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TERPENOID COUMARIN GLYCOSIDES OF *Ferula conocaula*

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Two new terpenoid glycoside coumarins have been isolated from the roots of *Ferula conocaula* Korov., and they have been named conferoside and cauferoside. On the basis of the study of chemical and spectral characteristics, their structures have been established as ferrocaulinin 4'-O-β-D-glucopyranoside and cauferin 4'-O-β-D-glycopyranoside, respectively.

Continuing a chemical study of the coumarins of *Ferula conocaula* Korov. [1-3], from the water-soluble fraction of an ethanolic extract of the roots we have isolated by chromatography two new coumarin derivatives which we have called conferoside (I) and cauferoside (II).

Conferoside (I) has a composition $C_{30}H_{38}O_{10}$, mp 195-197°C, $[\alpha]_D^{20} -110^\circ$ (c 1.0; ethanol). The UV spectrum of (I) is characteristic for the 7-hydroxycoumarin chromophore. The IR spectrum contains absorption bands due to the presence of an aromatic nucleus, the carbonyl of an α-pyrone, and a hydroxy group.

The acid hydrolysis of conferoside formed umbelliferone and D-glucose, and it is consequently a glycosylated coumarin.

Exhaustive acetylation with acetic anhydride in pyridine led to a tetraacetate of (I) with the composition $C_{38}H_{46}O_{14}$, M^+ 726 (III). The formation of the latter shows that the glycoside is a monoside.

The PMR spectrum of (I) (deuteropyridine) contained the signals from three tertiary methyl groups, 1.04 s, 3 H; 1.29 s, 6 H; from a vinyl methyl group at 1.74 s, 3 H; from the protons of the methylene group in a -CH₂-O-Ar fragment, and also from the protons of a sugar residue at 3.05-5.40 ppm, from an olefinic proton at 5.6 br.s, 1 H, and from the protons of a coumarin nucleus at 6.05-7.70 ppm.

According to its PMR spectrum and its composition, the terpenoid moiety of conferoside must have a bicyclic structure. In actual fact, the enzymatic cleavage of (I) with β-glycosidase [4] gave D-glucose and an aglycone with the composition $C_{24}H_{28}O_5$, M^+ 396. By a comparison of spectral characteristics (IR, NMR) and physical constants, the aglycone of conferoside was identified as ferrocaulinin [1].

The presence in the IR spectrum of (I) of four absorption bands at 1100, 1080, 1040, and 890 cm⁻¹ shows that the glucose residue has the pyranose form and is bound by a β-glycosidic bond [5-7]. This was confirmed by enzymatic hydrolysis and by the absolute value of the molecular rotation difference between the glycoside and the aglycone [5]. Thus, conferol is ferrocaulinin 4'-O-β-D-glucopyranoside (I).

Cauferoside (II) has the composition $C_{30}H_{40}O_{10}$, mp 176-177°C, $[\alpha]_D^{20} -140^\circ$ (c 1.0; ethanol). Its UV spectrum has the maxima characteristic for 7-hydroxycoumarin. Its IR

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spectrum contains absorption bands due to the presence of an aromatic nucleus and of the carbonyl of an α -pyrone and a strong absorption band of a hydroxy group.

The acid hydrolysis of cauferoside, just like that of conferoside, formed umbelliferone and D-glucose, which shows its glycosidic nature. When (II) was acetylated with acetic anhydride in pyridine, a pentaacetate (IV) with composition $C_{40}H_{50}O_{15}$, $M^+ 770$, was obtained.

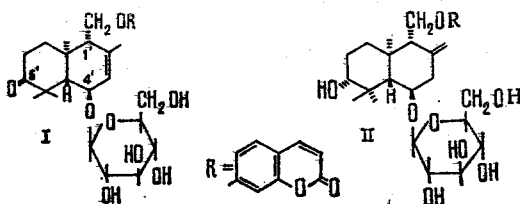
The PMR spectrum of (II) (deuteropyridine) showed the signals from three tertiary methyl groups at 0.79 s, 3H, and 1.40 s, 6 H; and the signals of a hemihydroxylic proton, of the protons of an exomethylene group and of the methylene group in the $-\text{CH}_2\text{-O-Ar}$ fragment, and also of the proton of a sugar residue at 3.10-5.92 ppm and of a coumarin nucleus at 6.05-7.92 ppm.

The enzymatic hydrolysis of (II) with β -glycosidase yielded D-glucose and an aglycone with the composition $C_{24}H_{30}O_5$, $M^+ 398$. A comparison of physicochemical and spectral (IR, PMR) characteristics showed that the aglycone of cauferoside was identical with caufferin [2].

Since caufferin has two secondary hydroxy groups, the position of the sugar residue was established by comparing the PMR spectra of cauferoside and its pentaacetate. In the PMR spectrum of the latter (IV), as compared with that of (II), the quartet signal of the hemihydroxylic proton at C_6' had shifted downfield. This shows that the hydroxy group in cauferoside at C_6' is free and, therefore, the sugar residue is located at C_4' .

The IR spectrum of (II) has absorption bands that are characteristic for the pyranose form of glucose and for a β -glucosidic bond. This was also confirmed by enzymatic hydrolysis and by the absolute value of the difference in the molecular rotations of the glycoside and of the aglycone.

On the basis of the facts given above, it may be assumed that cauferoside is caufferin 4'-O- β -D-glucopyranoside (II).



EXPERIMENTAL

The individuality of the substances was checked by thin-layer chromatography of Silufol plates in the chloroform-ethanol (4:1) system. The conditions of recording the PMR, IR, and UV spectra have been described previously [1]; the mass spectra were taken on an MKh-1310 instrument.

Isolation of the Glycosides. The butanolic fraction (30 g) obtained from an ethanolic extract of the roots of *Ferula conocaula* by the method described previously [3] was chromatographed on silica gel in the chloroform-ethanol (8:1) system, with the collection of 300-ml fractions.

Conferoside (I). The evaporation of fractions 49-57 yielded 2.10 g (0.021%) of a substance $C_{30}H_{38}O_{10}$ with mp 195-197°C, $[\alpha]_D^{20} -110^\circ$ (c 1.0; ethanol). UV spectrum, λ_{\max} , nm: 220, 242, 253, 297, 325 ($\log \epsilon$ 4.27, 3.70, 3.63, 4.01, 4.22). IR spectrum, ν_{\max} , cm^{-1} : 3430, 1733, 1712, 1617, 1560, 1520, 1100, 1080, 1040, 890.

Cauferoside (II). The concentration of fractions 63-71 yielded 1.70 g (0.017%) of a substance $C_{39}H_{40}O_{10}$, mp 176-177°C, $[\alpha]_D^{20} -140^\circ$ (c 1.0; ethanol). UV spectrum, λ_{\max} , nm: 217, 245, 254, 297, 326 ($\log \epsilon$ 4.35, 3.74, 3.47, 4.01, 4.24). IR spectrum, ν_{\max} , cm^{-1} : 3430, 1730, 1710, 1615, 1560, 1512, 1100, 1080, 1040, 890.

Acid Hydrolysis of Conferoside. A solution of 110 mg of substance (I) in 2 ml of ethanol was treated with 2 ml of 10% sulfuric acid, and the mixture was heated in the water bath for 6 h. Then it was diluted with water (1:2) and extracted with ether (5×20 ml). The extract was dried over sodium sulfate, and the solvent was distilled off. The dry residue was chromatographed on a column (25 \times 2 cm) containing silica gel, elution being performed with chloroform. Evaporation of the eluate and recrystallization from water yielded crystals with mp 230-231°C. D-Glucose was found in the hydrolyzate by paper chromatography.

Acetylation of Conferoside. A mixture of 80 mg of substance (I) and 3 ml of a 1:1 mixture of acetic anhydride and pyridine was kept at room temperature for 48 h. After the usual working up, 75 mg of a substance $C_{38}H_{46}O_{14}$ was obtained with M^+ 726.

Enzymatic Hydrolysis of Conferoside. A solution of 200 mg of substance (I) in 50 ml of water was treated with 0.1 g of enzyme [4]. The reaction mixture was placed in a thermostat and was kept at 30°C for 6 h. Then it was extracted with ethyl acetate (5 × 20 ml), the extract was dried over sodium sulfate, and the solvent was distilled off. This gave 124 mg of a substance $C_{24}H_{28}O_5$, M^+ 396, mp 84–86°C, $[\alpha]_D^{20}$ -40° (c 1.0; ethanol); yield 62%.

Acid Hydrolysis of Cauferoside. Substance (II) (95 mg) was hydrolyzed by the method described above. Umbelliferone and D-glucose were found.

Acetylation of Cauferoside. Substance (II) (85 mg) was acetylated by the method described above. This gave 78 mg of a substance $C_{40}H_{50}O_{15}$, M^+ 770.

Enzymatic Hydrolysis of Cauferoside. Substance (II) (160 mg) was subjected to enzymatic hydrolysis by the method described above. This gave 102 mg of a substance $C_{24}H_{30}O_5$, M^+ 398, mp 104–106°C, $[\alpha]_D^{20}$ -50° (c 1.0; ethanol); yield 64%.

SUMMARY

Two new terpenoid coumarin glycosides have been isolated from the roots of *Ferula cono-caula* Korov., and they have been called conferoside and cauferoside.

On the basis of a study of chemical and spectral characteristics, their structures have been determined as ferocaulinin 4'-O-β-D-glucopyranoside and cauferin 4'-O-β-D-glucopyranoside, respectively.

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