GLOGOSIDE – A NEW FLAVONOID FROM *Crataegus pentagyna*

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We have separated the flavonoid compounds from the leaves and flowers of Crataegus pentagyna, W.K. growing in the environs of the town of Burgas (Bulgarian People's Republic, May, 1970) by column chromatography on polyamide and cellulose powders, as described previously [1]. The fractions eluted from the polyamide column with 20% ethanol, on reseparation on polyamide and cellulose columns gave a new flavonoid, with yields of 0.003% (leaves) and 0.005% (flowers), which we have called glogoside.

Glogoside, $C_{22}H_{22}O_{12}$, mp 224-226°C (from 50% ethanol), $[\alpha]_D^{20} - 6.6°$ (c 0.1; ethanol), λ_{max} (nm) 270, 325, and 360 sh. in methanol, 285 and 410 with the addition of sodium acetate, 285, 355, and 420 in the presence of zirconyl chloride, and 415 in alkali; no changes in the differential spectra were observed with boric acid and sodium acetate or with zirconyl chloride and citric acid [2].

Quantitative acid hydrolysis gave D-glucose and the aglycone with a yield of 60%. Consequently, the flavonoid is a monooside. An enzyme preparation from the fungus Aspergillus oryzae split glogoside into D-glucose and the aglycone.

The aglycone, $C_{16}H_{12}O_6$, had mp 283-286°C (from 70% ethanol), λ_{max} (nm) 270, 325, and 375 in methanol, 415 in the presence of sodium acetate, 465 in the presence of zirconyl chloride, 420 in alkali, and 415 in the presence of boric acid and sodium acetate.

The IR spectrum of the aglycone had the bands characteristic for a hydroxy group (3250-3370 cm^{-1}), for the carbonyl group of a γ-pyrone ring (1660 cm^{-1}) and for aromatic rings (1633, 1610, 1575, 1525, and 1515 cm^{-1}) and also two other bands, at 2850 and 2960 cm^{-1}, which are specific for the asymmetric and symmetric valence vibrations of the C–H bonds of a methoxy group.

The NMR spectrum of the trimethylsilyl ether of the aglycone showed the signals of H-2' and H-6' protons (8.03-7.89 ppm, 2H), the H-3' and H-5' protons (6.86-6.71 ppm, d, 2H), and the H-6 proton (6.09 ppm, s, 1H), the protons of a methoxy group (3.73 ppm, s, 3H), and the proton of a 5-hydroxy group (11.86 ppm, s, 1H).

Thus, according to NMR spectroscopy the aglycone has substituents in the 3, 4', 5, 7, and 8 positions. According to the UV spectra, hydroxy groups are present in the 4', 5, 7, and 8 positions and the 3-hydroxy group is probably methylated.

The aglycone was demethylated with pyridinium hydrochloride on heating [6], and in the flavonol obtained the 3-hydroxy group was detected by the bathochromic shift of the maximum of the long-wave band in the UV spectrum of the zirconyl complex (495 nm), which shifted to 450 nm in the presence of citric acid [3]. Consequently, the aglycone of glogoside can be characterized as 4',5,7,8-tetrahydroxy-3-methoxyflavone, and its demethylated product is herbacetin (3,4',5,7,8-pentahydroxyflavone) [4]. This is the first time that 3-O-methylherbacetin has been isolated from plant material.

The position of the carbohydrate substituent in glogoside at C_8 was shown by bathochromic shifts: glycoside hydroxy groups were found in the 4', 5, and 7 positions and in the aglycone in the 4', 5, 7, and 8

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positions (with boric acid and sodium acetate the long-wave maximum shifted bathochromically by 40 nm which can be explained by complex formation with boric acid at the 7,8-dihydroxygrouping).

The results of a polarimetric analysis ($[M]_D \cdot K_P = -17.0°$, $[M]_D$ for phenyl β-D-glucopyranoside = −182°) permit the assumption that the carbohydrate residue has the β configuration of the glycosidic bond and exists in the pyranose form [3]. The low value of the optical activity of the glycoside is probably due to the steric influence on it of the neighboring substituents of the aglycone, in particular the 7-hydroxy group. In the IR spectrum of the glycoside there is a band at 898 cm^{-1} which is characteristic for a β-glycosidic bond [3], and in the NMR spectrum of the trimethylsilyl ether of the glycoside a doublet at 5.77 and 5.66 ppm (1H) confirms the β configuration of the glycosidic bond [5].

Thus, according to the results of the chemical study and of UV, IR, and NMR spectroscopy glogoside can be characterized as 3-O-methylherbacetin 8-β-D-glucopyranoside.

EXPERIMENTAL

The melting points of the substances were determined on a Kofler block. The UV spectra were taken on an SF-4A spectrophotometer (methanol), the IR spectra on a Shimadzu IRG-1 instrument (potassium bromide tablets), and the NMR spectra on a Perkin-Elmer R=20A, 60 MHz, spectrometer (CCl_4) after the conversion of the substances into the trimethylsilyl ethers by the method of Mabry et al. [5]. The individuality of the substances was checked by chromatography on "medium" paper of the Volodarskii Leningrad Mill in the systems: 1) 15% acetic acid; 2) ethyl acetate–formic acid–water (10:2:3); and 3) benzene–ethyl acetate–acetic acid (70:30:2; paper impregnated with formamide).

Isolation of Glogoside. The flavonoids from 2 kg of the comminuted leaves or flowers of Crataegus pentagyna were extracted with 96% ethanol (three times with 10 liters at 80°C for 1 h). The extracts were concentrated and dissolved in water. The flavonoids were chromatographed on a column of polyamide sorbent (1000×50 mm, 600 g, eluents water and 45% ethanol). The purified mixtures of flavonoids (3.0% from the leaves and 3.5% from the flowers) were reseparated on columns of polyamide sorbent. The fractions eluted by 20% ethanol were additionally purified on a column of cellulose powder (with water as the eluent), giving glogoside. The individuality of the glycoside was checked by paper chromatography, R_f 0.62. It formed a faint yellow spot in visible light and a dark olive spot in filtered UV light. With metallic magnesium and HCl it formed a dark red pigment not extracted by octanol.

Quantitative Acid Hydrolysis. The glycoside (100 mg) was hydrolyzed with a 5% solution of HCl in 50% methanol (50 ml) on the water bath for 1 h. The aglycone, which was obtained with a yield of 60% on the glycoside, was crystallized from 50% ethanol. The aqueous residue of the hydrolyzate yielded D-glucose with mp 146–148°C (mp of the phenylosazone 208–209°C).

The individuality of the aglycone was determined chromatographically: R_f 0.08. It gave a spot appearing yellow-brown in UV light.

Stepwise Acid Hydrolysis. Glogoside (10 mg) was hydrolyzed with 0.05% HCl in 50% methanol (10 ml, 100°C), samples being taken for chromatographic analysis of the products formed every 15 min for 4 h. It was found that the complete cleavage of the glycoside to the aglycone took 3 h without the formation of intermediate products.

Enzymatic Hydrolysis. A mixture of 10 mg of glogoside and 30 ml of water was incubated with 5 mg of an enzyme preparation from the fungus Aspergillus oryzae at 40°C for 5 h, the process of hydrolysis being monitored every 30 min. It was found that the complete cleavage of the glycoside to the aglycone without the formation of intermediate products took place in 4 h.

Demethylation of the Aglycone. A mixture of 20 mg of the aglycone and 2 g of pyridinium hydrochloride was heated in an oil bath at 190–200°C for 1 h, the process being monitored every 15 min by paper chromatography. Complete demethylation took 45 min. The reaction mixture was dissolved in methanol (30 ml) and the product formed was isolated by preparative chromatography on paper. The individuality of the substances was checked by chromatography; R_f 0.06 (1). In UV light the spot appeared dark olive and with zirconyl chloride it became yellowish dark green and with alkali dark blue-green. With a 2% methanolic solution of zirconyl chloride the substance gave a pink coloration, in contrast to the yellow produced by kaempferol and quercetin and the yellow-orange of the initial aglycone.

Alkaline Degradation of the Aglycones. The initial aglycone (10 mg) was dissolved in 1 g of fused anhydrous KOH (220–240°C). After 5 min, the melt was cooled and dissolved in 20 ml of water, and then it was

neutralized with conc. HCl to pH 5-6. The demethylation product of the initial aglycone was cleaved under similar conditions. The degradation products were extracted with ethyl acetate and were analyzed by paper chromatography. Both aglycones yielded p-hydroxybenzoic acid.

SUMMARY

From the flowers and leaves of Crataegus pentagyna W. K. we have isolated a new flavonoid glycoside – glogoside. From the results of chemical and spectroscopic investigations it has been established that glogoside is 3-O-methylherbacetin 8-β-D-glucopyranoside.

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