COUMARINS OF Ferula kopetdaghensis AND THE STRUCTURE OF KOPEOLIN AND KOPEOSIDE

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We have previously reported the isolation of galbanic acid from the roots of Ferula kopetdaghensis Eug. Kor. collected in the Kara-Kala gorge in the upper reaches of the R. Akinzhir [1]. Continuing a study of the lactones of this plant, from ethereal and aqueous methanolic fractions by chromatography on KSK silica gel, with elution by petroleum ether–benzene and chloroform–propanol we have isolated another five coumarins – umbelliferone and four new ones which we have called kopetin, kopeodin, kopeolin, and kopeoside.

Umbelliferone (I), $C_9H_6O_3$, mp 231-232°C (from water), R_f 0.95 [chloroform–ethyl acetate–methanol (15:7:3)], M^+ 162, identified by its IR spectrum and by a mixed melting point.

Kopetin (II), $C_{24}H_{32}O_5$, mp 145-146°C [petroleum ether–benzene (5:1)], $[\alpha]_D^{25}$ −25.4° (c 0.78; ethanol), R_f 0.40 [hexane–benzene–methanol (5:4:1)], M^+ 400, UV spectrum: λ_{max} 244, 255, 327 nm (log ε 3.56, 3.42, 4.17). According to the UV spectrum and the products of acid cleavage, kopetin is an ether of umbelliferone and a sesquiterpene triol with the composition $C_{15}H_{28}O_3$. Its IR spectrum has absorption bands at (cm^{-1}): 3450-3530 (hydroxy groups), 1730 (carbonyl of an α-pyrone ring), and 1620, 1560, and 1515 (aromatic nucleus).

Kopeodin (III), $C_{24}H_{30}O_4$, mp 111-112°C (from benzene), $[\alpha]_D^{25}$ +14.8° (c 0.84; ethanol), M^+ 382, R_f 0.2, UV spectrum: λ_{max} 244, 255, and 327 nm (log ε 3.62, 3.61, 4.29). The IR spectrum has absorption bands at (cm^{-1}) 3450 (hydroxy group), 1700 (carbonyl of an α-pyrone), and 1620, 1565, and 1515 (aromatic nucleus). This substance is also an ether of umbelliferone, in this case with a sesquiterpene diol having the composition $C_{25}H_{26}O_2$.

Kopeolin (IV), $C_{24}H_{32}O_5$, mp 146-147°C (from benzene), $[\alpha]_D^{25}$ −15.9°(c 0.98; ethanol), R_f 0.19, M^+ 400, UV spectrum: λ_{max} 244, 253, 327 nm (log ε 3.64, 3.59, 4.13). In the IR spectrum there are absorption bands at (cm^{-1}): 3450-3510 (hydroxy groups), 2885-2935 (C-methyl groups), 1725 (carbonyl of an α-pyrone) and 1625, 1515, and 1470 (aromatic nucleus). The chemical and spectroscopic results show that it is an ether of umbelliferone with a sesquiterpene triol, $C_{15}H_{28}O_3$.

Kopeoside (V), $C_{30}H_{42}O_{10}$, mp 177-178°C (from aqueous methanol), $[\alpha]_D^{25}$ −22.1° (c, 1.47; ethanol), M^+ 400, UV spectrum: 244, 253, 327 nm (log ε 3.34, 3.12, 4.12). IR spectrum (cm^{-1}): 3250-3550 (hydroxy groups), 2900-2950 (C-methyl groups), 1720 (carbonyl of an α-pyrone ring), and 1625, 1515, and 1465 (aromatic nucleus). Kopeoside is also an ether of umbelliferone and a glycosylated sesquiterpene triol with the composition $C_{15}H_{26}O_3$.

Thus, the coumarins of Ferula kopetdaghensis consist of a complex mixture of ethers of umbelliferone and sesquiterpene alcohols (diols and triols), and also their glycosides.

As follows from the empirical formula of kopeolin (IV), the terpenoid part of its molecule has the composition $C_{15}H_{27}O_2$ and is the residue of a sesquiterpene triol $C_{15}H_{28}O_3$.

On catalytic hydrogenation over PtO_2, substance (IV) added 1 mole of hydrogen, forming a dihydro derivative with M^+ 402. This shows that the triol contains one double bond. The presence of such a bond was confirmed by an absorption band at 830 cm^{-1} in its IR spectrum (Fig. 1), corresponding to a trisub-

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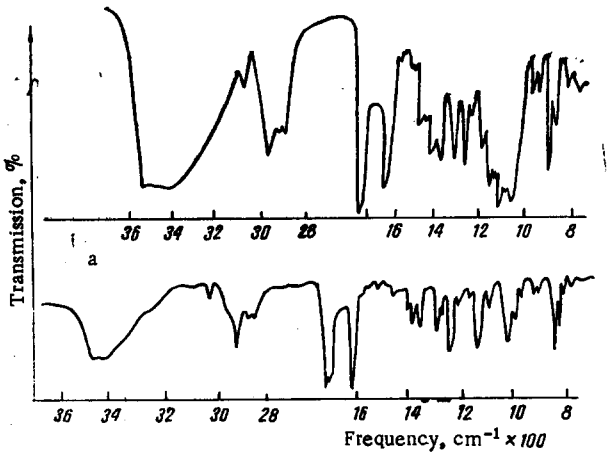

Fig. 1. IR spectra in KBr of kopeolin (a) and kopeoside (b).

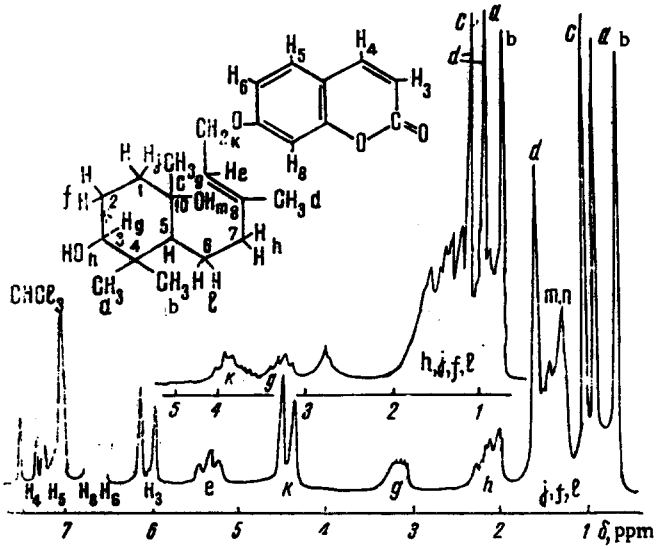

Fig. 2. NMR spectrum of kopeolin (bottom), and of dihydrokopeolin (top).

stituted ethylene group $R-C(H)=C(R_1)(R_2)$ which disappeared in the spectrum of the dihydro derivative. The facts mentioned are in harmony with the NMR spectrum, which has a one-proton signal of an olefinic proton. With the given composition and one double bond the triol can have only a monocylic structure. The IR spectrum of (IV) also exhibits a broad absorption band at 3400-3500 cm^{-1} relating to hydroxy groups. In the mass spectrum of (IV), in addition to the peak of a molecular ion, M^+ 400, peaks appear with m/e 239, 162, and 163, due, respectively, to the terpenoid moiety of (IV) and to the molecular and protonated ions of umbelliferone. Peaks with m/e 221 and 203 correspond to the fragments formed as the result of the successive splitting out of two molecules of water from the terpenoid residue [2], which shows the presence of free hydroxyls in it.

Thus, the developed formula of (IV) may be represented in the following way: $C_9H_5O_2-OCH_2-C_{14}H_{23}(OH)_2$.

The NMR spectrum of (I) (Fig. 2) has the signals of the protons of a 7-hydroxycoumarin residue: doublets at 7.54 and 6.14 ppm, J = 10.5 Hz, quartet at 6.72 ppm, J_1 = 9.5 Hz, J_2 = 2.0 Hz, doublets at 7.28 ppm, J = 9.5 Hz, and 6.82 ppm, J = 2.0 Hz, corresponding to the H-4, H-3, H-6, H-5, and H-8 protons.

In the strong-field region there are singlets at 0.97 and 0.73 ppm (3H each) (gem-dimethyl groups on a quaternary carbon atom), a singlet at 1.12 ppm (3H) (methyl group on a carbon atom to which a hydroxyl is attached), and a broadened singlet at 1.72 ppm (3H) (methyl group on a double bond). The broadening of the last signal is caused by the allyl interaction of the protons of the methyl group with the olefinic proton, which appears in the form of a triplet at 5.42 ppm, J = 7.0 Hz.

A doublet in the 4.49-ppm region (2H, J = 7.0 Hz) is due to the equivalent methylene protons in the $Ar-OCH_2-$ grouping undergoing interaction with an olefinic proton in the vicinal position, and a multiplet in the 2.13-ppm region (2H) is due to methylene protons attached to a carbon atom adjacent to a double bond. The multiplicity of the latter shows that there is a methylene group adjacent to it. It follows from this that the kopeolin molecule has the fragment $Ar-OCH_2-\overset{H}{\underset{|}{C}}=\overset{CH_3}{\underset{|}{C}}-CH_2-CH_2-$, i.e., the first isopentenyl link attached to the umbelliferone forms an open chain.

In the spectrum of the dihydro derivative, the signals of the olefinic proton and of the methyl group on the double bond have disappeared, and a three-proton doublet has appeared at 1.02 ppm, J = 8.5 Hz, corresponding to a methyl group on a secondary carbon atom. The signal of the methylene protons in the $Ar-OCH_2$ grouping has changed from a doublet into a multiplet with its center at 3.22 ppm, and the protons vicinal to the double bond have shifted upfield. These results show the presence of the fragment given above in the kopeolin molecule. The presence of methyl groups on tertiary carbon atoms, and also biogenetic considerations, show that the second and third isopentenyl links form part of a six-membered ring; i.e., substance (IV) has the carbon skeleton of farnesiferol C [3]. Both the hydroxy groups are present in this ring. As has been shown, one of them is located in the geminal position to a methyl group and is consequently tertiary; the second can only be secondary.

When kopeoside (see below) was treated with acetic anhydride and sodium acetate, the tertiary hydroxyl underwent acetylation, forming an acetyl derivative with mp 151-152°C.

In the NMR spectrum of the latter product, the signal of the geminal methyl group is shifted downfield by 0.24 ppm because of an electron-accepting influence, which shows the geminal positions of the hydroxyl and methyl groups.

The secondary hydroxyl can be attached to one of three carbon atoms: C_1, C_2, or C_3. The geminal methine proton appears in the NMR spectrum of (IV) in the form of a poorly resolved quartet at 3.22 ppm, the half-width of which is 18 Hz, which excludes the C_2 position of the hydroxyl, since in this case (five-spin system) the signal of the methine proton would be a multiplet and would have a half-width of 12 (equatorial) or 40 Hz (axial). The nature of the multiplicity and of the half-width of this signal in the spectrum of (IV) shows that the methine proton is located at C_1 or C_3, i.e., in the axial position.

The treatment of (IV) with acetic anhydride in pyridine formed a monoacetate with mp 110-111°C, composition $C_{26}H_{34}O_5$, M^+ 442. In its NMR spectrum, the signal of one of the gem-dimethyl groups has undergone a paramagnetic shift by 0.12 ppm, and the other a diamagnetic shift by 0.07 ppm; the signal of the C_{10} methyl has shifted downfield by 0.04 ppm. This shows that the secondary hydroxy group is most probably located at C_3 [2]. The different behaviors of the signals of the protons of the gem-dimethyl groups on the conversion of substance (IV) into the acetate is apparently due to steric factors (one of them is equatorial and the other axial). On the basis of these results, kopeolin has the structure shown in Fig. 2.

Kopeoside (V), as shown above, is an ether of umbelliferone and a glycosylated alcohol. The developed formula $C_9H_5O_2-C_{15}H_{25}O_2(OH)-C_6H_{11}O_5$ is proposed for it. The NMR spectrum of this coumarin is similar to that of kopeolin in relation to the chemical shift of the signals and their intensities and splittings. The main difference consists of the signals of the protons of the sugar residue and the superposition of the signals of the $C_{10}-CH_3$ protons on the signal of one of the gem-dimethyl groups, and also a downfield shift of the C_3 proton to 3.55 ppm. This unambiguously shows that kopeoside is a 3-O-glycoside of kopeolin. This conclusion is supported by the NMR spectrum of kopeoside pentaacetate. In the latter, in addition to the appearance of the signals of CH_3CO groups (15H) in the 1.87-1.98 ppm region, a paramagnetic shift by 0.24 ppm of the signal of the protons of the tertiary methyl group (geminal to a hydroxy group) is observed and, consequently, the tertiary hydroxyl in kopeoside is free and the glucose residue is located at C_3. The absence of the peak of the molecular ion and the presence of fragment with m/e 400 are due to the splitting off of the sugar component from the glycoside on electron impact, in analogy with flavonoid glycosides [4].

The acid and enzymatic [5] hydrolysis of kopeoside led to its cleavage, forming 1 mole of glucose, identified by paper chromatography, and a coumarin with mp 144.5-145°C, R_f 0.19 [hexane–benzene–methanol (5 : 4 : 1)] which was identified by a mixed melting point and by IR and NMR spectroscopy as kopeolin. The IR spectrum of kopeoside has bands at 1085, 1060, and 1018 cm^{-1} and, therefore, the D-glucose is present in the pyranose form. The presence of a band at 893 cm^{-1} and the absence of one at 850 cm^{-1} give grounds for considering that the sugar component is attached by an α-glycosidic bond [6]. Thus, kopeoside is kopeolin 3-O-β-D-glucopyranoside.

EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer (KBr); the NMR spectra on a JEOL instrument at 60 MHz (in $CDCl_3$ and deuteropyridine), the chemical shifts being given in the δ scale from the signal of HMDS taken as 0; and the mass spectra on an MKh-1303 instrument. The purity of the substances was checked by thin-layer chromatography on silica gel in the hexane–benzene–methanol (5 : 4 : 1) (1) and chloroform–ethyl acetate–methanol (15 : 7 : 3) (2) systems.

Isolation of Kopetin, Kopeodin, and Kopeolin. The dried and comminuted roots of Ferula kopetdaghensis (4.5 kg) were steeped with methanol three times (25, 20, and 20 liters). The extracts were combined, evaporated in volume to 0.5 liter, and diluted with water (1 : 2). The liquid obtained was treated with ether (3 × 1 liter), whereupon four substances, with R_f 0.18, 0.20, 0.35, and 0.40 (system 1) passed into the extract, while substances with R_f 0.0 remained in the methanolic fraction. The ethereal extract was washed first with 10% sodium carbonate solution to eliminate the galbanic acid (R_f 0.18) and then with water, and it was dried over anhydrous sodium sulfate and was evaporated to dryness. This gave 290 g of residue in the form of a brown resin. About 100 g of this was chromatographed on a column filled with KSK silica gel (particle size 0.25 mm, weight 2 kg). Elution was performed with petroleum ether (bp 70°C)–benzene (1 : 1), 750-ml fractions being collected. The first fractions, which did not contain coumarins, were discarded, and the latter contained a mixture of purified substances. These fractions were rechromatographed under the conditions described above, and the substances were eluted with petroleum ether–benzene.

Concentration of the eluate from fractions 4-6 gave kopetin (0.4 g), fractions 10-11 yielded kopeodin (0.9 g), and the subsequent fractions yielded kopeolin (0.5 g).

Isolation of Umbelliferone and Kopeoside. The aqueous methanolic fraction was evaporated to dryness. This gave 380 g of a brown substance; 115 g of this was transferred to a chromatographic column filled with silica gel (2 kg, particle size 0.2 mm). On chloroform elution, fractions 1-10 yielded umbelliferone; on subsequent elution of the column with propanol–chloroform (5 : 15); fractions 14-15 yielded kopeoside (1.5 g).

Acid Hydrolysis of Kopeolin. A solution of 0.5 g of the substance in 5 ml of ethanol was treated with 2 ml of 10% HCl and the mixture was heated in the water bath for 10 min and was then left at room temperature for two days. Then it was diluted with water (1 : 2) and treated with ether (3 × 100 ml), and the extract was washed with water and distilled. The dry residue was chromatographed on a column (h = 20 cm; d = 3 cm) of type KSK silica gel, with chloroform elution. The evaporation of 1 liter of the eluate and recrystallization from water yielded small colorless crystals with mp 231-232°C.

Dihydrokopeolin. A solution of 0.040 g of the substance in 10 ml of ethanol was treated with 0.05 g of PtO_2, and hydrogenation was performed at room temperature. The amount of hydrogen consumed was 2.5 ml (1 mole). The liquid was filtered and evaporated in vacuum, giving an oily substance with M^+ 402, R_f 0.2 (system 1).

Kopeolin Monoacetate. A solution of 0.12 g of the substance in 5 ml of pyridine was treated with 10 ml of acetic anhydride and heated in the water bath for 1 h. Colorless crystals deposited with mp 110-111°C (from ether).

Kopeoside Pentaacetate. A solution of 0.15 g of kopeoside in 20 ml of acetic anhydride was treated with 2.0 g of fused sodium acetate and the mixture was heated in the water bath for 4 h. A crystalline substance with mp 151-152°C, R_f 0.9 (system 2) deposited.

SUMMARY

The roots of Ferula kopetdaghensis Eug. Kor. have yielded five coumarins – umbelliferone and four new compounds (kopetin, kopeodin, kopeolin, and kopeoside).

On the basis of NMR, mass, and IR spectroscopy, derivatives, and transformation products, the structures of two of the new coumarins kopeolin and kopeoside have been established. It has been shown that kopeolin is a derivative of farnesiferol C and kopeoside is kopeolin 3-O-β-D-glucoside.

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