FLAVONOIDS FROM Phlomis tuberosa

N. K. Vavilova and É. V. Gella

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Phlomis tuberosa L. Sp. Pl (tuber Jerusalem sage), family Labiatae, is a perennial herbaceous plant of wide distribution. In the USSR, it is found in the European part, Siberia, Central Asia, and the Far East. In addition to phenolcarboxylic acids [1], flavonoid compounds have been detected in the plant by qualitative reactions. The qualitative composition of the combined flavonoids from the environs of the town of Kursk in July, 1971, was determined by one-dimensional and two-dimensional paper chromatography. Three compounds of glycosidic nature (I, II, and III) were isolated. By sorption chromatography on Kapron, the combined flavonoids gave two compounds in the crystalline state.

With ethanolic ferric chloride solutions, these glycosides formed dark green solutions, and with solutions of alkalis they gave yellow colorations. These results show the presence of free hydroxy groups in the substances under investigation [2-4]. For a more accurate determination of the positions of the carbohydrate substituents and of the free hydroxy groups we used a comparison of the UV spectra of the initial glycosides and their aglycones with the addition of ionizing and complex-forming reagents [3, 5] and the analysis of the products of alkaline hydrolysis, and for substance (I) hydrolysis in Kiliani's modification [6]. Analysis of the hydrolysis products showed that the two glycosides have the same aglycone.

The positive reaction of the glycosides and their aglycone with an ammoniacal solution of silver nitrate and a 22-30-nm bathochromic shift with boric acid in the presence of sodium acetate shows the presence of a free ortho-dihydroxy grouping in ring B.

The formation by substances (I) and (II) and their aglycone of complexes with zirconyl chloride causes bathochromic shifts of the long-wave bands by 46-62 nm, showing that the hydroxy group in position 5 is free.

Because of the negative reaction with zirconyl chloride and citric acid and also because of the distance between the maxima of the first and second bands (90-94 nm), substances (I) and (II) were assigned to the class of flavones. Under the influence of sodium ethoxide, the first band shifted by 60 nm, which shows the presence of an unsubstituted hydroxyl in position 4'.

The difficulty of the acid hydrolysis of compound (II) and the absence of a bathochromic shift in the UV spectrum of the glycoside on the addition of sodium acetate, in contrast to the aglycone, shows the presence of a sugar residue at C_7 in substance (II).

The results of a study of acid hydrolysis and Kiliani hydrolysis for substance (I), the appearance of a bathochromic shift by 30 nm with sodium acetate, and the absence of a hydroxy group in the aglycone molecule after hydrolysis permit the conclusion that the carbohydrate component is present in position 8 and is attached to the aglycone by a C–C bond [7]. The monoglycoside nature of the substances investigated was confirmed by the specific rotation of their aglycone.

The IR spectra of the glycoside showed bands at 3380 cm^{-1} (OH^-), 1670 cm^{-1} (C=O), 928 and 786 cm^{-1} (carbohydrate substituents in the pyranose form), and 890 cm^{-1} (β bond) [8, 9].

After alkaline fusion, protocatechuic acid and phloroglucinol were obtained, which is in harmony with the results of qualitative reactions and of UV spectroscopy concerning the positions of the hydroxy groups.

Thus, of the substances isolated from the epigeal part of Phlomis tuberosa, (I) is luteolin 8-C-β-D-glucopyranoside and substance (II) is luteolin 7-O-β-D-glucopyranoside.

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EXPERIMENTAL

The following solvent systems were used for chromatographic investigation on "M" ["slow"] paper of the Volodarskii Mill, Leningrad (ascending method): 1) 15% solution of CH_3COOH; 2) n-butanol–acetic acid–water (4:1:5); 3) 40% solution of CH_3COOH.

Isolation of the Flavonoids. The air-dry raw material (300 g) was subjected to extraction with 80% ethanol until the cyanidin reaction for flavonoids was negative, after which the extracts were evaporated in vacuum and the dry residue was dissolved in water and the solution was treated with diethyl ether. The aqueous extract (60 ml) was deposited on a column of polyamide (diameter 4 cm, height 47 cm). The separation process was monitored by paper chromatography in system 2. First the column was eluted with water until the aromatic acids had been eliminated, and then elution was continued with mixtures of water and ethanol with a gradually increasing concentration of the latter. Fractions 7-13 of 50 ml each, eluted with 40-50% ethanol, contained substance (II), and their concentration led to the deposition of yellow crystals with mp 256-257°C, $[\alpha]_D^{20} - 79.6°$ (c 0.1; methanol), R_f 0.15 (system 1), 0.40 (system 2). Fractions 32-35, obtained at an ethanol concentration of 70-80%, contained compound (I) and on evaporation they deposited light yellow acicular crystals with mp 267-269°C $[\alpha]_D^{20} + 20.4°$ (c 0.6; methanol), R_f 0.15 (system 1), 0.32 (system 2).

Acid Hydrolysis. A mixture of 0.1 g of glycoside (I) and 3 ml of 10% H_2SO_4 was heated on the water bath for 3 days. The completeness of hydrolysis was checked by paper chromatography in system 2. Only one spot was found, with R_f 0.32. Enzymatic hydrolysis did not lead to the splitting off of a sugar, and therefore we used Kiliani's method of hydrolysis. The glycoside (0.05 g) was dissolved in a mixture consisting of 35 parts of acetic acid, 35 parts of water, and 10 parts of hydrochloric acid. Hydrolysis was performed on the water bath for 4 h. Then the mixture was diluted with water to 50 ml and was passed through AV-17 ion-exchange resin (OH^- form). The eluates obtained were chromatographed on a column of Kapron, and the aglycone was eluted with methanol and was then crystallized from aqueous ethanol. Mp 329-331°C, R_f 0.12, 0.78, and 0.58 (in system 1, 2, and 3, respectively).

Glycoside (II) (0.1 g) was heated with 3 ml of 10% H_2SO_4 solution on the water bath for 6 h. The completeness of hydrolysis was checked in the above-mentioned solvents, the chromatograms each showing a single spot with R_f 0.12, 0.78, and 0.58 (in systems 1, 2, and 3, respectively). The precipitate that deposited from the diluted methanol eluate was filtered off, washed with water, and dried. After recrystallization, the aglycone had mp 329-331°C.

On paper chromatography in system 2, the spot of the aglycone of the glycosides (I) and (II) studied coincided with that of authentic samples of luteolin [10].

D-Glucose was found in the mother liquor from the separation of the aglycone from substances (I) and (II) after neutralization to pH 5.0 and chromatography on paper followed by treatment of the chromatogram with aniline phthalate.

Acetyl Derivative of the Aglycone. A mixture of 0.05 g of the aglycone, 0.1 g of calcined sodium acetate, and 3 ml of acetic acid was heated on the water bath for 1.5 h. The mixture was poured into ice water and was left in the refrigerator for 48 h. After recrystallization from ethanol, white crystals deposited in the form of needles with mp 227-228°C, which agrees with the literature figure [10].

Alkaline Degradation of the Aglycone. A mixture of 0.5 g of caustic potash and 0.5 g of the aglycone was fused in a porcelain crucible. Then the mixture was dissolved in water, the solution was neutralized with H_2SO_4, and the reaction products were extracted with ether. The ethereal extract was shown by paper chromatography in system 2 to contain protocatechuic acid and phloroglucinol.

Alkaline Hydrolysis of the Glycosides. The hydrolysis of the glycosides (40 mg) was carried out with 10 ml of a 0.5% solution of KOH by a method described previously [11]. No biose was found in the products of the alkaline cleavage of glycosides (I) and (II).

SUMMARY

The epigeal part of Phlomis tuberosa L. Sp. Pl. has yielded two flavonoid glycosides, which have been characterized as luteolin 8-C-β-D-glucopyranoside (I) and luteolin glucopyranoside (II).

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