COUMARINS OF Ferula kopetdaghensis – KOPETDAGHIN AND KOPEODIN (FARNESIFEROL B)

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Continuing a study of the coumarins of the roots of Ferula kopetdaghensis Eug. Kor. we have isolated, in addition to galbanic acid [1], another two isomeric coumarins; both have the composition $C_{24}H_{30}O_4$, but one has mp 125-125.5°C $[\alpha]_D^{18}$ +28° (c 1.0; chloroform) and the other has mp 111-112°C, $[\alpha]_D^{20}$ +14.5° (c 0.84; ethanol), which we have called, respectively, kopetdaghin and kopeodin (farnesiferol B). The UV spectrum of kopetdaghin (I) [λ_{max} 244.255 and 327 nm (log ε 3.22; 2.92; 3.15)] is characteristic for the chromophore of 7-hydroxycoumarin.

IR spectrum of (I) (Fig. 1): ν_{max} 3450 cm^{-1} (hydroxy group), 1690 cm^{-1} (carbonyl of an α-pyrone), 1610, 1560, 1510 cm^{-1} (aromatic nucleus). The presence of a free hydroxy group in the substance was confirmed by the formation of a monoacetate $C_{26}H_{32}O_5$ (II) with M^+ 424. The mass spectrum of kopetdaghin has the peak of the molecular ion, M^+ 382, and high-intensity peaks among which those with m/e 162 and 163 are due to the molecular and protonated molecular ions of umbelliferone [2], and a peak with m/e 220 is due to the terpenoid part of the molecule. Peaks with m/e 202 and 203 correspond to the fragment formed by the splitting off of one molecule of water from the terpenoid residue, which also confirms the presence of one hydroxy group. On catalytic hydrogenation over PtO_2, kopetdaghin adds two moles of hydrogen and forms a tetrahydro derivative $C_{24}H_{34}O_4$ (III) with M^+ 386, the UV spectrum of which contains the absorption maximum characteristic of the initial chromophore, which shows the addition of the hydrogen to two double bonds present in the sesquiterpene residue. With the composition $C_{24}H_{30}O_4$ and the presence of two double bonds, the sesquiterpene moiety of kopetdaghin can have only a monocyclic structure.

In the NMR spectrum of kopetdaghin (Fig. 2) there are doublets at 7.57 and 6.16 ppm, J = 10 Hz, relating to the H-4 and H-3 protons, a quartet at 6.75 ppm, J_1 = 9 Hz, J_2 = 2.0 Hz, and doublets at 7.35 ppm, J = 9 Hz, and 6.82 ppm, J = 2.0 Hz due, respectively, to the H-6, H-5, and H-8 protons of the coumarin nucleus [3-4].

In the strong-field region there are the signals of gem-dimethyl groups on a quaternary carbon atom – singlets at 0.94 and 0.79 ppm (3H each). In addition, the molecule of kopetdaghin contains two methyl groups present on double bonds, as is shown by two broadened singlets at 1.73 and 1.66 ppm (3H each). The broadening of the latter is caused by long-range allyl coupling of the protons of the methyl groups with the olefinic protons [5], which appear in the form of a triplet at 5.42 ppm, J = 6.0 Hz, for one, and a multiplet at 5.18 ppm, for the other. The half-width of the multiplet is 10 Hz and is somewhat greater than that of a methyl group. Consequently, there is a secondary or tertiary atom in the vicinal position, $CH_3-C=CH-CH_2-$. A doublet in the 4.53-ppm region (2H), J = 6.0 Hz, is due to equivalent methylene protons in the $Ar-OCH_2-CH$ grouping. The equal spin–spin coupling constants of this doublet and of the signal of the olefinic proton (triplet 5.42 ppm, J = 6.0 Hz) shows that these protons are attached to adjacent carbon atoms in the $Ar-O-CH_2-CH=C(CH_3)$ grouping. It follows from this that the molecule of kopetdaghin contains the fragments $Ar-OCH_2-CH=C(CH_3)-CH_2-$ and $-CH_2-CH=C(CH_3)-CH$.

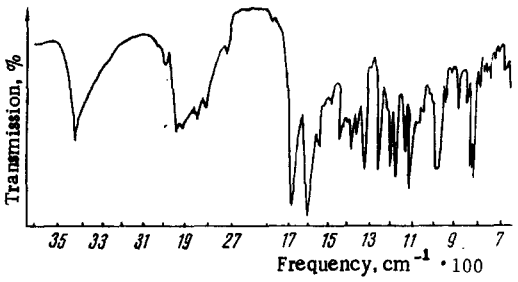

Fig. 1. IR spectrum of kopetdaghin.

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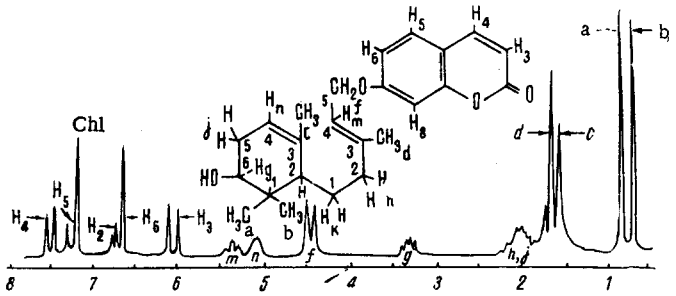

Fig. 2. NMR spectrum of kopetdaghin (in deuterochloroform).

The presence in it of triply substituted ethylenic groups is confirmed by the appearance in the IR spectrum of the substance of a band at 810 cm^{-1} which disappears on hydrogenation. In the NMR spectrum of (III), the signals of the olefinic protons and of the methyl groups on double bonds disappear, and two three-proton doublets appear at 1.20 and 0.97 ppm, J = 7.2 Hz, corresponding to methyl groups on a secondary carbon atom, and the signal of the methylene protons in the $Ar-OCH_2-CH$ group is converted from a doublet into a multiplet with its center at 3.92 ppm.

The facts given confirm the presence of the fragments discussed and show that one of them, connected with an aromatic nucleus, forms an open chain, and the second, by analogy with farnesiferol B, forms part of a six-membered ring. On the basis of these considerations it may be concluded that kopetdaghin has the skeleton shown in Fig. 2.

The hydroxy group in kopetdaghin is secondary. This follows from the capacity of the substance for undergoing acetylation under mild conditions and from the presence in the NMR spectrum of the signal of a hemihydroxylic methine proton in the form of a well-resolved quartet at 3.41 ppm, $J_1 = 10$ Hz, $J_2 = 5$ Hz. It follows from the nature of the multiplicity of this signal that the hemihydroxylic proton is located in the axial position and the hydroxyl in the equatorial position. In the spectrum of the acetate (II), the signal of the methine proton is shifted downfield by 1.09 ppm, the signal of one of the gem-dimethyl protons, upfield by 0.11 ppm, and that of the other downfield by 0.02 ppm. These facts unambiguously show that the hydroxyl is located on carbon atom 6 (for system of numbering, see Fig. 2).

Consequently, kopetdaghin has the most probable structure shown in Fig. 2. This is also confirmed by the fact that its tetrahydro derivative is identical, according to its IR and NMR spectra, with tetrahydrofarnesiferol B (see below).

Kopeodin (IV), $C_{24}H_{30}O_4$, with mp 111-112°C (from benzene) is also an ether of umbelliferone and a sesquiterpene diol with the composition $C_{15}H_{26}O_2$. On catalytic hydrogenation it added two moles of hydrogen, forming a tetrahydro derivative $C_{24}H_{34}O_4$ (III) with M^+ 386. With this composition and two double bonds, the terpenoid part of the molecule can be only monocyclic.

The NMR spectrum of kopeodin has the signals of the protons of a 7-monosubstituted coumarin: doublets at 7.50 and 6.45 ppm, J = 9.7 Hz (1H each); quartet at 6.72 ppm, $J_1 = 7.3$ Hz, $J_2 = 2.5$ Hz (1H); doublets at 7.22 ppm, J = 9 Hz, and 6.62 ppm, J = 2.5 Hz (1H each), corresponding to the H-4, H-3, H-6, H-5, and H-8 protons.

In the strong-field region the signals of gem-dimethyl groups on a quaternary carbon atom are observed – singlets at 0.95 and 0.67 ppm (3H each) and that of one methyl group on a double bond – broadened singlet at 1.70 ppm (3H). A doublet in the 4.5-ppm region (2H), J = 6 Hz, is due to the equivalent methylene protons in an $Ar-OCH_2-CH=C$ grouping. The signal of the olefinic proton in this grouping is represented by a triplet at 5.45 ppm, J = 6 Hz (1H). Thus, the first isopentyl link is present in an open chain, both in kopetdaghin and in kopeodin.

A quartet at 3.28 ppm (1H), $J_1 = 9.5$ Hz, $J_2 = 3.5$ Hz, is due to a hemihydroxylic methine proton and shows that the hydroxy group is secondary and is present in the equatorial position. Two singlets at 4.72 ppm (1H) and 4.52 ppm (1H) are due to exocyclic methylene protons. The NMR spectrum of kopeodin acetate (V) (M^+ 424, mp 65.5-67°C) showed a singlet at 1.92 ppm (3H) due to an acetyl group, and the signal of the hemihydroxylic proton had shifted downfield by 1.15 ppm. The signal of one of the gem-dimethyl groups had undergone a diamagnetic shift (by 0.11 ppm) and that of the other, a paramagnetic shift (by 0.15 ppm),

which unambiguously shows the position of the hydroxyl in the immediate vicinity of the gem-dimethyl groups, i.e., in position 6. In view of the presence of the fragment $Ar-OCH_2-CH=C(CH_3)-CH_2-$ and of gem-dimethyl groups in a six-membered ring, the exocyclic methylene group can be present only at carbon atom 3.

On the basis of what has been said, kopeodin is most probably identical with farnesiferol B [6]. This conclusion is in harmony with the melting points, optical activities, and the complete identity of the NMR spectra of the tetrahydro derivatives of (IV) and (I) obtained on catalytic reduction over PtO_2. The melting point of the acetate (V) agrees with that given in the literature. The hydrogenolysis of (IV) with sodium in liquid ammonia gave a diol (VI) in the form of a yellowish oil with R_f 0.6, in the IR spectrum of which the absorption bands of the α-pyrone carbonyl and of the aromatic nucleus had disappeared, and bands had appeared at 3300-3550, 1100, 1035 cm^{-1}, corresponding to the stretching and deformation vibrations of secondary and primary hydroxy groups, while in the mass spectrum peaks appeared with m/e 220 $(M-H_2O)$ and 202 $(M-2H_2O)$. The oxidation of the diol with chromium trioxide in pyridine formed a hydroxy ketone (VII) with R_f 0.9, in the IR spectrum of which there was an absorption band at 1725 cm^{-1} of the carbonyl of a nonconjugated ketone in a six-membered ring.

The melting point of its semicarbazone (VIII) corresponded to that given in the literature for the derivative obtained from farnesiferol B, which confirms the identity of (IV) and farnesiferol B. In addition, it must be noted that according to its NMR spectrum farnesiferol B is not an individual substance, but consists of a mixture of the two isomers (I) and (IV).

Thus, the signals of the vinyl protons have an integral intensity not of 2, but of only 1.4 proton units, while the intensities of the signals of the olefinic proton and of the methyl group of the double bond are increased by approximately 30%. Consequently, farnesiferol B is a mixture (possibly a molecular compound) of two moles of the isomer with structure (IV) (farnesiferol B proper) [6] and one mole of (I), which we have isolated for the first time and have called kopetdaghin.

The physicochemical properties given in the literature for farnesiferol B relate to this mixture.

EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer (KBr), the NMR spectra on a JNM-100/100 4H instrument at 100 MHz ($CDCl_3$), the chemical shifts being given in the δ scale from the signal of HMDS taken as 0, and the mass spectra were taken on an MKh-1303 instrument. The purity of the substances was checked by thin-layer chromatography on KSK silica gel [petroleum ether–ethyl acetate (4 : 1) system].

Isolation of Kopetdaghin (I) and Kopeodin (IV). After the removal of the galbanic acid [1], the ethereal solution was evaporated in vacuum to dryness. This gave 100 g of resinous residue. Of this, 25 g was mixed with silica gel and transferred to a chromatographic column (d = 3 cm, h = 70 cm) which was eluted with petroleum ether–ethyl acetate (5 : 1), 750-ml fractions being collected. After concentration and standing, fraction 6 deposited a crystalline substance with mp 125-125.5°C and fraction 7, a substance with mp 111-112°C.

Acid Hydrolysis of Kopetdaghin. A solution of 0.13 g of the substance in 20 ml of ethanol was treated with five drops of glacial acetic acid and 1 ml of concentrated sulfuric acid, and the mixture was heated on the water bath under reflux for 3 h. The liquid was diluted with water (1 : 2) and treated with ether (3 × 100 ml), and the extract was washed with water and evaporated. The dry residue was chromatographed on a column (h = 20 cm, d = 3 cm) containing KSK silica gel, and was eluted with chloroform. After the evaporation of one liter of eluate and recrystallization of the residue from water, colorless crystals were obtained with mp 231-232°C showing no depression of the melting point with an authentic sample of umbelliferone (IX).

Tetrahydrokopetdaghin (III). The hydrogenation of 0.1 g of kopetdaghin in 20 ml of acetic acid was performed in the presence of 0.05 g of PtO_2 (according to Adams) at room temperature. About 25 ml of H_2 was absorbed. The catalyst was filtered and the filtrate was evaporated in vacuum. A noncrystallizing substance, $C_{24}H_{34}O_4$, with R_f 0.22, M^+ 386 was isolated.

Kopetdaghin Acetate (II). A solution of 0.050 mg of kopetdaghin in 2 ml of acetic anhydride was treated with 1 ml of pyridine and left for 10 h. Then the reaction mixture was evaporated, the residue was treated with ether, and the solvent was evaporated off. This gave about 0.060 mg of an oily residue with R_f 0.45, M^+ 424.

Oxidation of Kopeodin (Farnesiferol B). To 0.22 g of the substance in 20 ml of acetone were added 5 ml of Beckmann's mixture, and the resulting mixture was left at room temperature for 10 min. Then it was diluted with 50 ml of water and treated with ether. The solvent was distilled off. About 0.2 g of an oily substance with R_f 0.22 was isolated. The 2,4-dinitrophenylhydrazone was obtained by a known method and formed red acicular crystals with mp 89-91°C (from ethanol).

Kopeodin Acetate (V). To 0.25 g of the substance were added 1 ml of pyridine and 5 ml of acetic anhydride, and the mixture was left for a day. The solvent was evaporated in vacuum to dryness, and the residue was transferred to a chromatographic column (d = 2 cm, h = 10 cm) filled with KSK silica gel. The acetate was eluted with petroleum ether–ethyl acetate (5 : 1). This gave a substance difficult to crystallize, with mp 65.5-67°C (from petroleum ether), R_f 0.55.

The Diol (VI). A solution of 0.35 g of kopeodin (farnesiferol B) in 2 ml of absolute methanol was added dropwise to the mixture obtained by dissolving 0.5 g of metallic sodium in 20 ml of liquid ammonia. After 3 h, 25 ml of a 3% solution of KOH in ethanol were added to the reaction mixture, and it was heated in the water bath for 3 h. Then it was diluted with water (1 : 3) and treated with ether, and the extracts were washed with water and evaporated. This gave an oily substance with R_f 0.6.

The Hydroxy Ketone (VII). A solution of 0.2 g of CrO_3 in 2 ml of pyridine was added to a solution of 0.25 g of the diol in 2.5 ml of pyridine, and the mixture was heated on the water bath for 30 min. The solvent was distilled off in vacuum and the residue was treated with pentane. About 0.2 g of hydroxy ketone with R_f 0.9 separated out. The semicarbazone (VIII) was obtained by the usual method. After recrystallization from methanol, yellowish acicular crystals with mp 139.5-141°C were obtained.

The acid hydrolysis and hydrogenation of kopeodin (farnesiferol B) were performed by the method described above.

SUMMARY

From the roots of Ferula kopetdaghensis Eug. Kor. a new coumarin has been isolated which has been called kopetdaghin. On the basis of the hydrolysis products, the results of hydrogenation and acetylation, and spectroscopy, it has been established that kopetdaghin is an ether of 7-hydroxycoumarin and 1-(3-hydroxy-2,2,6-trimethylcyclohex-5-enyl)-3-methylpent-2-en-1-ol, i.e., an isomer of farnesiferol B.

Furthermore, farnesiferol B has been shown to consist of a natural mixture of kopetdaghin (30%) and a component with the structure previously proposed for it (70%).

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