6-O-GALLOYLARBUTIN FROM *Rhodiola coccinea*

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We have previously shown the presence in the epigeal parts of *Rhodiola coccinea* (Royle) A. Boris of arbutin and hydroquinone and have reported the isolation of a crystalline mixture of substances [1]. By rechromatography on a column of polyamide sorbent with discrete-gradient elution with chloroform–ethanol (9:1; 8:2) we have isolated in the individual state a phenolic glycoside with the composition $C_{19}H_{20}O_{11} \cdot 1.5\ H_2O$, forming snow-white crystals with mp 245-246°C (from water), $[\alpha]_D^{24}$ −32° (c 0.5; ethanol), readily soluble in ethanol and acetone, highly soluble in hot water, slightly soluble in ethyl acetate, and insoluble in ether, chloroform, and benzene. In the UV spectrum of the glycoside, absorption maxima are observed at 218 and 280 nm (log ε 4.32; 3.90) while on the addition of 0.1 N KOH the maxima appear at 241 and 280 nm (log ε 4.03, 3.77). In its physicochemical properties, apart from the melting point, the glycoside is close to one of the galloyl derivatives of arbutin [2].

The discrepancy between the melting point found and that given in the literature required an accurate determination of the structure of the glycosides that we had isolated.

The IR spectrum of the glycoside shows the following absorption bands (cm^{-1}): 3300-3500 (OH group), 1710 (ester C=O), 1625, 1553, 1521 (aromatic ring), 1230, 1215 (phenolic hydroxyls), 1110, 1075, 1040 (pyranose form of the sugar residue), 877 (β-glycosidic bond), 832, 774 (p- or o-substitution in a benzene nucleus). The β-configuration of the glycosidic bond was confirmed by the results of enzymatic cleavage of the glycoside with emulsin. Similar absorption bands have been found in the IR spectrum of an authentic sample of arbutin. The UV spectrum of arbutin ($\lambda_{max}^{CH_3OH}$ 220, 283 nm) shows the presence of a chromophoric group in common with the substance under investigation.

The products of the acid hydrolysis of the substance were identified as hydroquinone, gallic acid, and D-glucose. On incubation with an enzyme preparation from *Aspergillus oryzae* [3], in addition to the substances mentioned, arbutin and galloylglucose were detected.

The alkaline cleavage of the glycoside formed arbutin, but the gallic acid split off was not detected because of the occurrence of oxidative degradation, as was confirmed in model experiments with arbutin + gallic acid.

The mass spectrum of the substance showed the peaks of ions with m/e 314, 262, 218, 200, 170, 153, 144, 125, 110. The molecular peak was not recorded. The fragment with m/e 314 arises from the molecular ion by the splitting out of a molecule of hydroquinone.

The PMR spectrum (Fig. 1) of the trimethylsilyl ether of the glucoside contains two doublets in the region of aromatic protons at 6.82 ppm (J = 9.5 Hz) and 6.58 ppm (J = 9.5 Hz), the first of which is due to the H-2' and H-6' protons and the second to H-3' and H-5' of a substituted hydroquinone. A two-proton singlet at 7.14 ppm relates to the aromatic protons H-2" and H-6" of gallic acid. The protons of D-glucose give signals at 3.52 ppm (2a, 3a, 4a), 4.17, 4.44, and 4.68 ppm. The last includes the signal of the anomeric proton, but it appeared impossible to determine the spin–spin coupling constant, obviously because of the existence of free rotation of the D-glucose, which leads to a complex multiplet signal.

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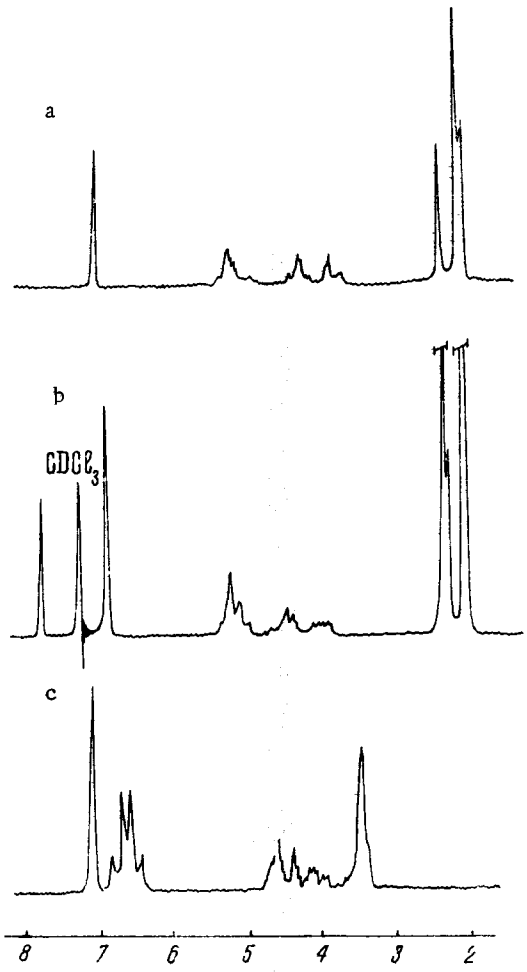
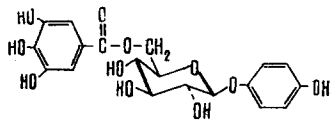


Fig. 1. PMR spectra of arbutin acetate (CCl_4) (a), 6-O-galloylarbutin-acetate-$CDCl_3$) (b), and the trimethylsilylether of 6-O-galloylarbutin (CCl_4) (c).

The position of attachment of the gallic acid residue was established by studying the PMR spectra of the acetyl derivatives of the galloylarbutin isolated and of free arbutin. The spectrum of arbutin acetate contained singlets characteristic for one phenolic acetyl of hydroquinone (2.21 ppm, s), for three acetyls of D-glucose in positions 2e, 3e, and 4e (2.00 ppm, s), and an acetyl D-glucose in position C_6 (1.97 ppm, s). The spectrum of the galloylarbutin acetate showed singlets relating to three acetyls of the galloyl residue (2.27 ppm, s), one hydroquinone acetyl (2.22 ppm, s), and three acetyls of D-glucose in positions 2e, 3e, and 4e (2.01 ppm, s). The absence of a singlet at 1.97 ppm shows that the galloyl radical was present at C_6 of the D-glucose. Consequently, the glycoside investigated is 1-O-p-hydroxyphenyl-6-O-galloyl β-D-glucopyranoside and has the following strcture:



EXPERIMENTAL METHOD

The UV spectra were obtained on a Spectromom 202 spectrophotometer, the IR spectra on UR-10 instrument (KBr tablets), the mass spectra on an MKh-1303 instrument, and the PMR spectra on a Hitachi R-20A spectrometer. The optical activities were determined on an SM circular polarimeter. Chromatography was performed on paper of type "M" ["slow"] (Leningrad paper mill No. 2) in a nonfixed thin layer of alumina (activity grade II, neutral) in the following solvent systems: 1) butan-1-ol—ethanol—water (5:1:2); 2) 15% acetic acid; 3) 60% acetic acid; 4) butan-1-ol—pyridine—water (6:4:3); and 5) ethyl acetate—pyridine—water (2:1:2); the usual reagents were used for showing up the spots of the phenolic substances and carbohydrates [4].

Isolation of 6-O-Galloylarbutin. The comminuted roots of *Rhodiola coccinea* (1.6 kg) were treated successively with petroleum ether, chloroform, and diethyl ether in order to free them from lipophilic substances, and they were then exhaustively extracted with 96% ethanol. The ethanolic extract was evaporated in vacuum on the boiling water bath, the residue was dissolved in water, and the solution was treated repeatedly with ethyl acetate. The combined organic extract was evaporated, and the resulting dry residue (169 g) was separated chromatographically on a column of polyamide sorbent (85 × 5 cm). The eluting solvents were water and then mixtures of water and ethanol. Arbutin, hydroquinone, and fraction (I) containing a mixture of phenolic compounds (25 g) were isolated successively; fraction I was rechromatographed on polyamide which had been pretreated with a 1% solution of caustic potash [5]. Elution of the column with chloroform—ethanol (9:1 → 7:3) yielded fraction II containing three phenolic substances (TLC, R_f (1) 0.40, 0.50, and 0.60). The dry residue obtained after the evaporation of fraction II was dissolved in a small amount of water. On standing, crystals of galloyl arbutin separated out, and these were recrystallized: $C_{19}H_{20}O_{11}$, mp 245-246°C (from water) PC, R_f 0.61 (2) and 0.55 (3).

Subsequent elution with chloroform—ethanol (6:4) yielded gallic acid, $C_7H_6O_5$, mp 240-241°C, R_f 0.54 (2).

Acid Hydrolysis. A mixture of 40.8 mg of galloylarbutin and 5 ml of 10% H_2SO_4 was heated at 96-100°C for 3 h. The hydrolysis products were extracted with chloroform and then with ether. On evaporation, the chloroform extract deposited crystals of hydroquinone (mp 169-170°C); hydroquinone and gallic acid were found in the ethereal solution [TLC, R_f (1) 0.86 and 0.03, respectively]. The hydrolyzate was neutralized with EDE-10P anion-exchange resin (OH^-) and was analyzed by paper chromatography (systems 4 and 5). The carbohydrate component was identified as D-glucose.

Enzymatic Hydrolysis. With heating, 22.2 mg of galloylarbutin was dissolved in 3 ml of water, and after cooling a solution of an enzyme preparation from the fungus *Aspergillus oryzae* (1:2) was added, followed by three drops of toluene, and the mixture was thermostated at 37°C. Every 3 h, samples were taken and were analyzed by paper chromatography and also in a thin layer. Arbutin and gallic acid were detected at first, and then hydroquinone, D-glucose, and, probably, galloylglucose [PC, R_f (2) 0.67].

Alkaline Cleavage. A mixture of 45 mg of galloylarbutin and 4 ml of a 0.5 N solution of KOH was heated at 100°C in a current of nitrogen for 35 min. The reaction mixture was neutralized with dilute HCl and extracted with ether. Arbutin was found among the cleavage products.

Pentaacetylgalloylarbutin. A mixture of 29 mg of galloylarbutin, 1.5 ml of freshly distilled anhydride, and 1.5 ml of anhydrous pyridine was left at room temperature for 24 h. Then it was poured into ice water and the white amorphous precipitate that deposited was filtered off and washed with water and, after drying, it was crystallized from chloroform–petroleum ether (1:4). Acicular crystals, $C_{33}H_{34}O_{18}$, mp 139-140°C.

SUMMARY

A phenolic glycoside for which the structure of 1-O-p-hydroxyphenyl-6-O-galloyl-β-D-glucopyranoside has been established has been isolated from the epigeal organs of the *Rhodiola coccinea* (Royle) A. Bor.

Arbutin and its 6-O-galloyl derivative are the dominating phenolic components of the plant.

LITARATURE CITED

1. T. G. Khoruzhaya and E. A. Krasnov, Khim. Prirodn. Soedin., 677 (1972).
2. G. Britton and L. Haslam, J. Chem. Soc., 7312 (1965).
3. P. I. Gvozdyak and V. I. Litvinenko, Med. Prom. SSSR, No. 5, 16 (1964).
4. I. Hais and K. Macek, Paper Chromatography, 3rd English ed., Academic Press, New York (1963).
5. V. N. Spiridonov, Khim. Farmats. Zh., No. 5, 43 (1968).