FLAVONOIDS OF Asperula oppositifolia

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From the herb Asperula oppositifolia Rgl. et Schmalh., family Rubiaceae, gathered in June, 1969 in the environs of the settlement of Daraut-Kurgan, Kirghiz SSR, we have isolated a new glycoside, and we have named it oppositifolin.

The IR spectrum of oppositifolin has, in addition to bands at 1670 cm^{-1} (=C=O) and 2940 cm^{-1} ($-OCH_3$) a band at 1715 cm^{-1} characteristic for an ester grouping [1]. When the glycoside was saponified with a dilute solution of alkali, desacetyloppositifolin was formed. No aromatic acids were found in the saponification products. Assuming that the acyl substituent may be represented by the residue of an aliphatic acid, we performed the hydroxylaminolysis [2] of oppositifolin; in the cleavage products we found acethydroxamic acid by chromatography. The hydroxylaminolysis of ethyl acetate was performed in parallel as a control. Similar results were obtained.

Hydrolysis of oppositifolin gave an aglycone with the composition $C_{16}H_{12}O_6$, appearing on a chromatogram in the form of a dark brown spot, which permitted its provisional assignment to the group of flavones and also D-mannose and D-glucose.

On the basis of the elementary composition of the aglycone and its triacetate [3], it may be assumed that it contains one methoxy group and three free hydroxy groups. A quantitative determination by the Zeisel method [4] showed the presence of one methoxy group, and luteolin was isolated from the reaction mixture [5].

By UV spectroscopy with various ionizing and complex-forming reagents [6], free hydroxy groups were found in the aglycone in the 4', 5, and 7 positions (Table 1).

The products of alkaline cleavage [7] were found to contain phloroglucinol and vanillic acid, which shows the presence of a methoxy group at C_3' of the aglycone.

Thus, the structure of the aglycone may be represented as 4',5,7-trihydroxy-3'-methoxyflavone (chrysoeriol). A comparison of physicochemical properties and the absence of a depression of the melting point of a mixture of the aglycone and an authentic sample of chrysoeriol [5] confirmed their complete identity.

To determine the position and sequence of addition of the sugar residues in oppositifolin we used the results of a comparison of the UV spectra of the bioside and the aglycone [6], and also stepwise acid hydrolysis and exhaustive methylation of the initial glycoside [8].

The absence of a bathochromic shift in the UV spectra of the glycoside, in contrast to the aglycone, or the addition of sodium acetate and the formation of 7-hydroxy-3',4',5-trimethoxyflavone as a result of the hydrolysis of the product of complete methylation of oppositifolin shows that the sugar residue is located at C_7.

Stepwise acid hydrolysis of the bioside gave as intermediate product a glucoside of chrysoeriol and D-mannose. Its subsequent treatment with rhamnodiastase led to the formation of the aglycone and D-glucose. Consequently, the D-glucose is attached to the aglycone directly, and the D-mannose is the terminal sugar.

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TABLE 1. Spectral Characteristics of the Flavonoids of Asperula oppositifolia

Substance	Absorption bands	Ethanolic solution	The same + zirconyl nitrate		The same + $Zr(NO_3)_4$ + citric acid		The same + sodium ethoxide		The same + sodium acetate		The same + sodium acetate + boric acid	
		λ_{max}, nm	λ_{max}, nm	$\Delta\lambda$	λ_{max}, nm	$\Delta\lambda$	λ_{max}, nm	$\Delta\lambda$	λ_{max}, nm	$\Delta\lambda$	λ_{max}, nm	$\Delta\lambda$
Oppositifolin	I	350	395	45	355	5	425	75	355	5	355	5
	II	255	290	35	250	−5	265	10	255	0	255	0
Desacetyloppositifolin	I	355	395	40	355	0	425	70	355	0	360	5
	II	255	285	30	250	−5	265	10	255	0	255	0
Monoside	I	350	390	40	350	0	425	75	350	0	355	5
	II	255	285	30	255	0	265	10	255	0	255	0
Aglycone	I	350	395	45	355	5	420	70	355	5	350	0
	II	250	290	40	250	0	270	20	270	20	250	0

To determine the position of attachment of the D-mannose to the D-glucose, we used the periodate oxidation of the glycoside under investigation, and also its enzymatic and alkaline hydrolysis. The resistance of oppositifolin to cleavage with rhamnodiastase, in contrast to glycosides with a 1 → 6 linkage between the sugars [9], the destruction of both sugars on periodate oxidation [10], showing the absence of a 1 → 3 bond, and its stability to alkaline hydrolysis [11] give grounds for assuming that the D-mannose is attached to the D-glucose by a 1 → 2 linkage.

The sizes of the oxide rings and the configurations of the glycosidic bonds were determined by a comparison of molecular rotations and by IR spectroscopy [12, 13]. It was established that the D-glucose is attached to the aglycone and the D-mannose to the glucose by β-glycosidic linkages, and both sugars have pyranose oxide rings. The latter conclusion is satisfactorily confirmed by the rate of acid hydrolysis [14].

The results of a spectroscopic investigation of the glycoside and of the alkaline cleavage of the aglycone show that the acetyl group in oppositifolin is attached to one of the sugar components, and the high negative specific rotation [2] and the absence from the IR spectrum of the glycoside of a band at 1020-1030 cm^{-1} [15] permit the assumption that it is located at C_6 of the glucose. Thus, the most probable structure of oppositifolin may be represented as chrysoeriol 7-O-β-D-mannopyranosyl-(1 → 2)-β-D-(6-acetylglucopyranoside).

EXPERIMENTAL

The following systems of solvents were used for the analysis of oppositifolin: 1) water-saturated phenol; 2) 15% acetic acid; 3) ethyl acetate–formic acid–water (10 : 2 : 3); 4) chloroform–formamide; 5) chloroform–acetic acid–water (13 : 6 : 1); and Filtrak No. 12 chromatographic paper. The UV spectra were recorded on an SF-4A spectrophotometer and the IR spectra on a UR-20 spectrophotometer. The optical rotations were measured on an SPU-M instrument. The melting points were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Isolation of Oppositifolin. The comminuted air-dry raw material (500 g) was extracted to exhaustion by heating it on the water bath with 70% ethanol. The extracts were combined, evaporated to an aqueous residue, purified with 4 liters of diethyl ether (8 × 0.5 liter) and 4 liters of chloroform (8 × 0.5 liter), and deposited on a column of Kapron (80 × 7 cm).

The column was first washed with water until colorless eluates were obtained, and then the combined flavonoids were eluted with 96% ethanol. The ethanolic eluates were evaporated to small volume. The crystalline precipitate of oppositifolin that deposited (0.5 g) was separated off. The glycoside is readily soluble in aqueous alcohol, dimethylformamide, pyridine, and alkalis, sparingly soluble in water, ethanol, and methanol, and insoluble in ethyl acetate, acetone, diethyl ether, chloroform, and petroleum ether. In system 2 it has R_f 0.42 and in system 3 R_f 0.44; mp 200-205°C; $[\alpha]_D^{20}$ 188° [c 0.1; ethanol–dimethylformamide (8 : 2)]. The spectral characteristics are given in Table 1.

Hydroxylaminolysis of Oppositifolin. The glycoside (0.01 g) was gently heated with two drops of hydroxylamine reagent [2], and then one drop of an ethanolic solution of potassium hydroxide was added and the mixture was brought to the boil. After cooling, it was acidified with 1 N hydrochloric acid and chromatographed on paper in systems 2 and 3. When the chromatogram was treated with a 1% ethanolic solution of ferric chloride, acethydroxamic acid was revealed in the form of a lilac spot with R_f 0.91 (2) and 0.50 (3).

Preparation of Desacetyloppositifolin. With heating, 0.5 g of the substance was dissolved in 50 ml of 0.5% potassium hydroxide solution. The mixture was cooled, brought to a feebly acid reaction with 0.5% hydrochloric acid, deposited on a column of Kapron, and eluted with water until neutral eluates were obtained. Then the desacetyloppositifolin was eluted with ethanol and was crystallized from 50% ethanol. This gave 0.42 g of light yellow crystals with mp 178-181°C; $[\alpha]_D^{20}$ - 33° [c 0.1; ethanol–dimethylformamide (8:2)]; R_f 0.22 (2) and 0.26 (3).

Acid Hydrolysis of Oppositifolin. A solution of 0.25 g of the substance in 25 ml of 3% aqueous sulfuric acid was heated in the boiling water bath for 8 h. The completeness of hydrolysis was checked by paper chromatography in system 3. The hydrolyzate yielded 112 mg of aglycone in the form of golden yellow plates with mp 324-325°C (from ethanol).

After neutralization with AV-17 ion-exchange resin (OH form), the hydrolyzate was evaporated to 0.5 ml and chromatographed on paper in system 1 with authentic samples of monosaccharides. The treatment of the chromatogram with a solution of aniline phthalate [16] showed the presence of D-mannose and D-glucose.

Stepwise Acid Hydrolysis of Oppositifolin. A solution of 1 g of the substance in 75 ml of 1.5% aqueous sulfuric acid was heated in the boiling water bath for 3 h and was then rapidly cooled and deposited on a column of Kapron (60 × 5 cm). The acid was washed out with water, and the residues of desacetyloppositifolin were eluted with 25% ethanol, and the monoside with 50% ethanol. The fractions containing the monoside were combined, evaporated in vacuum, and crystallized. Crystals deposited in the form of light yellow needles with mp 164-169°C (from ethanol), $[\alpha]_D^{20}$ - 65° [c 0.04; ethanol–water (9:1)], R_f 0.10 (2) and 0.47 (3).

Enzymatic Hydrolysis of the Monoside. A solution of 20 mg of the substance in 5 ml of 40% ethanol was diluted with water to 30 ml, treated with 20 mg of rhamnodiastase, and left at 38°C for 12 h. Then the reaction mixture was treated with diethyl ether, the ether was distilled off, and the residue was crystallized from ethanol. This gave 8 mg of the aglycone.

The aqueous residue was evaporated, and the enzyme was precipitated with a tenfold amount of boiling ethanol; the precipitate was filtered off, and the concentrated filtrate was chromatographed on paper in system 1. D-Glucose was found.

Acetylation of the Aglycone. The aglycone (0.2 g) was acetylated by a published method [3]. The crystals obtained were colorless flaky needles with mp 220-221°C (from ethanol). By the Partridge method [17], three acetyl groups (32.08%) were found.

Alkaline Cleavage of the Aglycone. The aglycone was cleaved by the method of Deryugin et al. [7]. The cleavage products were shown by paper chromatography in system 5 to contain phloroglucinol and vanillic acid (the chromatogram was treated with diazotized sulfanilic acid).

Demethylation of the Aglycone. The aglycone (50 mg) was demethylated by the Kuhn–Roth method [18]. This gave 25 mg of crystals (yellow needles) with mp 326-327°C. A mixture of the demethylated product with an authentic sample of luteolin [5] gave no depression of the melting point.

Methylation of Oppositifolin. The substance (0.5 g) was methylated as described by Mzhel'skaya and Abubakirov [8]. The methylation process was monitored by paper chromatography in system 4. The partially methylated product was separated on a column of silica gel (30 × 2 cm). The column was washed with benzene, with mixtures of benzene and chloroform with gradually increasing concentrations of the latter, and then with chloroform. The fractions containing the completely methylated product were combined and evaporated. The residue (97 mg) was dissolved in 5 ml of methanol, 5 ml of 3% sulfuric acid was added, and hydrolysis was performed at 100°C for 6 h. After the end of hydrolysis, 10 ml of water was added to the reaction mixture, and the methanol was evaporated off in vacuum. This lead to the deposition of colorless crystals of the methylated product with the composition $C_{18}H_{16}O_6$, mp 285-288°C.

SUMMARY

A flavonoid glycoside which has been called oppositifolin has been isolated from the herb Asperula oppositifolia Rgl. et Schmalh. It has been assigned the most probable structure of chrysoeriol 7-O-β-D-mannopyranosyl-(1 → 2)-β-D-(6-acetylglucopyranoside). Desacetyloppositifolin [chrysoeriol 7-O-β-D-mannopyranosyl-(1 → 2)-β-D-glucopyranoside] and the corresponding monoside (chrysoeriol 7-O-β-D-glucopyranoside) have been obtained and isolated by chromatography on Kapron.

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