STRUCTURE OF DELPHOSIDE – A NEW ISOCOUMARIN GLYCOSIDE

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In an investigation of the epigeal parts of four species of Delphinium – D. flexiosum M.B., D. tamarae Kem.-Nath., D. elisabethae N. Busch, and D. dzavachischvilii Kem.-Nath. collected in the environs of the villages of Dzhava and Bakuriani (Georgian SSR) we detected the presence of glycosides. By adsorption chromatography we succeeded in isolating an individual component with the composition $C_{16}H_{18}O_9$, mp 236-238°C (from methanol), $[\alpha]_D^{20}$ −56° (c 0.1; pyridine), mol. wt. 354 (mass spectrometrically), readily soluble in pyridine, dimethylformamide, and methanol (on heating), sparingly soluble in chloroform, and insoluble in water, which we have called delphoside.

The IR spectrum of the substance had λ_{max} 230, 251, 257, 290 and 317 nm (log ε 4.06, 4.16, 4.19, 3.67 and 3.48) and λ_{min} 217, 236, 254, 272, 308 nm (log ε 3.97; 4.05; 4.13; 3.58; 3.48), which shows the presence of the chromophore of 6,8-dihydroxyisocoumarin [1]. This conclusion was confirmed by the IR spectrum (Fig. 1), which showed absorption bands at (cm^{-1}) 1670 (carbonyl of an 8-hydroxyisocoumarin), 1630 and 1585 (aromatic nucleus), and 3100-3600 (hydroxy groups).

The acid hydrolysis of delphoside gave an aglycone with the composition $C_{10}H_8O_4$, mp 263-265°C (from methanol), M^+ 192, UV spectrum: λ_{max} 228, 249, 257, 296 and 322 (inflection) nm (log ε 4.23; 4.30; 4.30; 3.98; 3.83), λ_{min} 221, 235, 253, 271 nm (log ε 4.22; 4.19; 4.29; 3.78). D-glucose was found in the hydrolyzate by paper chromatography.

Delphoside is a monoside. This is confirmed by the preparative yield of the aglycone on its hydrolysis (the yield was 58% while the calculated figure was 54.2%), by the value of the molecular extinction E at λ 290-296 nm of the glycoside and of the aglycone (4765 and 9600, respectively), and by the mass spectrum, which contained, in addition to the peak of the molecular ion, peaks with m/e 192 and 162 corresponding to the aglycone and a glucose residue.

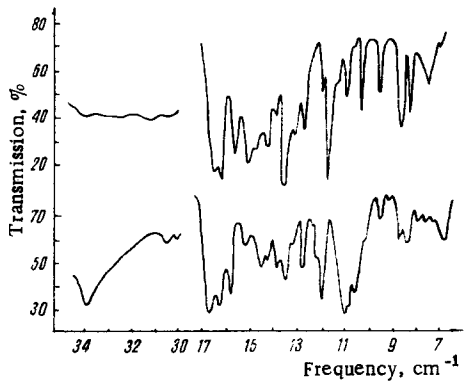

Fig. 1. IR spectrum of the aglycone of delphoside (a) and its glycoside (b) in KBr.

From the value of $M_D \cdot K_P$ calculated according to Klyne [2] (found: $M_D \cdot K_P$ 142.7°; literature data: 182°) it may be concluded that the glucose residue is present in the pyranose form and is attached to the aglycone by a β-glycosidic bond.

The NMR spectrum of delphoside (Fig. 2) shows doublets at 6.64 and 6.58 ppm (J = 2 Hz) due to aromatic meta-interacting protons, H-5 and H-7, and singlets at 5.87 (1 H) and 1.88 ppm (3 H) caused by the H-4 olefinic proton and a methyl group on a double bond (C_3) [1, 3, 4]. These results unambiguously show that the substance under investigation is a 6,8-disubstituted 3-methylisocoumarin.

It follows from the composition of delphoside that one of the substituents is a hydroxy group and the other is a D-

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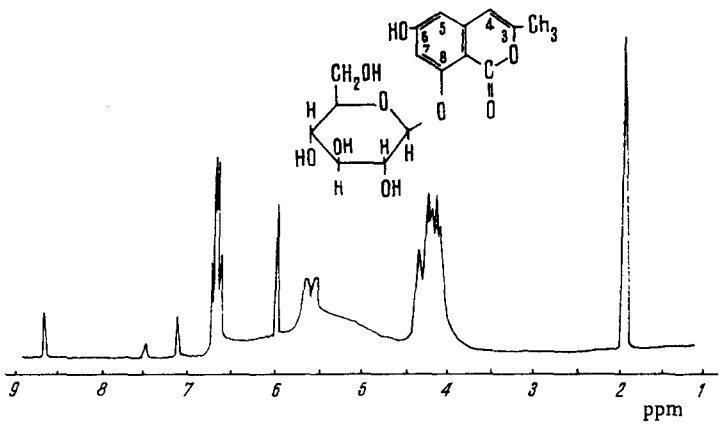

Fig. 2. NMR spectrum of delphoside.

glucose residue. The protons of the latter are represented in the spectrum by a six-proton multiplet in the 3.9-4.4 ppm region (hemihydroxylic protons and $H_{5'}$, by a doublet at 5.6 ppm ($J = 5$ Hz) (β-anomeric proton of the glucose), and by a signal in the 4.6-5.9 ppm region (5 H) due to the protons of the hydroxyls of the glucose and of a phenol. The presence of hydroxy groups is confirmed by the formation of a diacetate of the aglycone, $C_{14}H_{12}O_6$, with mp 244°C and by a pentaacetate of the glycoside, $C_{26}H_{28}O_{14}$, with mp 259-261°C.

In the NMR spectrum of delphoside pentaacetate, in addition to the signals of the protons of the acetyl residues there are two groups of signals in the 5.1 to 4.2 ppm regions with intensities of 3 and 2 H, respectively, due to the hemiacyl protons of the acetylated D-glucose residue. The ratio of their intensities (3 : 2) corresponds to that given by Mabry et al., [5] and confirms that the D-glucose in delphoside is present in the pyranose form.

The position of the sugar residue in the glycosides followed from the results of a comparison of the NMR spectra of the glycoside and its aglycone, in which the signals of the protons of the sugar components have disappeared and a broadened signal has appeared at 13.25 ppm (1 H) due to the proton of a hydroxy group at C_8. The position of this signal in such a weak field can be explained by the formation of a hydrogen bond between this proton and the carbonyl of the isocoumarin, which is present in the peri position.

On the basis of what has been said, it may be concluded that the glucose residue in delphoside is present in position 8 and the hydroxy group in position 6 and, consequently, it is 8-O-β-D-glucopyranosyl-6-hydroxy-3-methylisocoumarin, with the structure given in Fig. 2.

In addition to delphoside, from the herbage of these species of plants we have also isolated an isocoumarin $C_{10}H_8O_4$ with mp 263-265°C, which, according to a mixed melting point, and IR spectroscopy, proved to be identical with the aglycone of delphoside (6,8-dihydroxy-3-methylisocoumarin). This compound, which has been obtained previously by synthesis [6], had mp 250-253°C and lower values of log ε of the long-wave maxima in the UV spectrum, which is apparently due to the presence of impurities in it.

EXPERIMENTAL

The NMR spectra were taken on a Jeol spectrometer at 60 MHz using solutions in deuteropyridine using the δ scale from the signal of HMDS taken as 0; the UV spectra were taken on a Hitachi instrument, the IR spectra on a UR-10 instrument, and the mass spectra on a MHk-1303 instrument. The purity of the substances was checked by chromatography on Silufol in the ethyl acetate–methanol–water (100 : 16.5 : 13.5) system; yellow fluorescence in UV light. The results of elementary analysis corresponded to the calculated figures.

Isolation of Delphoside. The air-dry comminuted leaves (400 g) were steeped five times successively with 70% methanol at 70°C for 3 h. The extracts were combined, concentrated until the ethanol had been eliminated, and treated with chloroform (removal of chlorophyll) and with ethyl acetate. Then the extract was dried and the residue was chromatographed on a column of polyamide (40 × 5 cm). When the column was washed with water (3 liters) and the eluate was concentrated, a crystalline precipitate deposited which was recrystallized from methanol. A colorless substance with mp 236-238°C, R_f 0.25, was isolated in a yield of 0.5%.

Delphoside pentaacetate was obtained by a known method by heating the substance with acetic anhydride in pyridine for 2 h. Colorless crystals with mp 259-261°C (from methanol), R_f 0.40.

Acid Hydrolysis of Delphoside. A) Preparation of the Aglycone. A mixture of 0.1 g of the substance and 15 ml of 10% HCl was heated in the water bath for 1.5 h. The liquid was cooled, the aglycone was extracted with ether, and the extract was washed with water, dried, and distilled. This gave 0.058 g of a substance with mp 263-265°C, R_f 0.78.

B) Identification of the D-Glucose. The acid hydrolyzate was passed through a column of AV-17 anion-exchange resin, evaporated to a volume of 5 ml, and chromatographed in the BAW (4 : 1 : 2) system. On treatment with aniline phthalate, D-glucose was revealed (R_f 0.30).

The diacetate of the aglycone was obtained by a known method by heating a solution of the substance in pyridine with acetic anhydride for 2 h. Colorless crystals with mp 244°C, R_f 0.85.

SUMMARY

The epigeal parts of four species of Delphinium have yielded a new isocoumarin glycoside, $C_{16}H_{18}O_9$, mp 236-238°C, $[\alpha]$ −56°C, which we have called delphoside. It has the structure of 8-O-β-D-glucopyranosyl-6-hydroxy-3-methylisocoumarin. Its aglycone has also been isolated in the free state from the plants.

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