5.3 (7 H, m); 7.14 (2 H, s).

In the hydrolysate, delphinidin was detected by paper chromatography: R_f 0.36 (2 N HCl), λ_{max} 554 nm (0.1% HCl in ethanol).

Cleavage of Rhodikhim. Rhodikhim (50 mg) was hydrolyzed by the method described above. After suitable working up, the hydrolysate was chromatographed on Sephadex LH-20. This gave (±)-gallocatechin gallate, mp 206°C, $[\alpha]_D^{20}$ ±0° (acetone–water (1:1)), R_f 0.68 (system 3).

In the hydrolysate, pelargonidin was detected by the PC method: R_f 0.81 (2 N HCl), λ_{max} 518 nm (0.1% HCl in ethanol).

Thiolytic Cleavage of Rhodikhinoside. A mixture of 100 mg of rhodikhinoside, 1.5 ml of phenyl marcaptan, and 1 ml of acetic acid in 10 ml of ethanol was left at room temperature for 16 h and was then concentrated. An oily residue was obtained which was chromatographed on Sephadex LH-20. The substance was eluted with ethanol. This gave (–)-epigallocatechin gallate (9 mg) and an amorphous substance – a thioether (29 mg).

Cleavage of the Thioether from (I). The thioether (29 mg) was mixed with 1 ml of ethanol–acetic acid (9:1). Then Raney nickel catalyst was added to the reaction mixture and it was kept at 50°C for 30 min. After this, it was filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20. Elution was performed with 80% aqueous ethanol. This gave 11 mg of a substance with mp 210-212°C, which was identified as (–)-epigallocatechin gallate.

Thiolytic Cleavage of Rhodikhim. The substance (132 mg) was cleaved and the products were separated by the method described above. This gave (±)-gallocatechin gallate (17 mg) and a thioether (92 mg).

Cleavage of the Thioether from (II). The thioether (92 mg) was cleaved by the method described above. The reaction mixture was chromatographed on Sephadex LH-20 (60% ethanol). This gave two compounds: (+)-afzelichin gallate (8 mg), $[\alpha]_D^{23}$ + 18.7° (c 0.43; acetone–water), λ_{max} 214, 292 nm, PMR (acetone-d_6): 4.65 (1H, d, J = 7.5 Hz; H-2); 5.43 (1H, m, $W_{1/2}$ = 16 Hz, H-3); 2.65 (2H, dd, J_1 = 7.5 Hz, J_2 = 16 Hz, 2H-4); 5.88 (1H, d J = 2.5 Hz, H-6); 6.03 (1H, d, J = 2.5 Hz, H-8); 6.76 (2H, d, J = 8.5 Hz, H-3, H-5); 6.92 (2H, d, J = 8.5 Hz, H-2, H-6); 6.97 (2H, s, H-2, H-6 of galloyl), and (–)-epiafzelchin gallate (19 mg), $[\alpha]_D^{23}$ –176.2° (c 0.33; acetone–water), λ_{max} 207, 276 nm, PMR (acetone-d_6): 5.1 (1H, br. s. H-2); 5.45 (1H, m, $W_{1/2}$ = 3 Hz, H-3); 2.74 (2H, br. s., 2H-4); 5.96 (2H, d, J = 2 Hz, H-6, H-8), 6.94 (2H, d, J = 8.5 Hz, H-3, H-5); 7.33 (2H, d, J = 8.5 Hz, H-2, H-6); 6.98 (2H, s, H-2, H-6 of galloyl).

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