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COUMARINS OF *Hapolophyllum obtusifolium*. STRUCTURES OF TWO NEW COUMARIN GLYCOSIDES.

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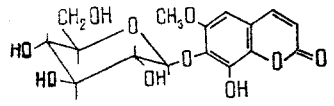
Two coumarin glycosides have been isolated from an aqueous ethanolic extract of the epigeal part of *Haplophyllum obtusifolium*: (I) — $C_{16}H_{16}O_{10}$, mp 164–166°C, $[\alpha]_D$ −52.4° (dimethylformamide); and (II) — $C_{26}H_{26}O_{12}$, mp 206–208°C, $[\alpha]_D$ −110.5° (pyridine). It has been established on the basis of chemical transformations and spectral characteristics that (I) has the structure of fraxetin 7-O-β-D-glucopyranoside and (II) that of scopoletin 7-O-(6'-O-feruloyl-β-D-glucopyranoside).

Continuing a study of *Haplophyllum obtusifolium*, by chromatography of an aqueous ethanolic extract [1] on a column of silica gel with elution by chloroform–methanol we have isolated two new coumarin glycosides.

Glycoside (I) has mp 164–166°C and the composition $C_{16}H_{18}O_{10}$. Its UV spectrum ($\lambda_{max}^{ethanol}$ 228, 257, 306 nm; lg ε 4.02, 3.54, 3.86) is similar to those of capensin and obtusicin [2]. When the UV spectrum was taken in an alkaline medium, a bathochromic shift of the long-wave maximum (Δλ + 28 nm) was observed. Consequently, compound (I) contains a free phenolic hydroxyl.

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3130–3475 (hydroxy groups), 1732 (carbonyl of an α-pyrone), and 1626, 1585, and 1500 (C=C bonds of an aromatic system).



I

The acid hydrolysis of glycoside (I) formed D-glucose and an aglycone identical with fraxetin, in equimolar proportions. This means that (I) is a monoglucoside of fraxetin. To establish the position of the glucose to the aglycone, glycoside (I) was methylated with dimethyl sulfate in the presence of potassium carbonate. Subsequent hydrolysis of the methylation product led to the formation of a substance $C_{11}H_{10}O_5$ with mp 144–145°C, which was identified by direct comparison with an authentic sample as isofraxidin [3, 4]. Thus, the glucose is attached to the hydroxyl in the C-7 position of fraxetin. The acetylation of (I) gave a pentaacetyl derivative the PMR spectrum of which showed the signals of the protons of four aliphatic and one aromatic acetyl groups, and also those of H-3, H-4, H-5, and CH_3O. The signals of the hemiacyl methine protons and of the anomeric protons of the glucose residue appear at 4.96–5.24 ppm (4 H, multiplet). The signal of the H-5' atom (1 H, m) appears at 3.58 ppm, and the signals of the $-CH_2-OAc$ protons at 3.91 ppm (J_{gem} = 12 Hz, $J_{5',6'}$ = 2 Hz) and 4.26 ppm (J_{gem} = 12 Hz, $J_{5',6'}$ = 4.5 Hz), in the form of two

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quartets. A similar pattern is observed in the case where the glucose residue has a pyranose oxide ring in the C1 conformation [5, 6]. Glycoside (I) was cleaved by emulsin, which shows the β-glycosidic linkage of the glucose with the aglycone. This was also confirmed by the results of a polarimetric analysis ($M_D \cdot K_f = -135.7°$) and by the IR spectrum [7]. Thus (I) has the structure of 7-β-D-glucopyranosyloxy-8-hydroxy-6-methoxycoumarin.

A coumarin glycoside, which has been called isofraxoside, has been isolated together with fraxin (fraxetin 8-O-β-D-glucoside) from *Diervilla lonicera* Mill. (family Caprifoliaceae) [8, 9]. According to the author concerned, the acid hydrolysis of isofraxoside led to fraxetin and glucose. In the presence of $AlCl_3$, the long-wave maximum in its UV spectrum underwent a bathochromic shift by 61 nm, in contrast to fraxin, for which the shift was only 18 nm. On the basis of these facts, the structure of fraxetin 7-O-glucoside was put forward for isofraxoside. The UV spectrum of the coumarin that we isolated did not change when a solution of $AlCl_3$ was added, and, in addition, its physicochemical constants differ from those of isofraxoside. For these reasons, the suggested structure of isofraxoside is a matter of doubt.

Glycoside (II) has the composition $C_{26}H_{26}O_{12}$ (M^+ 530), $\lambda_{max}^{ethanol}$ 229, 249 sh., 297, 332 nm (log ε 4.42, 4.14, 4.15, 4.30). A positive reaction with $FeCl_3$ solution and a bathochromic shift of the long-wave band under the action of alkali (Δλ 60 nm) showed the presence of a phenolic hydroxyl in its molecule. The IR spectrum of (II) showed the absorption bands of hydroxy groups (3210–3540 cm^{-1}) of an α-pyrone carbonyl group (1736 cm^{-1}), and of aromatic C=C bonds (1639, 1610, 1575 cm^{-1}).

It was established on the basis of the products of acid hydrolysis, the results of GLC, and of PMR spectroscopy that (II) included residues of scopoletin, D-glucose, and ferulic acid in a molar ratio of 1:1:1. Glycoside (II) formed an acetyl derivative with the composition $C_{34}H_{34}O_{16}$, M^+ 698 (III) the PMR spectrum of which contains the signals of four acetyl groups, one of which is,from the values of its chemical shift, aromatic. The PMR spectra of (II) and (III) (Fig. 1) contains the signals of five aromatic and four olefinic protons, which were assigned to the scopoletin and trans-ferulic acid residues ($J_{\alpha,\beta}$ = 16 Hz). There are also the signals of the protons of two $-OCH_3$ groups, of an anomeric proton (J = 6 Hz), and of two protons of the sugar moiety. The facts given show that (II) is an acylated coumarin glycoside. In actual fact, the mild saponification of (II) with 0.5% caustic potash solution led to ferulic acid and scopoletin 7-O-β-D-glucopyranoside [10]. The position of attachment of the ferulic acid residue was found by an analysis of the PMR spectrum of (II): a two-proton multiplet was detected with its center at 4.83 ppm (J_{gem} = 12 Hz), which shows the attachment of the acyl residue to the CH_2O group of the glucose moiety [5, 11]. A combination of these facts shows that (II) corresponds to the structure of scopoletin 7-O-(6'-O-feruloyl-β-D-glucopyranoside).

The mass spectrum of (II) agrees well with the suggested structure, containing in addition to the peak of the molecular ion with m/z 530, the peak of the M − 192 (m/z 338) ion arising from the splitting out of scopoletin from M^+. The peaks of ions corresponding to the molecular ions of ferulic acid (m/z 194) and of scopoletin (m/z 192) and also of fragmentary ions derived from them, were also detected.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrophotometer, IR spectrum on a UR-20 instrument (tablets with KBr), mass spectra on a MKh-1310 mass spectrometer, and PMR spectra on JNM-4H-100 and JNM-C-60L spectrometers. Chemical shifts are given in the δ scale (HMDS − 0).

The purity of the substances and the courses of the reactions were monitored by TLC on Silufol UV-254 plates in the following solvent systems: 1) chloroform–methanol (5:1); 2) benzene–methanol (4:1); and 3) benzene–methanol–acetic acid (90:16:8).

Isolation of the Coumarin Glycosides. The aqueous ethanolic extract [1] (340 g) was passed through a column of silica gel (8 × 45 cm) and the glycosides were eluted with methanol. Evaporation of the solvent yielded 50.0 g of a viscous residue, which was chromatographed on a column (4.5 × 100 cm) of silica gel L 100/160 (Chemapol) at a ratio of substance to sorbent of 1:15. The glycosides were eluted with chloroform containing methanol in increasing concentrations. The volume of each fraction was about

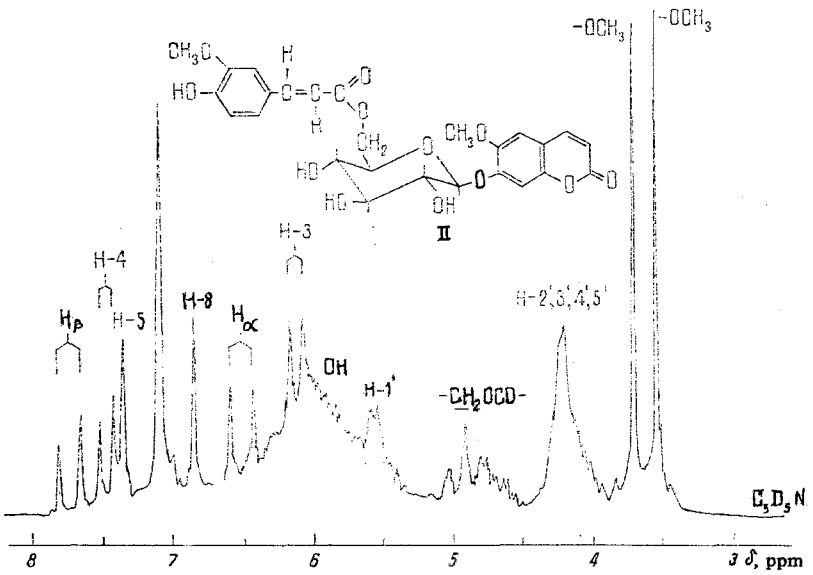

Fig. 1. PMR spectrum of scopeletin 7-O-(6'-O-feruloyl-β-D-glucopyranoside) in deuteropyridine.

600 ml. At a composition of the solvent mixture of 95:5 (by volume), 0.183 g of (II) was isolated. The same mixture in a ratio of 92:8 eluted 0.210 g of (I).

Fraxetin 7-O-β-glucopyranoside (I), mp 164–166°C, (ethyl acetate), $[\alpha]_D$ –52.4° (c 0.42; dimethylformamide); R_f 0.21 (system 1).

Acid Hydrolysis of (I). With heating, 20 mg of the substance was dissolved in 5 ml of 5% sulfuric acid solution, and heating on the boiling water bath was continued for 3 h. The reaction product was extracted with ethyl acetate, the extract was washed with water and dried, and the solvent was distilled off. The residue was recrystallized from dilute ethanol, giving a substance with mp 228–229°C, identical with fraxetin [2]. Glucose was detected by PC and GLC in the $BaCO_3$-neutralized and evaporated solution.

Methylation and Hydrolysis of (I). A solution of 60 mg of the glycoside in 15 ml of acetone was treated with 1 ml of dimethyl sulfate and 300 mg of anhydrous potassium carbonate. The mixture was boiled for 3 h until the reaction with $FeCl_3$ solution was negative. Then it was filtered and evaporated. The residue was dissolved in 8.5 ml of ethanol, 1.5 ml of concentrated hydrochloric acid was added, and the mixture was boiled on the water bath for 2 h, after which the ethanol was distilled off and the residue was diluted with water. The reaction product was extracted with chloroform, and the residue after the solvent had been distilled off was recrystallized twice from methanol. This gave a substance with mp 144–145°C, R_f 0.62 (system 2), which proved to be identical with isofraxidin (TLC, mixed melting point).

Acetylation of (I). With heating, 35 mg of compound (I) was dissolved in 1.5 ml of pyridine, and 2 ml of acetic anhydride was added. The mixture was heated further at 70–75°C for 30 min, after which an acetyl derivative with mp 199–201°C (ethanol) was isolated by the usual method.

PMR spectrum ($CDCl_3$; ppm): 1.95 (6 H, s, 2 CH_3CO), 1.98 and 2.01 (3 H, s, each, 2CH_3CO); 2.31 (3 H, s, Ar–OCOC$\underline{H}_3$); 3.58 (1 H, m, H-5'); 3.81 (3 H, s, CH_3O); 3.91 (1 H, q, J_1 = 2 Hz, J_{gem} = 12 Hz); 4.26 (1 H, q, J_1 = 4.5 Hz, J_{gem} = 12 Hz, –C$\underline{H}_2$OAc); 4.96–5.24 (4 H, m, H-1', 2', 3', 4'); 6.20 (1 H, d, 9.5 Hz, H-3); 6.70 (1 H, s, H-5); 7.46 (1 H, d, 9.5 Hz, H-4).

Scopoletin 7-O-(6-O-Feruloyl-β-D-glucopyranoside) (II). mp 206-208°C (methanol), $[\alpha]_D$ –110.5° (c 0.38; pyridine); R_f 0.42 (system 1).

PMR spectrum (Py-d_5; ppm): 3.54 and 3.74 (3 H, s, each, 2 CH_3O); 3.95-4.45 (4 H, m, H-2', 3', 4', 5'); 4.83 (2 H, m, J_{gem} = 12 Hz, CH_2OAc); 5.50 (1 H, d, 6 Hz, H-1'); 6.08 (1 H, d, 10 Hz, H-3); 6.47 (1 H, d, 16 Hz, H_α); 6.81 (1 H, s, H-5); 7.07 (3 H, s, H-2", 5", 6"); 7.32 (1 H, s, H-8); 7.43 (1 H, d, 10 Hz, H-4); and 7.70 (1 H, d, 16 Hz, H_β).

Mass spectrum, m/z (%): M^+ 530(6), 338(7), 194(16), 193(14), 192(100), 178(7), 177(66), 164(24), 150(22), 149(48), 145(6), 135(16), 121(22), 107(11), 105(7), 97(7), 95(6), 83(7), 81(12), 79(21), 77(10), 69(30).

Acid Hydrolysis of (II). A mixture of 30 mg of (II) and 10 ml of 10% hydrochloric acid was heated on the water bath for 30 min. The course of the reaction was monitored by the TLC method in system 1. After 6 hours' heating, the initial compound had disappeared completely. The residual algycone was filtered off, recrystallized, and shown to be identical with scopoletin. Ferulic acid and D-glucose were detected in the evaporated aqueous solution by TLC and GLC with markers.

Acetylation of (II). The acetate was obtained in the usual way, mp 109–110°C, M^+ 698.

PMR spectrum (Py-d_5; ppm); 1.92 (6 H, s, 2 $CHCO_3$); 1.98 (3 H, s, CH_3CO); 2.12 (3 H, s, Ar–$OCOCH_3$); 3.56 and 3.65 (3 H, s, each, 2 CH_3O); 4.36 (1 H, m, H-5'); 4.48 (2 H, m, $-CH_2OAc$); 5.25–5.80 (4 H, m, H-1', 2', 3', 4'); 6.17 (1 H, d, 9.5 Hz, H-3); 6.59 (1 H, d, 16 Hz, H_α); 6.84 (1 H, s, H-5); 6.97–7.18 (3 H, H-2", 5", 6"); 7.25 (1 H, s, H-8); 7.44 (1 H, d, 9.5 Hz, H-4); and 7.72 (1 H, d, 16 Hz, H_β).

Alkaline Hydrolysis of (II). A solution of 40 mg of (II) in 6 ml of 0.5% KOH solution was heated at 60°C for 35 min. Then the mixture was neutralized with 4% hydrochloric acid and extracted with ether. On standing, ferulic acid crystallized out from the ethereal extract, with mp 165–167°C, R_f 0.57 (TLC on silica gel in system 3). The aqueous solution was evaporated at 40–50°C, and the residue was chromatographed on a column of silica gel. Elution with chloroform–ethanol (48:2) yielded scopoletin 7-O-β-glucopyranoside [10] with mp 206–208°C. The substances mentioned were identified by direct comparison with authentic samples.

SUMMARY

Two new coumarin glycosides have been isolated from an aqueous ethanolic extract of the epigeal part of *Haplophyllum obtusifolium*. On the basis of chemical transformations and spectral characteristics the structures of fraxetin 7-O-β-D-glucopyranoside and scopoletin 7-O-(6'-O-feruloyl-β-D-glucopyranoside) have been established for them.

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