A NEW QUERCETIN DIGLYCOSIDE FROM *Campanula glomerata*

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Two flavonoids – rutin and quercetin – have been isolated previously from the herbage of *Campanula glomerata* L. (Danes'-blood bellflower) growing in the Perm oblast [1].

The present communication gives the results of a chemical investigation of a new flavonoid isolated from Danes'-blood bellflower collected in the flowering phase in the mountains of the Altai. The substance obtained has not been described in the literature, and we have called it campanuloside.

Hydrolysis of campanuloside with 5% sulfuric acid gave an aglycone and two monosaccharides – D-glucose and D-galactose.

In a spectroscopic investigation of the aglycone in the UV region with complex-forming and ionizing additives, free hydroxyls were found in positions 3, 3', 4', 5, and 7, which corresponds to the structure of quercetin.

The results of a comparison of the physicochemical properties and also the absence of a depression of the melting point of a mixture of the aglycone with an authentic sample of quercetin showed their complete identity.

A comparison of the UV spectra of campanuloside and its aglycone in the presence of additives was used to determine the position of attachment of the carbohydrate residues [2, 3]. The absence of bathochromic shifts with sodium acetate with zirconyl chloride plus citric acid gave grounds for assuming that campanuloside is a diglycoside in which the carbohydrate residues are present in positions 3 and 7.

The quantitative acid hydrolysis of campanuloside gave 46% of aglycone (calculated, 47.2%), and the ratio of the specific absorption ($E^{1\%}_{1\,cm}$) of the glycoside, 371, to the value of $E^{1\%}_{1\,cm}$ of the aglycone, 794, confirmed its diglycosidic nature [4].

The position of attachment of the carbohydrate residues was also determined from the products of acid and enzymatic hydrolysis with emulsin and rhamnodiastase.

Hydrolysis with 2% hydrochloric acid gave D-galactose and a monoglycoside (I) in which the hydroxyl at C_3 was free. Consequently, it may be assumed that the galactose is attached in position 3.

The difficulty of acid cleavage and the absence of a bathochromic shift in the UV spectrum (I) on the addition of sodium acetate showed the presence of a sugar component at C_7. The hydrolysis of (I) with 5% sulfuric acid and emulsin led to the formation of D-glucose and quercetin. Thus, the monoglycoside (I) can be characterized as quercetin 7-O-β-D-glucoside or quercimeritrin.

To obtain the monoglycoside (II) with D-galactose in position 3 we performed the partial hydrolysis of campanuloside with emulsin, which first splits off a sugar in position 7 [5]. In this way we obtained a monoside identical with quercetin 3-O-β-D-galactose or hyperoside.

In the hydrolysis of campanuloside with rhamnodiastase it was found that this enzyme, unlike emulsin, first splits off the D-galactose and forms the monoglycoside (I). The latter is subsequently split into quercetin and D-glucose.

In a study of the configuration of the glycosidic bonds and a determination of the sizes of the oxide rings in the carbohydrate part of the glycoside we used the results of enzymatic hydrolysis and a comparison of molecular rotations [6] (see Table 1).

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TABLE 1. Polarimetric Analysis of Flavonoids

Substance	M	$[\alpha]_D$ deg	$[M]_D$	Kp	$[M]_D \cdot Kp$	Type of bond	Form of the sugar
Campanuloside	640	−46,8	−293,0	0,66	−193,0	—	—
Monoglycoside (I)	464	−53,0	−245,0	0,55	−134,0	β	Pyranose
Monoglycoside (II)	464	−27,8	−129,0	0,55	− 71,0	β	
Phenylglucoside	256	−71,0	−182,0	1,0	−182,0	β	
Phenylgalactoside	256	−42,9	−110,0	1,0	−110,0	β	

As can be seen from Table 1, the D-glucose and D-galactose are attached to the quercetin by β-bonds in positions 3 and 7, respectively, and have the pyranose forms.

In order to confirm the results of polarimetric analysis we used differential IR spectroscopy [6]. Thus, in the IR spectrum of the monoglycoside (I) there are bands at 1085, 1055, and 1025 cm^{-1} (ring vibrations of pyranoses and C−O groups) and 895 cm^{-1} (β-anomer), and in the monoglycoside (II) there are bands at 1090, 1060, and 1022 cm^{-1} (ring vibrations of pyranoses and C−O groups) and 890 cm^{-1} (β-anomer).

On the basis of the results obtained, campanuloside can be characterized as quercetin 3-O-β-D-galactopyranoside-7-O-β-D-glucopyranoside.

EXPERIMENTAL METHOD

The UV spectra were taken on a SF-16 spectrophotometer (ethanol) and the IR spectra on a UR-20 instrument (in paraffin oil). The chromatographic analysis of the flavonoids and sugars was performed on Filtrak FN-4 paper in the following systems: 1) 15% acetic acid; 2) butan-1-ol−acetic acid−water (4:1:5); and 3) butan-1-ol−pyridine−water (6:4:3). The analyses of all the compounds corresponded to the calculated figures.

Isolation of Campanuloside. The comminuted herbage of Campanula glomerata (1 kg) was treated with 96 and 70% ethanols, the combined extracts were evaporated in vacuum, and the aqueous residue was shaken with chloroform. The purified extract was deposited on a column of polyamide sorbent (1000 × 45 mm, 450 g) and eluted first with water and then with mixtures of the ethanol and water. The fractions obtained on elution with water and with 5-10% ethanol were combined and rechromatographed on polyamide (column of sorbent 300 × 30 mm). When the column was eluted with 10% ethanol, campanuloside was isolated: $C_{28}H_{32}O_{17}$ (0.25 g), mp 222-223°C, $[\alpha]_D$ − 46.8° (c 0.3; DMFA); R_f 0.51 (1) and 0.19 (2). UV spectrum: λ_{max} (ethanol) 362, 258 nm; λ_{max} (sodium acetate) 365, 258 nm; (boric acid + sodium acetate) 389, 263 nm; λ_{max} (sodium ethoxide) 410, 272 nm; λ_{max} (zirconyl chloride) 430, 274 nm; λ_{max} (zirconyl chloride + citric acid) 362, 260 nm.

Acid Hydrolysis. Campanuloside (0.1 g) was heated with 5% sulfuric acid in the boiling water bath for 3 h. The aglycone separated out (46 mg), composition $C_{15}H_{10}O_7$, mp 310-312°C (from 70% ethanol), R_f 0.02 (1) and 0.64 (2).

A mixture of the aglycone with an authentic sample of quercetin gave no depression of the melting point.

The acid hydrolyzate was neutralized with barium carbonate and filtered, and the filtrate was evaporated to a syrup. Chromatography on paper by the descending method in systems 2 and 3 with markers showed the presence of D-glucose and D-galactose.

Alkaline Cleavage. The agylcone (10 mg) was added to a melt of 0.2 g of caustic potash with two drops of water at 250-270°C, and the mixture was heated for 10 min. After cooling, the alkaline melt was neutralized with sulfuric acid and extracted with diethyl ether. By paper chromatography with markers, the ethereal extract was shown to contain protocatechuic acid and phloroglucinol.

The Monoglycoside (I). Campanuloside was heated with a 2% solution of hydrochloric acid in the water bath for 20 min. A precipitate deposited with the composition $C_{21}H_{10}O_{12}$, mp 231-232°C (from 50% ethanol), $[\alpha]_D$ −53°C (c 0.2; methanol); R_f 0.07 (1) and 0.27 (2).

The Monoglycoside (II). A solution of 60 mg of campanuloside in 30 ml of water was treated with 60 mg of emulsin and left at 37°C for an hour. Then 30 ml of 96% ethanol was added to the liquid and it was heated to the boil. The mixture was filtered, and the filtrate was concentrated under vacuum and deposited on a column of Kapron [nylon-6] (100 × 25 mm, 20 g). Elution with 40% ethanol yielded yellow crystals with the composition $C_{21}H_{20}O_{12}$, mp 227-229°C, $[\alpha]_D$ −27.8° (c 0.3; methanol); R_f 0.33 (1) and 0.56 (2).

SUMMARY

A new quercetin diglycoside – campanuloside – has been isolated from the herbage of Campanula glomerata L., and for it the structure of quercetin 3-O-β-D-galactopyranoside-7-O-β-D-glucopyranoside has been proposed.

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