UDC 547.918+547.568.5

RHODIOLOSIDE FROM RHODIOLA ROSEA AND RH. QUADRIFIDA. I

A. T. Troshchenko and G. A. Kutikova

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 4, pp. 244–249, 1967

Rhodiola rosea L. (Sedum rosea) (family Crassulaceae) is used by the indigenous population of the Altai as an agent for increasing vital activity [1].

Pharmacological studies carried out in the Department of Pharmacology of Tomsk Medical Institute have confirmed this information. The similarity of the pharmacological action of extracts of this plant to that of preparations of the ginseng–eleutherococcus group has been established, the characteristic stimulating action of preparations of Rh. rosea being more pronounced [2].

We have studied the roots of Rhodiola rosea L. and Rh. quadrifida (Pall.) Fisch. et Mey; the two plants have very similar roots and they were studied in parallel.

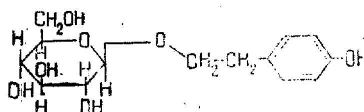
From an ethereal extract of Rh. rosea we isolated β-(p-hydroxyphenyl)-ethanol (tyrosol). The same substance was isolated from Rh. quadrifida.

Tyrosol is formed from tyrosine by bacteria [3], and by yeasts [4]; it is present in the Japanese beverage sake [5] and in plants of the family Oleaceae, Osmantus frangrans Lour. [6] and Ligustrum ovalifolium Hassk [7].

When an ethanolic extract of Rh. quadrifida was chromatographed on kapron, a second crystalline substance with the composition $C_{14}H_{20}O_7$ was obtained.

On the basis of the composition of the compound and its properties and IR spectrum, we have decided that it is a new glycoside and have called it rhodioloside. The hydrolysis of this substance with normal sulfuric acid gave tyrosol, while D-glucose was identified chromatographically.

To establish the structure of the glycoside, it was methylated exhaustively with methyl iodide in dimethylformamide [8] with the production of a pentamethyl derivative. Hydrolysis of the latter with normal hydrochloric acid gave β-(p-methoxyphenyl)ethanol and 2, 3, 4, 6-tetramethyl-D-glucose. Consequently, rhodioloside has the structure of the β-D-glucopyranoside of β-(p-hydroxyphenyl)ethanol.



The β-configuration of the glycosidic bond was established by comparing the specific rotation found with that calculated by Klyne's method [9], and also by an analysis of the differential IR spectra [10] (fig. 1). We have shown that rhodioloside is present in Rh. rosea in approximately the same amounts as in Rh. quadrifida. Rhodioloside is physiologically active, like an alcoholic extract of the roots of the plant.

Experimental

The IR spectra were taken on a UR-10 instrument in tablets of potassium bromide, and the UV spectra on a SFD-2 instrument in ethanol. The melting points were determined on a Boetius heated stage.

The glycoside and its genin were revealed on chromatograms by means of a 10% solution of antimony pentachloride in chloroform, and the monosaccharide and its methylated derivative with a solution of α-naphthol in 50% phosphoric acid.

Chromatography was carried out with type KSK silica gel (100–140 mesh) and the following systems of solvents: 1) toluene–ethyl acetate–ethanol (2 : 1 : 1); 2) 1-butanol–water–acetic acid (4 : 1 : 1); 3) acetone–1-butanol–water (7 : 2 : 1); 4) chloroform–methanol (80 : 1); and 5) petroleum ether–methyl ethyl ketone (3 : 1).

Isolation of tyrosol. Forty kilograms of the finely comminuted air-dry roots of Rh. rosea was extracted with ether to exhaustion. The residue after the elimination of the solvent weighed 1.1 kg. The extract (400 g) was treated with hot water (5 × 500 ml). The aqueous solution was extracted successively with chloroform, ether, ethyl acetate, and n-butanol. The residue from the ethereal solution (7.4 g) was extracted with hot chloroform (6 × 100 ml). On cooling, the extract deposited colorless needle-like crystals with mp 92–93° C.

The IR spectrum had absorption bands at 1650, 1520, and 3025 cm^{-1}, which are characteristic for a benzene ring, and also at 1240 and 1370 cm^{-1}, characteristic for a phenolic hydroxyl (Fig. 2). The UV spectrum had absorption maxima at 224 and 278 mμ (log ε 4.01 and 3.43, respectively), which are characteristic for a phenol.

On the basis of the elemental analysis, the substance has the composition $C_8H_{10}O_2$. These properties correspond to the literature data given for tyrosol [5].

The monoacetyl derivative of tyrosol. The substance obtained had mp 58° C. Literature data, mp 59° C. [6].

Dibenzoyl derivative of tyrosol. The derivative had mp 109–110° C. Literature data, mp 111° C [6].

The monophenylurethane of tyrosol. The substance melted at 148–149° C (from chloroform). Literature data, mp 149–150° C [4].

β-(p-Methoxyphenyl)ethanol. Tyrosol did not undergo methylation with diazomethane in ethereal solution. When it was methylated in n-heptane with an excess of diazomethane, a product with mp 23–24° C was formed. Literature data, mp 24° C [7].

Phenylurethane of β-(p-methoxyphenyl)ethanol. The phenylurethane was obtained in the usual way and had mp 127–128° C (from petroleum ether).

Found, %: N 5.24, 5.04, Calculated for $C_{16}H_{17}O_3N$, %: N 4.16.

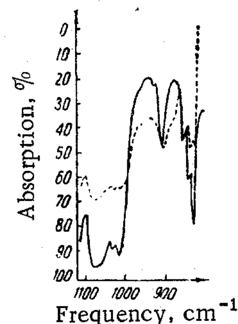


Fig. 1. Differential IR spectrum of the β-D-glucopyranoside of β-(p-hydroxyphenyl)ethanol against the spectrum of β-(p-hydroxyphenyl)ethanol. 1) The pure glycoside; II) the glycoside with an equivalent amount of β-(p-hydroxyphenyl)ethanol.

Isolation of tyrosol from Rh. quadrifida. The dry residue from the ethereal extract of 100 g of the comminuted roots was extracted with hot water. The aqueous solution was extracted with ether and the residue after the solvent had been driven off was recrystallized from chloroform. The weight of the substance was 0.1 g, mp 92–93° C. A mixture with an authentic sample gave no depression of the melting point. The IR spectrum of the substance and an authentic sample of tyrosol were identical.

Isolation of rhodioloside from Rh. quadrifida. 1. 300 g of the ground roots was extracted with ethanol in a Soxhlet apparatus. The extract was evaporated. The residue (120 g) was dissolved in 1 *l* of water. The aqueous solution was extracted with isobutanol. After the latter had been distilled off, the residue, weighing 28 g, was dissolved in methanol, mixed with 10 g of kapron, dried, and transferred to a column of kapron (4 × 30 cm, filled in water). Elution was carried out with distilled water, 500-ml fractions being collected. A total of six fractions was obtained. The process was monitored by thin-layer chromatography in systems 1 and 2.

The first two fractions mainly contained the glycoside with small amounts of four other substances. The weight of the material isolated was 3.47 g.

2. Sixteen kilograms of the ground roots was extracted successively by the infusion method with petroleum ether, chloroform, ethyl acetate, and methanol.

The residue from the methanolic extract (540 g) was dissolved in 2 *l* of water and filtered. The filtrate was treated with an excess of basic lead acetate. The filtrate from the lead salts was extracted with n-butanol (10 × 200 ml). The butanolic extracts were washed with 5% sodium sulfate solution (100 ml) and with water (2 × 100 ml), and the solvent was distilled off. The residue consisted of 38 g of a clear reddish mass; 30 g of the residue in 50 ml of water was transferred to a column of kapron (3 × 30 cm) and was eluted with distilled water; 100-ml fractions were collected and monitoring was carried out by thin-layer chromatography in system 1.

The glycoside and the four impurities were detected in the first five fractions. By its chromatographic behavior in systems 1, 2, and 3, one of the impurities was identified as arabinose. The combined fractions gave, after the elimination of the solvent, a residue weighing 22 g. For further purification, 10 g of this product in 15 ml of water was transferred to a column of Dowex-2 (HCO_3^- form) and was eluted with distilled water, 50-ml fractions being collected.

The arabinose and other impurities passed into the first three fractions and the glycoside into fractions 4–12; the latter was obtained, after elimination of the solvent, as crystals (7 g), mp 164–165° C [from absolute ethanol or from a mixture of absolute ethanol and benzene (50 : 100)]. It was very readily soluble in water and ethanol and sparingly

soluble in chloroform and benzene. In aqueous solutions it gave a deep blue coloration with ferric chloride. When it was deposited on a fresh slice of potato tuber, a pink coloration appeared. It had a bitter taste, $[\alpha]_D^{21}$ −32.38° (c 0.54; in water). According to Klyne: −22.11°.

The IR spectrum shows absorption bands at 1021 cm^{-1}, 1055, 1072 (pyranosides); 900 (β-glycosidic bond); 1520, 1600 (benzene ring); and 1250 cm^{-1} (phenolic hydroxyl) (Fig. 3).

The IR spectrum has absorption maxima at 222 and 278 mμ (log ε 3.88 and 3.26, respectively).

Found, %: C 56.11, 56.37; H 6.88, 6.31. Calculated for $C_{14}H_{20}O_7$, %: C 55.99; H 6.71.

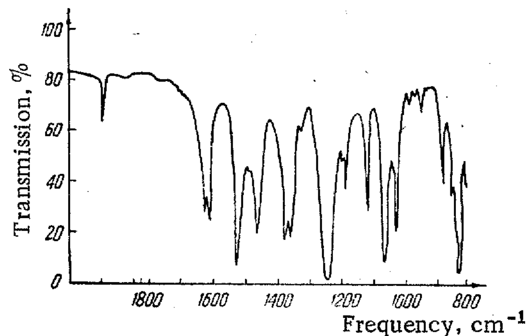


Fig. 2. IR spectrum of β-(p-hydroxyphenyl)ethanol in KBr, c 0.5.

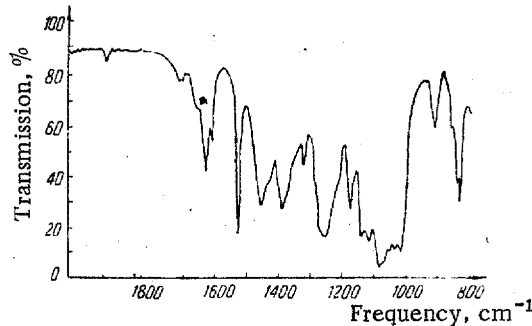


Fig. 3. IR spectrum of the β-D-glucopyranoside of β-(p-hydroxyphenyl)ethanol in KBr, c 0.5.

Hydrolysis of rhodioloside. A mixture of 0.1 g of the substance and 10 ml of 1 N sulfuric acid was heated in the boiling water bath for 2 hr 30 min. The reaction mixture was extracted with ether, and the ethereal extract was washed with a solution of sodium hydrogen carbonate and with water, and was dried and evaporated to dryness. Needle-like crystals with mp 92–93° C (from chloroform) were obtained. The melting point of a mixture with tyrosol showed no depression. Their identity was confirmed by thin-layer chromatography in systems 1 and 2 and also by their IR spectra, which were completely identical.

After being extracted with ether, the aqueous solution was neutralized with Dowex-2 (HCO_3^- form). On thin-layer chromatography in systems 1 and 2, only glucose was identified.

Methylation of rhodioloside. A mixture of 2 g of rhosioloside, 60 ml of dimethylformamide, 12 ml of methyl iodide, and 8 g of barium oxide was stirred at room temperature. After 50 min, the temperature of the reaction mixture began to rise. It was kept at 30° C and stirring was continued for 5 hr.

Chromatographic analysis in system 5 showed that methylation had taken place incompletely. The reaction mixture was filtered, the residue was washed with a few milliliters of dimethylformamide, and the filtrate was treated with 6 ml of methyl iodide and 5 g of silver oxide [8] and was stirred at room temperature for another 24 hr. On chromatographic analysis in system 4, it was found that methylation had now taken place completely.

The precipitate was filtered off and washed with a small amount of dimethylformamide. The filtrate was poured into a mixture of 150 ml of a saturated aqueous solution of sodium hydrosulfite and 150 ml of chloroform and it was then carefully stirred and filtered. The chloroform layer was separated off, and the aqueous layer was extracted with chloroform. The combined chloroform solutions were dried over sodium sulfate and evaporated. A clear, light colored oil was obtained; weight 1.3 g, yield 53%. On standing in the refrigerator, the product crystallized. After recrystallization, from petroleum ether, colorless plates were obtained with mp 33–33.5° C, $[\alpha]_D^{25}$ −15.0° (c 1; in chloroform). UV spectrum: 224, 276, 284 mμ (log ε 3.98, 3.18, 3.10, respectively).

Found, %: C 61.90, 61.58; H 8.32, 8.20; OCH_3 41.59, 42.02. Calculated for $C_{19}H_{30}O_7$, %: C 61.60; H 8.16; OCH_3 41.9.

Hydrolysis of pentamethylrhodioloside. A solution of 0.5 g of the methylation product in 50 ml of methanol was treated with 50 ml of N hydrochloric acid. The alcohol was slowly distilled off in the water bath. The residue (35 ml) was treated with 15 ml of water and the mixture was heated at 100° C for 5 hr. It was then extracted with chloroform (5 × 50 ml), the combined extracts were washed with water (2 × 20 ml) and dried with sodium sulfate, and the solvent was distilled off. The weight of residue was 0.45 g, which corresponds to 90% of theory.

In 30 ml of petroleum ether, 0.45 g of the mixture of products was transferred to a column of silica gel (1.5 × × 30 cm) and was successively eluted with 100 ml of a mixture of petroleum ether and ether (4 : 1), 200 ml of ether,

and then 200 ml of ether–methyl ethyl ketone (4 : 1). Fractions of 10 ml were collected. The methylated aglycone was found in the ether and the methylated sugar in the ether–methyl ethyl ketone fraction. The separation of the fractions was monitored by thin-layer chromatography in system 5. The hydrolysis products were identified chromatographically as β-(p-methoxyphenyl)ethanol and 2, 3, 4, 6-tetramethyl-D-glucose.

The β-(p-methoxyphenyl)ethanol was converted in the usual way into the monophenylurethane, a mixture of which with a sample obtained independently from tyrosol gave no depression of the melting point. The identity of the two samples was confirmed by a direct comparison of their IR spectra.

2, 3, 4, 6-Tetramethyl-D-glucose. The methylated sugar obtained by the hydrolysis of the pentamethylrhodioloside was isolated as described above and recrystallized from petroleum ether, mp 90–93° C (determined in a capillary), $[\alpha]_D^{23}$ +84.11° (c 0.931; in water). Literature data for 2, 3, 4, 6-tetramethyl-D-glucose: mp 93° and 96°C [11], $[\alpha]_D^{20}$ +83.3° [12].

Investigation of a methanolic extract of Rh. rosea. When Rh. rosea was extracted by method 1, this glycoside was found chromatographically in systems 1 and 3 in the same amount as in Rh. quadrifida.

Summary

1. Rh. quadrifida (Pall.) Fisch. et Mey has yielded a new glycoside which has been called rhodioloside and for which the structure of the β-D-glucopyranoside of β-(p-hydroxyphenyl)ethanol has been established.

2. Rhodioloside is also present in Rh. rosea L.

3. β-(p-Hydroxyphenyl)ethanol, which is the aglycone of the glucoside isolated, has been obtained from both plants.

REFERENCES

1. T. F. Morina and T. P. Prishchep, Izv. Sib. otd. AN SSSR, ser. biologo-med. nauk, part 1, no. 4, 49, 1964.
2. M. I. Zotova, G. V. Krylov, and A. S. Saratikov, Izv. Sib. otd. AN SSSR, ser. biologo-med. nauk, part 2, no. 8, 111, 1965; A. S. Saratikov, T. F. Morina, and I. M. Kaliko, Izv. Sib. otd. AN SSSR, ser. biologo-med. nauk, part 2, no. 8, 120, 1965.
3. W. Grimmer and B. Wiemann, Forsch. geb. Milchwirtsch. Molkereiwis., 1,2, 1921; C., 1, 775, 1921; W. Grimmer and B. Wonschkuhn, Milchwirtsch. Forsch., **20**, 110, 1939; C., **11**, 4390, 1939.
4. F. Ehrlich, Ber., **40**, 1047, 1907; **44**, 139, 1911; F. Ehrlich and P. Pistchimuka, Ber., **45**, 2428, 1912; P. S. Pishchimuka, ZhRFKhO, **48**, 1, 1916.
5. M. Yukawa, J. Coll. Agric., Tokyo, **5**, 291, 1944; C. A., **46**, 2747, 1952; Repts. Sci. Research Inst. (Japan), **27**, 257, 1951; C. A., **46**, 6319, 1952.
6. T. Ishiguro, N. Kogo, K. Takamura, and T. Muruome, J. Pharm. Soc., Japan, **75**, 781, 1955; C. A., **49**, 16358, 1955.
7. W. C. Veer, P. J. Ond, and J. E. Ribbers, Rec. Trav., Chim., **76**, 810, 1957.
8. R. Kuhn, H. Trischmann, and J. Low, Angew. Chem., **67**, 32, 1955; R. Kuhn and H. H. Boer, Ann., 611, 236, 1958; H. G. Walker, Jr., M. Gee, and R. M. McGreody, J. Org. Chem., **27**, 2100, 1962.
9. W. Klyne, Biochem., J., **47**, 41, 1950; E. A. Braude and F. C. Nachod, Determination of Organic Structure by Physical Methods, 94, 1955.
10. I. P. Kovalev and V. I. Litvinenko, KhPS [Chemistry of Natural Compounds], 233, 1965.
11. W. H. Haworth, C. W. Long, and J. H. G. Plant, J. Chem. Soc., 2909, 1927; J. C. Irvine and J. W. Oldham, J. Chem. Soc., **119**, 1748, 1921.
12. W. H. Haworth and G. G. Leith, J. Chem. Soc., **121**, 1921, 1922.

27 October 1966

Novosibirsk Institute of Organic Chemistry
Siberian Division AS USSR