PLANT POLYPHENOLS

I. Study of the Chemical Composition of the Leaves of Rhododendron Aureum Georgi

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Rhododendron aureum Georgi (Rh. chrysanthum Pall.) – goldmat rhodendron (kashkara) – is a small evergreen shrub of the mountain regions of Eastern Siberia and the Far East. An infusion of the leaves of this plant collected during the flowering season (May-June) is used in popular medicine for the treatment of rheumatism and other diseases [1]. Various types of rhodendron have been shown to contain ursolic acid [2], arbutin [3], rhodendrin [3], andromedotoxin [4, 5] and flavonoids and anthocyanins [6, 7].

Table 1

UV Spectra in Various Media

Substance	Ethanol							
	Absolute		+NaOAc		+H_3BO_3+NaOAc		+NaOEt	
	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε	λ_{max}, mμ	log ε
Flavonoid (hyperoside)	258	4.31	270	4.34	264	4.35	275	4.21
	—	—	326	4.03	—	—	—	—
	365	4.24	383	4.19	386	4.31	419	4.32
Aglycone (quercetin	257	4.28	277	4.29	260	4.39	331	4.23
	375	4.32	—	—	387	4.44	—	—
3', 4', 5, 7-Tetramethylquercetin	252	4.36	251	4.35	275	4.13	278	4.05
	360	4.38	361	4.23	360	3.80	345	3.42
Penta-acetate of the aglycone	255	4.27	—	—	—	—	—	—
	299	4.21	—	—	—	—	—	—

We have studied the leaves of the goldmat rhododendron provided by the botanical expedition of VILAR [All-Union Scientific Research Institute for Medicinal and Aromatic Plants]. By paper chromatography, the alcoholic extract was shown to contain two flavonoids and two substances of a phenolic nature. To isolate them, the raw material was extracted with methanol, and the residue after evaporation was dissolved in water and was extracted successively with ether and ethyl acetate. By paper chromatography the ethereal solution was found to contain quercetin and rhododendrol [8]. Two substances were isolated from the ethyl acetate solution:

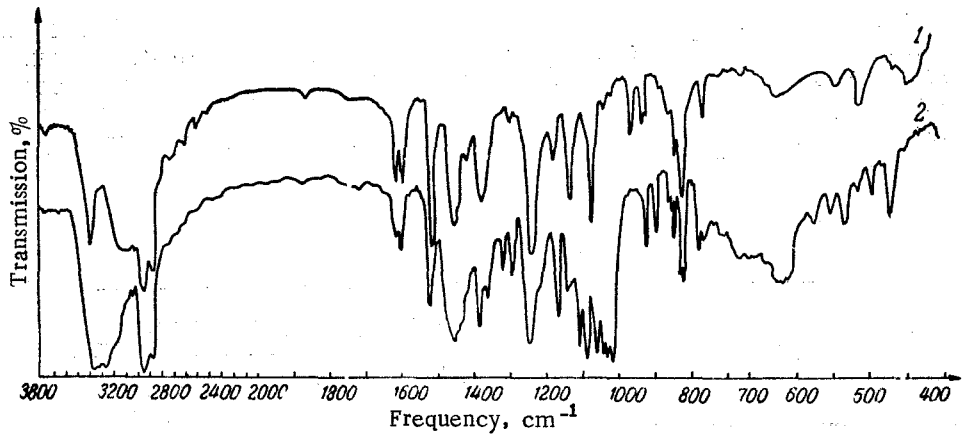
1) Bright yellow crystals with the composition $C_{21}H_{20}O_{12} \cdot 1/2H_2O$ with mp 233-234°. Hydrolysis in an acid medium gave the aglycone with the empirical formula $C_{15}H_{10}O_7$, mp 315-316°, which, from the R_f values, and the IR and UV spectra (Table 1), and also from the constants of the penta-acetate, was identical with quercetin, which we prepared from rutin. From the melting point of the osazone and the R_f values in three systems of solvent, the sugar was identified as galactose. Methylation of the glycoside with the subsequent splitting off of the carbohydrate residue led to 3', 4', 5, 7-tetramethylquercetin. From a comparison of the results obtained with the literature, it was established that the substance isolated was hyperoside [3];

2) Colorless crystals of composition $C_{16}H_{24}O_7$, mp 190.0-190.5°. The acid hydrolysis of this substance gave a compound with the empirical formula $C_{10}H_{14}O_2$ and D-glucose, identified chromatographically and by the production of the osazone. The physicochemical constants of the substance that we had isolated and its aglycone correspond to data given in the literature [8, 9] for rhododendrin, p-hydroxy-(γ-glucosyloxybutyl)-benzene, and rhododendrol, p-hydroxy-(γ-hydroxybutyl)-benzene.

The IR spectra given (figure) do not contradict this structure for the compounds obtained.

Experimental

Isolation of the total substances. 1.2 kg of the dried and comminuted leaves of the goldmat rhododendron was extracted successively with petroleum ether (three 6-liter portions) and methanol (three 6-liter portions). The combined methanolic extract was evaporated in vacuum, and the residue was treated with 0.6 liter of water and heated at 45-50° for 1 hr. The aqueous solution was left for two days at 2-5°. The precipitate which was deposited was separated off, and the filtrate was exhaustively extracted with diethyl ether (6 liters) and then with ethyl acetate (7.5 liters). The cyanidine reaction was positive for both extracts.



IR transmission spectra of rhododendrin (1) and rhododendrol (2).

Evaporation of the ethereal solution gave 16.2 g of a resinous residue in which, by paper chromatography in two systems with reference substances, quercetin and rhododendrol were identified (Table 2). The preparative separation of the ethereal fraction was not carried out because of the small amount of these substances in it.

The following systems of solvents were used for chromatography (descending chromatography): 1) n-butyl alcohol – acetic acid – water (4 : 1 : 5); 2) ethyl acetate – formic acid–water (10 : 2 : 3); 3) ethyl acetate-pyridine-water 80% acetic acid (8 : 3 : 8 : 0.5); 4) n-butyl alcohol – benzene – pyridine – water (5 : 1 : 3 : 3). The time of chromatography was 24-48 hr. Detecting agents: for flavonoids, irradiation with UV light and spraying with a 1% solution of aluminium chloride in alcohol; for sugars, aniline phthalate; for phenolic substances (rhododendrin and rhododendrol), diazotized sulfanilic acid + 20% aqueous sodium carbonate solution.

Isolation of hyperoside. After the evaporation of the solvent in vacuum, the ethyl acetate extract gave 42.5 g of a yellowish powder of the total substances, the recrystallization of which from alcohol gave 4.7 g (0.39%) of a flavonoid in the form of yellowish crystals having, after recrystallization, mp 233-234°; R_f in system 1, 0.55, in system 2, 0.64. The maxima of the UV spectra are given in Table 1.

Table 2

Results of Paper Chromatography

Substance	R_f in the systems		Coloration		
	1	2	In UV light	$AlCl_3$, in UV light	Diazonium reagent + Na_2CO_3
Quercetin	0.75	0.74	Yellow	Yellow-green	Brown
Rhododentrol	0.91	0.95	—	—	Bright red

The IR spectra had absorption bands characteristic for a carbonyl group (1660 cm^{-1}) and for hydroxyl groups (3330 and 3470 cm^{-1}).

Found, %: C 59.13, 59.10; H 3.57, 3.69. Calculated for $C_{15}H_{10}O_7$, %: C 59.61; H 3.33.

Hydrolysis. Production of quercetin. A mixture of 0.2528 g of the compound with mp 233-234° and 20 ml of 3% sulfuric acid was heated at 98° for 2 hr. After cooling, the deposit was separated off, washed with water to neutrality,

and dried in vacuum at 65° to constant weight. Yield 0.1634 g (64.6%, theoretical on the basis of 1 mole of hexose per 1 mole of quercetin, 65%). After recrystallization from 20% alcohol, a substance with mp 315-316° was obtained, the R_f values of this being given in Table 2 and the maxima of the UV spectra in Table 1.

Found, %: C 59.13, 59.10; H 3.57, 3.69. Calculated for $C_{15}H_{10}O_7$, %: C 59.61; H 3.33.

The penta-acetate of the aglycone (mp 197.5-198.5°) gave no depression of the melting point in admixture with quercetin penta-acetate.

Found, %: 58.60, 58.24; H 4.06, 3.88. Calculated for $C_{25}H_{20}O_{12}$, %: C 58.60; H 3.93.

The hydrolysis liquid was passed through a column of the anion-exchanger "Dowex" 1 × 4 in the HCO_3^- form. The neutral aqueous solution was concentrated in vacuum and deposited on a chromatogram in the presence of a series of reference sugars. The R_f values in systems 1, 3, and 4 of the sugar obtained coincided with the values for galactose in these systems.

The osazone, mp 187-188°, showed no depression of the melting point in admixture with the osazone of galactose.

Methylation and hydrolysis, 3', 4', 5, -7-tetramethylquercetin. A mixture of 0.5 g of the glycoside (mp 233-234°), 4 g of calcined potassium acetate, 50 ml of acetone, and 5 ml of freshly-distilled dimethyl sulfate was heated at the boil for 5 hr. After cooling, the solution was filtered, 50 ml of 3% sulfuric acid was added, and the mixture was heated to the boil for 3 hr. The acetone was distilled off and the aqueous solution was cooled. The precipitate was filtered off, washed on the filter with water (two 5-ml portions), and recrystallized from alcohol. Mp 197-198°; R_f in system 2, 0.84. The maxima of the UV spectra are given in Table 1.

Found, %: C 63.46, 63.40; H 5.27, 5.40; OCH_3 34.96, 34.22. Calculated for $C_{19}H_{18}O_7$, %: C 63.68; H 5.06; OCH_3 34.62.

Isolation of rhododendrin. The filtrate, after the removal of the hyperoside in the recrystallization of the total (ethyl acetate extract), was evaporated in vacuum, and the residue was recrystallized from 20% alcohol. This gave 3.4 g (0.28%) of colorless crystals with mp 190.0-190.5°, R_f 0.70 in system 1, 0.80 in system 2; $[\alpha]_D^{20}$ −43.4° (c 0.8; 10% alcohol). The IR spectrum is given in Fig. 1, curve 1.

Found, %: C 58.61, 58.10; H 7.66, 7.28; H_{act} 1.53, 1.48. Calculated for $C_{16}H_{24}O_7$, %: C 58.53; H 7.37; H_{act} 1.535.

Hydrolysis. Production of rhododendrol. A mixture of 3.10 g of rhododendrin (mp 190°) and 50 ml of 5% sulfuric acid was heated at 98° for 3 hr. The cooled solution was extracted with ether (five 25-ml portions). The combined extracts were dried with magnesium sulfate. The ether was driven off. This gave 1.50 g (48.5%, theoretical 50.6%) of material. After recrystallization from benzene, a substance was isolated with mp 81.5-82.0°; R_f in system 1, 0.91, in system 2, 0.95.

The IR spectrum (Fig. 1, curve 2) had a sharp peak at 3380 cm^{-1}, which is characteristic for a free OH group, and two other bands, confirming the structure of the compound.

Found, %: C 72.38, 72.26; H 8.41, 8.57, H_{act} 1.30, 1.23. Calculated for $C_{10}H_{14}O_2$, %: C 72.25; H 8.50; H_{act} 1.21.

The acid hydrolysis solution was neutralized on a column of the anion-exchanger "Dowex" 1 × 4 (HCO_3^-) and evaporated in vacuum. The results of paper chromatography of the sugar isolated in comparison with various samples of hexoses showed its identity with glucose in three systems of solvents (1, 3, 4).

The osazone (mp 202-202.5°) gave no depression of the melting point with glucose osazone.

The UV spectra were taken on a SF-4 spectrophotometer by Jurd's method [10, 11] and the IR transmission spectra on a UR-10 spectrometer in liquid paraffin.

In all cases, the paper chromatography was carried out with Leningrad type "M" paper.

Summary

Hyperoside and rhododendrin have been isolated from the leaves of Rhododendron aureum Georgi; the presence of quercetin and rhododendrol has been shown by paper chromatography.

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