COUMARINS OF Fraxinus mandschurica AND F. potamophila

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Plants of the genus Fraxinus are characterized by a high content of hydroxycoumarins and glucosides which possess spasmolytic activity [1]. From 29 species of ash, 11 coumarins have been isolated at the present time, and from the two species mentioned in the title two coumarins [2]. In a chromatographic evaluation of 10 species growing in the USSR, we found in them a complex mixture of these substances with at least 13 components.

We have investigated the hydroxycoumarins present in the leaves of Fraxinus mandschurica Rupr., collected in October, 1970, and those present in the bark of three-year shoots of F. potamophila Herd., collected in March, 1971, in the Tashkent region in the period of the swelling of the buds.

To isolate the coumarins, the raw material was treated with methanol. The extract obtained, after it had been freed from pigments and lipophilic substances, was chromatographed on columns of polyamide, silica gel, and acidic alumina, with elution by organic solvents. Three coumarins were isolated.

The first coumarin had the composition $C_9H_6O_4$, mp 270-271°C, M^+ 178 (mass spectrometrically), R_f 0.82. UV spectrum, λ_{max}: 230, 260, 303, 354 nm (log ε 4.31, 3.79, 3.89, 4.23); its IR spectrum exhibited the absorption bands characteristic of hydroxycoumarins. In its composition and physicochemical properties it corresponded to 6,7-dihydroxycoumarin (esculetin), and this was confirmed by its IR spectrum and a mixed melting point.

The second coumarin, $C_{11}H_{10}O_5$, mp 171-172°C, M^+ 222, R_f 0.9, as shown by its UV spectrum (λ_{max} 234, 315, 345 nm; log ε 3.99, 3.88, 3.82), is a derivative of 5,6,7-trihydroxycoumarin. According to its IR spectrum, it contains a free hydroxyl (absorption band at 3400-3200 cm^{-1}). In order to determine its position, we made use of information [3] on the change in the nature of the UV spectra of the hydroxycoumarins in the formation of salts. In the spectrum of the lactone taken in the presence of alkali there was a bathochromic shift of the maxima by 24, 12, and 99 nm, respectively with a decrease in the intensity of the long-wave band (log ε 4.14, 3.89, 3.68), which is characteristic for 6-hydroxycoumarins. In its NMR spectrum there are doublets at 7.81 and 6.11 ppm, J = 9.5 Hz (H-4 and H-3), three-proton singlets at 3.86 and 3.93 ppm (methoxy groups attached to an aromatic nucleus), and a singlet at 6.50 ppm (H-5 or H-8 proton). According to the results of calculation and from the OH and OCH_3 increments as a function of their positions [4], this signal corresponds to the H-8 proton. Thus, the second coumarin is 6-hydroxy-5,7-dimethoxycoumarin and is therefore identical with fraxinol.

The third coumarin has the composition $C_{10}H_8O_5$, mp 228-230°C, M^+ 208, R_f 0.69, and forms yellowish acicular crystals soluble in acetone, dimethyl sulfoxide, and ethanol, and insoluble in petroleum ether, benzene, chloroform, and ether; it does not fluoresce in UV light. From its physicochemical properties it is a new coumarin, and we have called it isofraxetin. Judging from its composition, it contains two hydroxy groups and one methoxy group. Its UV spectrum [277, 350 nm (log ε 3.45, 3.80)] shows the presence in it of the chromophore of a 5,6,7-trihydroxycoumarin. We attempted to use UV spectroscopy to determine the positions of the substituents. It is known [5] that the salts of ions of 4-, 5-, and 7-monohydroxycoumarins have the quinoid type of structure and those of the 6- and 8-hydroxy derivatives the coumarin type. Conse-

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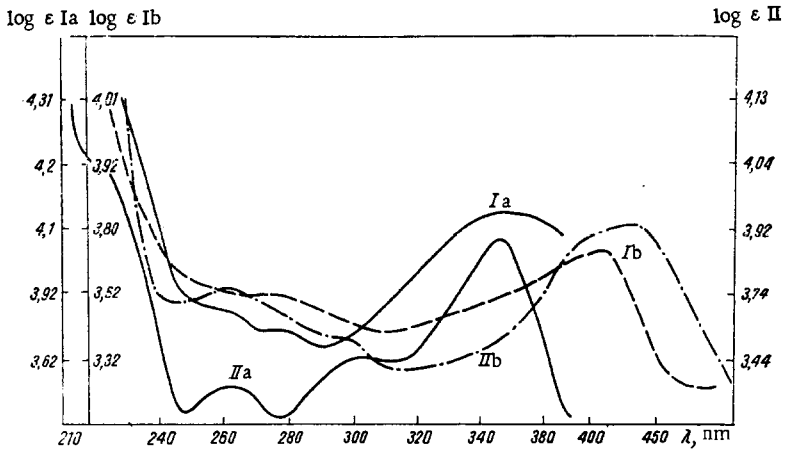

Fig. 1. UV spectra of isofraxetin (I) and of 6,7-dihydroxycoumarin (II) in neutral (a) and alkaline (b) media.

quently, when the UV spectra are taken in an alkaline medium the intensities of the maxima of the first group increase and in the second group they undergo bathochromic shifts with a simultaneous fall in log ε of the long-wave band. This characteristic was studied for the case of salts of monohydroxycoumarins, while isofraxetin is a dihydroxy derivative. When the hydroxy groups are located in the 5,6 or 6,7 positions, the corresponding ions may have either the quinoid or the coumarin structure, which explains the change in the UV spectrum in an alkaline medium. We have studied the spectrum of a model compound – 6,7-dihydroxycoumarin – under the conditions mentioned (Fig. 1). It was found that the spectrum of this substance changes like the spectrum of 6-hydroxycoumarin. Consequently, the ions of ortho-dihydroxy derivatives of this type possess the coumarin structure. The spectrum of isofraxetin changes in a similar manner to the spectrum of the 6-hydroxycoumarins and, therefore, the hydroxy groups in its molecule are present in the vicinal position and it is 5,6-dihydroxy-7-methoxycoumarin.

An analysis of the NMR spectra can confirm that isofraxetin is a 5,6,7-substituted coumarin. Doublets at 6.12 and 7.52 ppm, $J = 9.7$ Hz, correspond to the H-3 and H-4 protons, a singlet at 3.68 ppm (3 H) is due to a methoxy group attached to an aromatic ring, a broadened signal at 11.22 ppm (2 H) to phenolic hydroxyls, and a singlet at 6.52 ppm to the H-8 proton. The assignment of the latter signal is explained by the existence of long-range spin–spin coupling of the H-8 and H-4 protons as a result of which the peak intensity of the H-4 signal in the NMR spectrum is lower than that of H-3. A number of considerations permit a decision in favor of the position of the methoxy group at C-7. Thus, if it were present in position 5, the H-4 signal would shift downfield by analogy with the signal in coumarin, mexoticin, klozenin, and others [6-8] which is not observed in fact. Furthermore, the value of the chemical shift of H-8 is closer to the figure calculated using the method of increments [4] for 5,6-dihydroxy-7-methoxycoumarin. The methylation of isofraxetin with diazomethane gave a trimethoxy derivative with R_f 0.95 corresponding to a sample of 5,6,7-trimethoxycoumarin (it does not fluoresce in UV light, in contrast to 6,7,8-trimethoxycoumarin, which has a blue fluorescence).

EXPERIMENTAL

The UV spectra were taken on a Hitachi spectrophotometer, the IR spectra on a UR-10 instrument (KBr), the mass spectra on an MKh-1303 instrument, and the NMR spectra on a JEOL instrument at 60 MHz in $CDCl_3$ (I, II) and in deuteropyridine (III) (region from 1 to 9 ppm), the chemical shifts being given in the δ scale from the HMDS signal taken as 0.

Chromatography was performed on type "M" ["slow"] paper in the butan-1-ol–acetic acid–water (4:1:5) system; the spots were revealed with diazotized sulfanilic acid after the chromatograms had been sprayed with 10% sodium carbonate solution.

Isolation of Esculetin. The comminuted dried leaves of F. mandschurica (2 kg) were exhaustively extracted with methanol. The extract was concentrated to 500 ml, diluted with water (1:2), and treated successively with petroleum ether (pigments), chloroform, and n-butanol. After the solvents had been distilled off, 100 g of petroleum fraction (I), 10 g of chloroform fraction (II), and 49 g of butanol fraction (III) were obtained. The residual aqueous methanolic extract was called fraction (IV).

Fraction II was deposited on a chromatographic column filled with polyamide (h = 20, d = 10 cm) and was eluted with benzene–chloroform by the discrete method, and then with pure chloroform and with mixtures of chloroform and acetone with gradually increasing acetone contents.

Elution with a 30% solution of acetone in chloroform gave 0.050 g of a crystalline substance $C_9H_6O_4$ with mp 270-271°C (from methanol).

Isolation of Fraxinol. The dried and comminuted bark of F. potamophila (1 kg) was exhaustively extracted with methanol, the extract was evaporated to a viscous residue, and this was diluted with water (1 : 2) and successively extracted with petroleum ether (fraction I), chloroform (fraction II), and n-butanol (fraction III).

Fraction II was evaporated, giving 790 g of a brown mass of which 100 g was deposited on a chromatographic column containing acidic alumina of activity grade III (h = 40, d = 10 cm), and elution was performed with pure chloroform and then with acetone–chloroform (9 : 1), the total volume of the fractions amounting to 2.7 liters.

On concentration, the acetone–chloroform eluate gave 0.035 g of a crystalline substance $C_{11}H_{10}O_5$ with mp 171-172°C (from methanol).

Isolation of Isofraxetin. Another 10 g of fraction II was deposited on a column of KSK silica gel (h = 75, d = 4 cm), which was eluted with a mixture of acetone and chloroform (3 : 7). Distillation of the solvent yielded 0.0816 g of a substance $C_{10}H_8O_5$ with mp 228-230°C (from methanol).

Methylation of Isofraxetin. To 0.0300 g of the substance was added 30 ml of a solution of diazomethane in diethyl ether, and the mixture was left for 12 h (on methylation the substance dissolved in the ether). The solvent was distilled off to give a yellowish substance with R_f 0.95.

SUMMARY

The leaves of Fraxinus mandschurica Rupr. and the bark of F. potamophila Herd. have yielded esculetin, fraxinol, and a new coumarin $C_{10}H_8O_5$, mp 228-230°C which we have called isofraxetin. On the basis of NMR and UV spectroscopy it has been established that isofraxetin is 5,7-dihydroxy-7-methoxycoumarin.

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