GLYCOSIDES OF CINNAMYL ALCOHOL FROM THE RHIZOMES OF *Rhodiola rosea*

G. G. Zapesochnaya and V. A. Kurkin

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Three new cinnamyl alcohol glycosides have been isolated for the first time from the rhizomes of roseroot stonecrop *Rhodiola rosea* L. (*Sedum rosea*). On the basis of chemical transformations and the results of UV, IR, PMR, and mass spectroscopy the following structures are proposed for the compounds isolated: rozin — trans-cinnamyl O-β-D-glucopyranoside; rozavin — trans-cinnamyl O-(6'-O-α-L-arabinopyranosyl-β-D-glucopyranoside); and rozarin — trans-cinnamyl O-(6'-O-α-L-arabinofuranosyl-β-D-glucopyranoside).

In a chemical study of the substances extracted by ethanol from the rhizomes of *Rhodiola rosea* L. (*Sedum rosea*) (roseroot stonecrop), in addition to flavonoids [1], we have isolated a group of new glycosides to which we have given the names rozin (I), rozavin (II), and rozarin (III). They were all revealed on Silufol plates by their characteristic lilac coloration with 20% sulfuric acid after heating. We assume that all three substances had aglycones of similar structure since in the 7.5–6.2 ppm region their PMR spectrum contained identical signals which we assigned to a monosubstituted benzene ring and to two trans-olefinic protons in a 3-phenylallyl grouping. The IR spectra lacked the bands of ester groups, and therefore we assumed the presence of a cinnamyl alcohol residue in each of compounds (I–III). The UV spectrum of each of the compounds had an absorption maximum at 252 nm, which did not change on the addition of ionizing and complex-forming reagents.

Compounds (II) and (III) did not undergo enzymatic hydrolysis with β-glucosidase, while (I) was readily cleaved into glucose and the aglycone (IV), which was identified as trans-cinnamyl alcohol on the basis of the PMR and mass spectra of (IV) and its acetate.

The mass spectrum of (IV) contains ions characteristic for cinnamyl alcohol [2, 3]: M^+ 134, M − (H + H_2O) (m/z 115), M − CHO (m/z 105), M − C_2H_2O (m/z 92), M − C_3H_4O (m/z 78). The formation was noted of a metastable peak with m/z 63.2, corresponding to the transition 134 → 92 + 42. The fragmentation of the acetate of (IV) took place similarly: M^+ 176, m/z 134, 133, 115, 105, 92. The PMR spectrum of (IV) contains signals that characterize it as trans-cinnamyl alcohol (ppm): 7.3 (m, C_6H_5); 6.65 (d, 16 Hz, $-\underline{CH}=CH-CH_2$), 6.4 (dt, 6 and 16 Hz, $-CH=\underline{CH}-CH_2$), and 4.25 (m, $-CH-\underline{CH_2}O$). The spectrum of its acetate shows the signal of an aliphatic acetoxy group (2.14 ppm, s), and the signal of a $-\underline{CH_2}OAc$ group appears in the form of a doublet at 4.75 ppm (J = 6 Hz).

Glucose was identified in the products of the acid hydrolysis of all three glycosides, and also arabinose in those of compounds (II) and (III). The aglycones formed a complex mixture. In the case of (I), in addition to the aglycone (IV) a substance (V) with a higher chromatographic mobility was formed which may be cis-cinnamyl alcohol. These two substances (IV) and (V) were formed on the acid hydrolysis of (II) and (III), but, in addition to them, disappearing intermediate products (VI and VII) were formed which we isolated by chromatography on silica gel. In its chromatographic mobility, substance (VI) coincided with (I) and it gave similar products on hydrolysis with β-glucosidase (glucose + (IV)). Substance (VII) (it also appeared in the form of a disappearing spot in the acid hydrolysis of (I)) had a higher chromatographic mobility than (VI). Its enzymatic hydrolysis gave glucose and the aglycone (V), and it is probably a glycoside of cis-cinnamyl

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alcohol. Thus, arabinose is readily split off in the acid hydrolysis of (II) and (III) but the monoglycoside obtained is represented by the two isomers (VI) (I) and (VII).

What has been said permits us to consider that the structure of trans-cinnamyl O-β-D-glucopyranoside has been demonstrated for rozin (I).

Structurally, compounds (II) and (III) represent rozin glycosylated with arabinose. The differences in their properties may be caused by one or more of the following factors: different positions of attachment of the arabinose to the glucose, different configurations of the glycosidic bond, or different sizes of the oxide ring of arabinose.

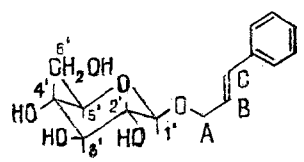
In the PMR spectra of (II) and its acetate and TMS ether it is readily possible to trace in the strong field (at 3.70, 3.06, and 3.17 ppm, respectively) a doublet of doublets with SSCCs of 13 and 2 Hz that is characteristic for the 5e-H atom of L-arabinopyranose in the $^4C_1$ conformation [4]. This signal is absent from the spectra of (III), and in the weak field there is the signal of the anomeric proton of α-L-arabinofuranose (δ 5.74 in pyridine-d_5, δ 5.05 in acetone-d_6, and 5.04 in CCl_4).

A conclusion relative to the position of attachment of the arabinose residue to the glucopyranose residue also followed from a comparison of the PMR spectra of the glycosides and their acetates. The region of resonance of the aliphatic protons in the spectra of the acetates (I—III) taken in deuterobenzene includes three groups of signals:

Compound	*5,6—5,1* ppm	*4,6—4,1* ppm	*4,0—3,0* ppm.
The acetate I	3H (2′, 3′, 4′)	5H (1′, 2H-6′, $2H_A$)	1H (5′)
The acetate II	6H (2′, 3′, 4′, 2″, 3″, 4″)	4H (1′, 1″, $2H_A$)	5H (2H-6′, H-5′, 2H-5″)
The acetate III	6H (2′, 3′, 4′, 2″, 3″, 1″)	5H (1′, 2H-5″, $2H_A$)	4H (2H-6′, H-5′, H-1″)

When (I) was acetylated, the signals of the gem-acyl methine protons (H-2', 3', 4') underwent the greatest paramagnetic shift (5.40—5.15 ppm); the signals of the anomeric glucose proton (H-1'), of the methylene group of cinnamyl alcohol ($2H_A$), and of the gem-acyl methylene protons of the glucose (2H-6') were located in the middle region (4.45—3.9 ppm). Only the signal of the H-5' proton of the glucose residue remained in the strongest field (m, 3.2 ppm). In the case of the acetate (II), the signals of the 2 H-6' atoms of glucose and the readily identified 2 H-5" signals of arabinopyranose remained in the strong field in addition to the signal of H-5', i.e., there was a total of signals from five protons in the 4.0—3.0 ppm region. In the case of the acetate of the glycoside (III) there were the signals of four protons here: 2H-6', H-5', and H-4" of an arabinofuranose residue. Thus, the 4.0—3.0 ppm region in the spectra of the acetates can be considered as diagnostic for deciding between the 6'-glycosylation of the glucopyranose residue and any other positions (2', 3', or 4') of the second glycosyl residue. If the arabinose residue were attached to any positions of the glucopyranose residue other than position 6, the signals of four protons should be expected in this region for (II) and the signals of three protons for (III).

On the basis of the facts given above, the structure of trans-cinnamyl O-(6'-O-α-L-arabinopyranosyl-β-D-glucopyranoside) or cinnamyl vicianoside (II) is proposed for rozavin, and that of trans-cinnamyl O-(6'-O-α-L-arabinofuranosyl-β-D-glucopyranoside) (III) for rozarin.



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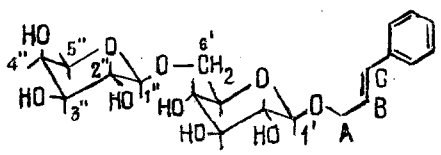

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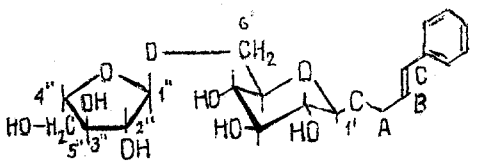

III

EXPERIMENTAL

For general information, see [1].

Isolation. The comminuted air-dry rhizomes of *Rhodiola rosea* L. were extracted with ethanol, and the evaporated extract was chromatographed on a column of Al_2O_3 with chloroform–methanol mixtures. The 15–20% methanol mixtures were rechromatographed repeatedly on silica gel likewise with mixtures of methanol and chloroform. The 5% methanolic eluates contained compound (I) (yield 0.1%); 10% methanol eluted compound (III) (yield 0.05%); and 15% methanol eluted substance (II) (yield 0.3%). Recrystallization from ethanol was used for the final purification of compound (II); (I) was additionally chromatographed on silica gel under the conditions described; and (III) was chromatographed on a column of Sephadex LH-20 with chloroform–methanol (93:7) as the eluent.

Cinnamyl O-β-D-Glucopyranoside (Rozin) (I). Colorless vitreous substance with the composition $C_{15}H_{20}O_6$, M^+ 296, $[\alpha]_D^{20}$ –44.8° [c 2.8; chloroform–methanol (1:1)], λ_{max}^{MeOH} 252 nm. TLC, R_f 0.57 (Silufol; chloroform–methanol–water (26:14:3)) (a); R_f 0.22 [Silufol; chloroform–methanol (6:1)] (b). PMR spectrum in acetone-d_6, ppm: 7.4–7.2 (m, 5 Ar–H); 6.7 (d, 16 Hz, H_C); 6.4 (dt, 6 and 16 Hz, H_B); 4.7–4.2 (dq, 6 and 13 Hz, 2 H_A); 4.46 (d, 7 Hz, H-1'); 4.0–3.3 (m, 6 H of a glucose residue).

The Tetraacetate of (I). PMR spectrum in benzene-d_6, ppm: 7.2–7.0 (m, 5 H); 6.5 (d, H_C); 6.2 (dt, H_B); 5.40–5.15 (m, 3 H); 4.5–3.9 (m, 5 H); 3.2 (m, H-5'); 1.75 (3 H); 1.72 (6 H); 1.70 (3 H) – singlets of aliphatic $-OCOCH_3$ groups.

Cinnamyl O-(6'-O-α-L-arabinopyranosyl-β-D-glucopyranoside) (Rozavin) (II). Colorless crystals with the composition $C_{20}H_{28}O_{10}$, mp 171–173°C, $[\alpha]_D^{20}$ –56.5° [c 0.7; chloroform–methanol (1:1)], λ_{max}^{MeOH} 252 nm (log ε 4.11). R_f 0.38 (a), 0.06 (b). PMR spectrum in pyridine-d_5, ppm: 7.4–7.2 (m, 5 Ar–H); 6.82 (d, 16 Hz, H_C); 6.5 (dt, H_B); 4.94 (d, 7 Hz, H-1'); 4.87 (d, 6 Hz, H-1"); 4.98–4.66 (m, 2 H_A); 4.55-3.95 (m, 10 H); 3.7 (q, 2 and 13 Hz, H-5"$_e$); TMS ether in benzene-d_6: 7.3–7.0 (5 Ar–H); 6.65 (H_C); 6.3 (H_B); 4.65 (dd, 6 and 12 Hz, 1 H_A); 4.5 (d, 6 Hz, H-1"); 4.4–3.3 (m, 12 H); 3.17 (q, 2 and 12 Hz, H-5"$_e$).

Hexaacetate of (II). Colorless crystals, mp 157–158°C (from ethanol), $[\alpha]_D^{20}$ –24.5° (c 1.0; acetone). PMR spectrum in benzene-d_6, ppm: 7.35-7.05 (5 Ar–H); 6.67 (H_C); 6.2 (H_B); 5.65–5.10 (m, 6 H); 4.55–4.10 (m, 4 H); 4.0–3.45 (m, 4 H); 3.06 (q, 2 and 12 H, H-5"$_e$); 1.85 (3 H); 1.80 (3 H); 1.77 (3 H); 1.74 (3 H); 1.69 (3 H); 1.64 (3 H) – singlets of aliphatic $-OCOCH_3$ groups.

Cinnamyl O-(6'-O-α-L-arabinofuranosyl-β-D-glucopyranoside) (Rozarin) (III). Colorless vitreous substance, $[\alpha]_D^{20}$ –76.1° [c 5.0; chloroform–methanol (1:1)], λ_{max}^{MeOH} 253 nm. R_f 0.44 (a), 0.11 (b). PMR spectrum in pyridine-d_5, ppm: 7.4–7.2 (m, 5 Ar–H); 6.78 (d, 16 Hz, H_C); 6.5 (dt, 6 and 16 Hz, H_B); 5.74 (d, 2 Hz, H-1"); 4.82 (d, 7 Hz, H-1'); 4.95–4.36 (m, 2 H_A); 4.3–3.9 (m, 11 H); in acetone-d_6: 7.50–7.25 (5 Ar–H); 6.74 (H_C); 6.4 (H_B); 5.05 (br.s, H-1");

4.6–3.2 (m, 14 H); TMS ether in benzene-d : 7.3–7.0 (5 Ar–H); 6.65 (H_C); 6.3 (H_B); 5.04 (d, 2 Hz, H-1"); 4.56 (dd, 6 and 12 Hz, 1 H_A); 4.4–3.3 (m, 13 H).

Hexaacetate of (III). PMR spectrum in benzene-d_6, ppm: 7.2–7.0 (5 Ar–H); 6.56 (H_C); 6.2 (H_B); 5.5–5.1 (m, 6 H); 4.6–4.0 (m, 5 H); 3.9–3.4 (m, 4 H); 1.76 (3 H); 1.74 (3 H); 1.72 (9 H); 1.64 (3 H) – singlets of aliphatic $-OCOH_3$ groups.

trans-Cinnamyl Alcohol (IV). Mass spectrum at 20°C, m/z (intensity, %): M^+ 134 (70), 115 (40), 105 (52), 92 (100), 91 (80), 78 (80), 77 (50). PMR spectrum in acetone-d_6, ppm: 7.4–7.2 (m 5 Ar–H); 6.65 (d, 16 Hz, H_C): 6.4 (dt, 6 and 16 Hz, H_B); 4.3 (m, 2 H_A).

Monoacetate of (IV). Mass spectrum at 20°C, m/z (intensity, %): M^+ 176 (44), 134 (70), 133 (57), 117 (66), 115 (100), 105 (87), 92 (62), 91 (63), 77 (60). PMR spectrum in chloroform-d, ppm: 7.4–7.2 (5 Ar–H); 6.7 (H_C); 6.3 (H_B); 4.75 (d, 7 Hz, 2 H_A); 2.14 (s, OCOCH).

Acetylation. This was performed in acetic anhydride in the presence of pyridine (20°C, 24 h). After the addition of ice water, the product was extracted with ether and was purified on a column of silica gel. The acetates of (I) and (IV) were eluted from the column with benzene and the acetate of (III) with benzene–acetone (9:1). The acetate of (III) was purified by recrystallization from ethanol without chromatography.

Enzymatic Hydrolysis. 1. A mixture of 50 mg of (I) and an aqueous solution of 15 mg of β-glucosidase was kept at 38°C for 24 h. Glucose was detected in the hydrolysate by paper chromatography. A chloroform extract was passed through a layer of silica gel, giving 20 mg of (IV) with R_f 0.83 (a), 0.64 (b).

2. A mixture of 3 mg of (VI) (or (VII)) with an aqueous solution of 1 mg of β-glucosidase was kept at 38°C for 24 h. In the hydrolysate of substance (VI) glucose and (IV) were detected, and in the hydrolysate of (VII) glucose and (V), with R_f 0.97 (a), 0.84 (b).

Acid Hydrolysis. 1. A mixture of 5 mg of (I) and 5 ml of 2% HCl was heated at 100°C for 30 min. Compounds (IV), (V), (VII), and glucose were detected by TLC and PC. Compounds (II) and (III) were hydrolyzed similarly. The same products were detected: (IV), (V), glucose, and arabinose.

2. A mixture of 30 mg of (II) (or (III)) was heated with 5 ml of 1% HCl for 15 min. In addition to (IV) and (V), two intermediate products were detected – (VI) and (VII) – and these were isolated by chromatography on silica gel. A 3% solution of methanol in chloroform eluted (IV) with R_f 0.61 (a), 0.39 (b); and 5% of methanol in chloroform eluted (VI) with R_f 0.59 (a), 0.24 (b). It must be mentioned that in all cases of acid hydrolysis the starting material was detected in the hydrolysate by TLC, but longer heating led to complete hydrolysis with the gradual disappearance of the highly volatile aglycone (IV).

SUMMARY

Three new glycosides of cinnamoyl alcohol have been isolated from the rhizomes of *Rhodiola rosea* L., and their structures have been established as follows: rozin (I) – trans-cinnamyl O-β-D-glucopyranoside; rozavin (II) – trans-cinnamyl O-(6'-O-α-L-arabinopyranosyl-β-D-glucopyranoside; and rozarin (III) – trans-cinnamyl O-(6'-O-α-L-arabinofuranosyl-β-D-glucopyranoside).

LITERATURE CITED

1. V. A. Kurkin, G. G. Zapesochnaya, and V. G. Klyazinka, Khim. Prir. Soedin., 581 (1982).
2. H. Schwarz, C. Köppel, and F. Bohlmann, Org. Mass. Spectrom., 7, 881 (1973).
3. C. Köppel and H. Schwarz, Org. Mass Spectrom., 11, 101 (1976).
4. G. G. Zapesochnaya, Khim. Prir. Soedin., 21 (1979).