THE STRUCTURES OF FEROSIDE AND OF REOSELIN A — NEW GLYCOSIDES FROM THE ROOTS OF *Ferula korshinskyi*

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From the roots of *Ferula korshinskyi* Eug. Korov (Korzhinskii's ferula) collected in the environs of lake Iskanderkul', Tadzhik SSR, we have isolated two coumarin glycosides with the compositions $C_{30}H_{42}O_{10}$, mp 110-111°C, $[\alpha]_D^{20} + 18{,}1°$ (*c* 1,1; CH_3OH) and $C_{36}H_{52}O_{15}$ with mp 160-161°C, $[\alpha]_D^{20} - 73{,}5°$ (*c* 1,1 CH_3OH). Both substances are new and we have called them feroside (I) and reoselin A (II), respectively.

Feroside (I) is readily soluble in alcohols, acetone, and pyridine, sparingly soluble in benzene, ethyl acetate, and water, and insoluble in ether. Its UV spectrum shows maxima at 330, 245, and 222 nm (log ε 4.06, 2.97, 3.99), which are characteristic for derivatives of 7-hydroxycoumarin [1, 2], and its IR spectrum shows absorption bands at (cm^{-1}) 1720 (carbonyl of an α-pyrone), 1710 (ketone carbonyl), 1520, 1560, 1620 (aromatic nucleus), and 3100-3650 and 1000-1150 (hydroxy groups). When compound (I) was subjected to acid hydrolysis, umbelliferone (III), $C_9H_6O_3$ with mp 231-232°C, was isolated from the acid fraction, and D-glucose (IV) from the neutral fraction, this being identified by the preparation of an osazone with mp 202-203°C and by paper chromatography.

The enzymatic hydrolysis of (I) with β-glycosidase gave an optically inactive aglycone with the composition $C_{24}H_{30}O_5$ (V) (M^+ 398), mp 59-60°C. These results showed that feroside is a monoglycoside of a terpenoid hydroxycoumarin (V). Its IR spectrum showed absorption bands of an α-pyrone carbonyl (1720 cm^{-1}), a ketone carbonyl (1710 cm^{-1}), an aromatic nucleus (1520, 1560, 1620 cm^{-1}), and a hydroxy group (3100-3600 cm^{-1}). In the determination of the structure of feroside it was necessary to determine the structure of the aglycone, the position of attachment of the sugar residue, and the size of the oxide ring, and also the configuration of the glucose.

The NMR spectrum of (V) contained the signals of two tertiary methyl groups (s, 1.1 ppm, 6H), of vinyl methyl groups (s, 1.62 and 1.70 ppm, 3H each), and of olefinic protons (m, 5.2 ppm, 2 H). The methylene protons in a $ArOCH_2$ grouping appeared in the form of a doublet at 4.35 ppm, J = 6.5 Hz, from which it follows that there is only one proton on the neighboring carbon atom [3]. Furthermore, in the 6.00-7.35 ppm region there were the signals of the protons of a 7-hydroxy-substituted coumarin nucleus.

The composition $C_{15}H_{25}O_2$ and the presence of two double bonds and of a carbonyl group showed that there must be an aliphatic hydrocarbon chain in the sesquiterpene substituent.

On comparing the physicochemical constants and spectral characteristics it can be seen that the aglycone is identical with karatavikin isolated previously from *F. karatavica* [4]. This was confirmed by a mixed melting point with an authentic sample of karatavikin which we isolated from *F. karatavica*.

N. P. Kir'yalov has suggested two alternative structures for karatavikin [4]. The mass spectrum of karatavikin contained a peak with $(M - 59)^+$ showing the presence of the $-C(OH)(CH_3)_2$ fragment, which permits a decision in favour of the alternative structure (I) (Figure 1a).

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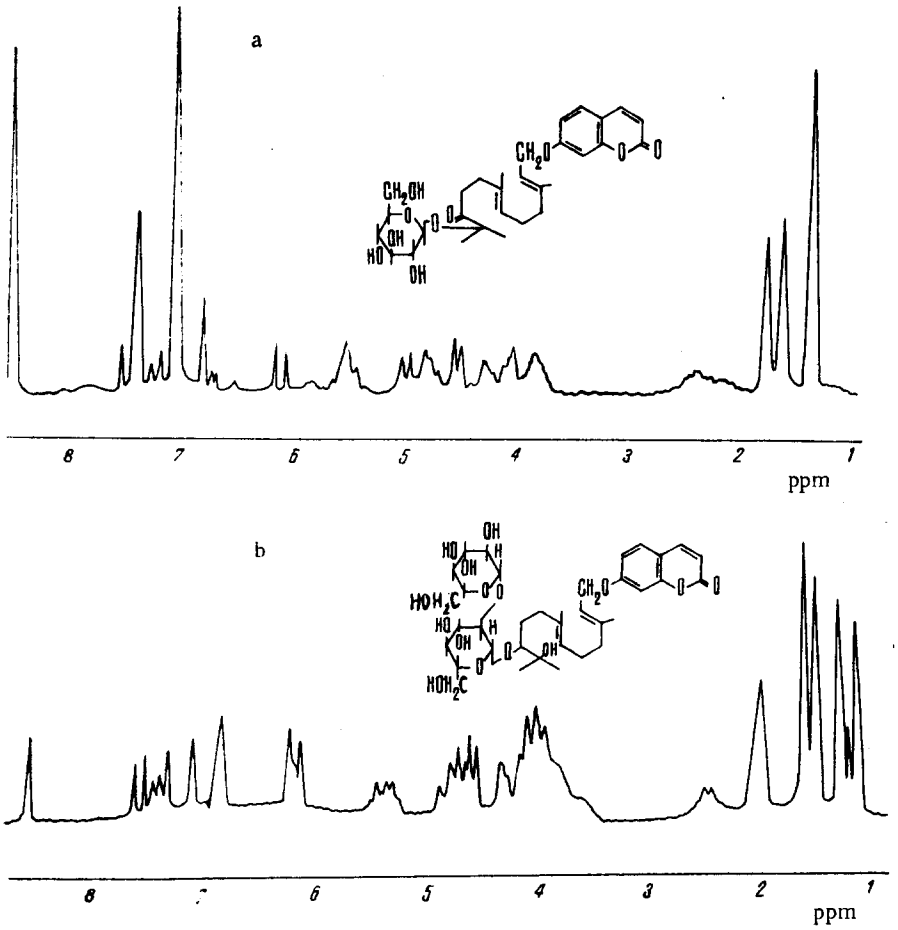

Fig. 1. NMR spectra (in deuteropyridine) of feroside (a) and of reoselin A (b).

Thus, feroside is a monoglycoside of karatavikin. The substance is readily hydrolyzed by β-glucosidase and consequently it is a O-β-glycoside at a tertiary hydroxy group. The existence of the β configuration in it is confirmed by the spin–spin coupling constant of the signal of the anomeric proton (d, 5.02 ppm, J = 6 Hz).

The IR spectrum of feroside shows absorption bands at 1010, 1040, and 1080 cm^{-1} which are characteristic of pyranosides [5]. Acetylation of the substance with acetic anhydride in pyridine yielded a tetraacetate (VI). Measurements of the integral intensities of the protons in the 3.5-4.5 and 4.5-5.5-ppm regions showed that they were present in a ratio of 2:1. This is characteristic of pyranosides [6-8]. The values obtained and also calculations of $[M]_D$ according to Klyne showed that the glucose has the pyranose form [9].

On the basis of what has been said above, we propose for feroside the structure shown in Fig. 1a.

Reoselin A (II), with the composition $C_{36}H_{52}O_{15}$, is readily soluble in ethanol, insoluble in benzene and ether, and sparingly soluble in water. Its UV spectrum contains maxima at 245, 255, and 326 nm (log ε 3.33, 3.03, and 4.04), which characterizes it as a derivative of a 7-hydroxy-substituted coumarin, and its IR spectrum contains absorption bands at (cm^{-1}) 3200-3600 (hydroxy groups), 1720 (α-pyrone carbonyl), and 1510, 1560, and 1615 (aromatic nucleus). Enzymatic hydrolysis of (II) with β-glycosidase gave an aglycone (III) with the composition $C_{24}H_{32}O_5$ (M^+ 400), mp 65-66°C, $[\alpha]_D^{20}$ ±0° (c 1.3; chloroform) and D-glucose. The latter was identified by paper chromatography and by the preparation of the osazone with mp 201-202°C. Judging from the compositions of the substance and the aglycone, the presence in the NMR spectrum of (II) of 14 protons in the 3.5-4.9 ppm region, and the results of quantitative hydrolysis, it may be concluded that the glycoside is a bioside.

The NMR spectrum of (III) shows the signals from the protons of tertiary methyl groups (s, 1.10 and 1.21 ppm, 3 H), vinyl methyl groups (s, 1.5 and 1.7 ppm, 3 H each), and hydroxy groups (s, 2.07 ppm, 2H, disappearing on deuteration) and also of olefinic protons (m, 5.05 and 5.30 ppm, $W_{1/2}$ = 17 Hz, 1 H each) and of a hemihydroxyl proton (m, 3.4 ppm, $W_{1/2}$ = 16 Hz, 1 H). The methyl protons in the $ArOCH_2$ grouping are shown in the form of a doublet at 4.5 ppm, $W_{1/2}$ = 6.5 Hz. This shows that like karatavikin (V), the molecule of (III) has a $ArOCH_2$—CH fragment. In addition, the signals of the protons of a 7-hydroxy-substituted coumarin can be seen in the 6.10-7.5 ppm region. Consequently, the aglycone is none other than the ether of 7-hydroxycoumarin and a sesquiterpene substituent with the composition $C_{15}H_{27}O_2$. With this composition and two double bonds, the terpenoid part of the aglycone must have an aliphatic hydrocarbon chain. On comparing the physicochemical constants of the aglycone obtained with those of coumarins of a similar type, it can be seen that they are identical with karatavikinol, isolated from *F. karatavica* [9]. This is confirmed by the IR and NMR spectra of the substances mentioned and by mixed melting points with an authentic sample isolated from *F. pseudooreoselinum* [3]. To determine the position of the sugar residue at the secondary or the tertiary hydroxyl, we used PMR spectroscopy. For this purpose, we prepared the octaacetate, in the IR spectrum of which the absorption bands of hydroxy groups had disappeared while the NMR spectrum showed the signals of eight acetyl residues in the form of singlets in the 1.9-2.1-ppm region. The signals of the gem-dimethyl groups were displaced downfield by 0.15 ppm, from which it follows that in the octaacetate seven of the acyl residues are bound to glucose and one to the tertiary hydroxyl of the terpenoid substituent. Consequently, in the glycoside molecule the glucose is attached through the secondary hydroxyl.

The size of the oxide ring of the D-glucose can be judged from the NMR spectrum of the octaacetate (II), in which the integral intensities of the signals of the hemiacyl protons of the hexose in the 3.5-4.5 and 4.5-5.5-ppm region are in a ratio of 2:1, which corresponds to literature information for pyranosides [6-8]. The anomeric protons of the sugar appear in the form of a doublet at 4.65 ppm (2 H, J = 7 Hz), which shows their β-configuration.

Thus, it can be seen from a consideration of the NMR spectra given that the glucose residues have six-membered rings.

Acid hydrolysis of the products of exhaustive methylation yielded 3,4,6-trimethyl- and 2,3,4,6-tetramethylglucoses, which were identified chromatographically from their R_f values and by means of color reactions. The formation of the latter indicate a 1→2 bond. The methylated diglycoside was stained orange on the chromatograms by diphenylamine—p-anisidine and yellow by diphenylamine—aniline, which also indicates a 1→2 bond.

This conclusion is also confirmed by the similar multiplicities of the signals in the NMR spectrum of reoselin A in the 3.5-5.0 ppm region and by literature information reported for diglycosides with a 1→2 bond [6].

Ferula pseudooreoselinum has previously yielded reoselin [9], which is a diglycoside of karatavikinol and two D-glucose residues connected by a 1→4 bond [3].

The glycoside that we have isolated is an isomer of reoselin with respect to the arrangement of the bond, and therefore we propose to call it reoselin A.

On the basis of the facts presented, reoselin A has the structure of karatavikinol 10'-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (Fig. 1b).

EXPERIMENTAL

The conditions for taking the NMR spectra have been described elsewhere [1]. The purity of the substances was checked by thin-layer chromatography on KSK silica gel in the systems 1) hexane—benzene—methanol (5:4:1), and 2) chloroform—methanol (4:1).

Isolation of Feroside and Reoselin A. The dried comminuted roots of Korzhinskii's ferula (10 kg) were steeped in methanol three times (45, 40, and 40 liters). The extracts were combined and evaporated in vacuum to small volume (1 liter) and the residue was diluted with water (1:2) and treated with ether (3 × 1 liter). The aqueous ethanolic mother solution was extracted with butan-1-ol (4 × 0.5 liter). The butanol solution was distilled. This gave 95 g of a viscous extract which was deposited on a chromatographic column (80 × 6 cm) filled with type KSK silica gel and was eluted with two liters of chloroform. When the solvent was distilled off, 4.2 g of a mixture of substances with R_f 0.40, 0.51, 0.62, 0.80, and 0.90

(system 1) was isolated. After this, the column was washed with a mixture of chloroform and propanol (4:1), 300-ml fractions being collected. When fractions 1-10 were concentrated, 3.3 g of an oily residue was obtained which was crystallized from isopropanol. This yielded 1.7 g of a colorless cyrstalline substance with the composition $C_{30}H_{42}O_{10}$, mp 110-111°C. On subsequent elution of the column with the same solvent, fractions 18-27 yielded 3.9 g of a substance with R_f 0.6. On crystallization from isopropanol, 2.6 g of a colorless crystalline substance with the composition $C_{36}H_{52}O_{15}$, mp 160-161°C deposited.

Enzymatic Hydrolysis of Feroside. A few drops of ethanol were added to a solution of 0.6 g of the substance in 20 ml of hot water and, after cooling, 0.3 g of β-glucosidase was added and the mixture was left in the thermostat at 37°C for 5 days. The liquid was diluted with ethanol (1:2) and heated on the water bath. The ethanol was distilled off, the liquid was diluted with water, and the aglycone was extracted with ether (4 × 50 ml). After the elimination of the solvent, a colorless crystalline substance with mp 59-60°C (ether–ethyl acetate) was obtained. Yield 70%.

Acid Hydrolysis of Feroside. To a solution of 0.16 g of the substance in 10 ml of ethanol was added 5 ml of 10% HCl, and the mixture was heated on the water bath for 30 min. Then the liquid was treated with chloroform, and the extract was distilled. The residue was crystallized from methanol. Crystals deposited with mp 231-232°C, a mixed melting point of which with an authentic sample of umbelliferone showed no depression of the melting point. D-glucose was found in the mother solution by paper chromatography in the butanol–acetic acid–water (4:1:5) system.

Feroside Tetraacetate. A mixture of 0.17 g of the substance, 5 ml of pyridine, and 5 ml of acetic anhydride was heated in the water bath for 4 h. The acetate was obtained by a known method in the form of an amorphous substance with R_f 0.7 (system 1).

The enzymatic hydrolysis of reoselin A was performed by the method described above. A colorless cyrstalline substance with mp 65-66°C (from ether) was isolated. Yield 55%.

The acid hydrolysis of reoselin A was performed by the method described above. Umbelliferone was obtained with mp 231-232°C.

Heptaacetate of Reoselin A. By a method analogous to that of feroside, a colorless cyrstalline substance with mp 161-162°C (from ether) was obtained.

The octaacetate of reoselin A was isolated by heating the substance with fused sodium acetate and acetic anhydride in pyridine on the water bath for 6 h. After appropriate working up, a crystalline substance deposited with mp 167-168°C (from ether).

D-Glucosephenyl osazone was obtained by a known method; yellow crystals with mp 201-202°C. A mixture with an authentic sample showed no depression of the melting point.

Isolation of Karatavikin. The concentrated methanolic extract of the comminuted herbage (1.2 kg) of *Ferula karatavica* collected in the environs of Chimkent, KazSSR, was diluted with water (2:1) and treated with ether (3 × 0.5 liter).

The ethereal extract was evaporated, giving 12 g of an oily residue consisting of a mixture of coumarins with R_f 0.3, 0.4, 0.6, and 0.7 [hexane–benzene–methanol (5:4:1) system] which was deposited on a column (80 × 5 cm) filled with type KSK silica gel. When the column was washed with gasoline–ethyl acetate (9:2), fractions 5-9 deposited a colorless cyrstalline substance with mp 59°C (petroleum ether–ethyl acetate) with an IR spectrum identical to that of karatavikin [4].

SUMMARY

Two new glycosides – feroside and reoselin A – have been isolated from the roots of *Ferula korshinskyi* Eug. Korov. On the basis of their chemical properties and spectra it has been established that feroside is karatavikin 11'-O-β-D-glucopyranoside and reoselin A is karatavikinol 10'-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranoside. The structure of karatavikin has been refined.

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