COUMARINS OF Seseli tenuisectum

A. M. Aminov and G. K. Nikonov

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In a preceding paper [1] we reported the isolation from the roots and herbage of Seseli tenuisectum Rgl. et Schmalh. of the pyranocoumarin anomalin and the presence in them of two other lactones – C-2 and C-3.

In the present paper we give the results of a further study of these components and also of a lactone C-4 detected in the mother liquors after the separation of anomalin.

The lactones C-2 and C-3 appeared on paper in the form of two spots close to one another. On chromatography on alumina, they were eluted simultaneously. Numerous attempts to separate them by fractional crystallization and by rechromatography also proved unsuccessful. We have studied a purified binary mixture of these lactones by the PMR method.

The NMR spectrum of this mixture (Fig. 1) showed the following doublets: 7.51 ppm, superposed on 7.43 ppm, and 6.07 ppm superposed on 5.98 ppm, J = 10.5 Hz (1 H each), 7.20 and 6.61 ppm, J = 9 Hz (2 H each), due, respectively, to the H-4 and H-3 protons of the pyrone ring and to the H-5 and H-6 of the aromatic nuclei of the molecules of the two coumarins. This shows that both substances belong to the group of 7,8-disubstituted coumarins.

Two pairs of singlets at 1.40 and 1.33 ppm (12 H) correspond to the protons of gem-dimethyl groups in a six-membered ring, and two pairs of doublets at 6.33 and 3.94 ppm, J = 4.0 Hz and 5.2 and 4.91 ppm, J = 4.0 (1 H each) to methine protons in a pyran ring. This shows that these substances are derivatives of khellactone. It is known that the signal of the methine proton at the benzyl carbon atom in position 4' is always located in a weaker field than the analogous proton in position 3'. When there is an acyl group in position 4', the $H_{4'}$ signal shifts downfield from 5.20 to 6.3 ppm, which is due to the electron-accepting influence of the carbonyl of the ester group. If the acyl group is in position 3', the signal of the $H_{3'}$ proton likewise shifts downfield from 3.9 to 4.9 ppm.

By analogy with the esters of khellactone [2-4], the first pair of doublets corresponds to the methine proton of a khellactone acylated in position 4', and the second pair of doublets to the same proton of a khel-

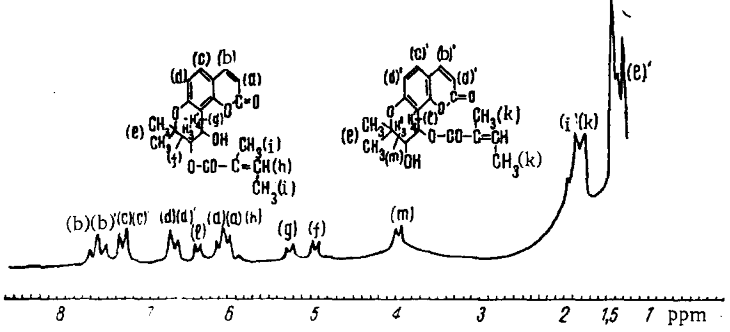

Fig. 1. NMR spectrum of a mixture of the lactones C-2 and C-3.

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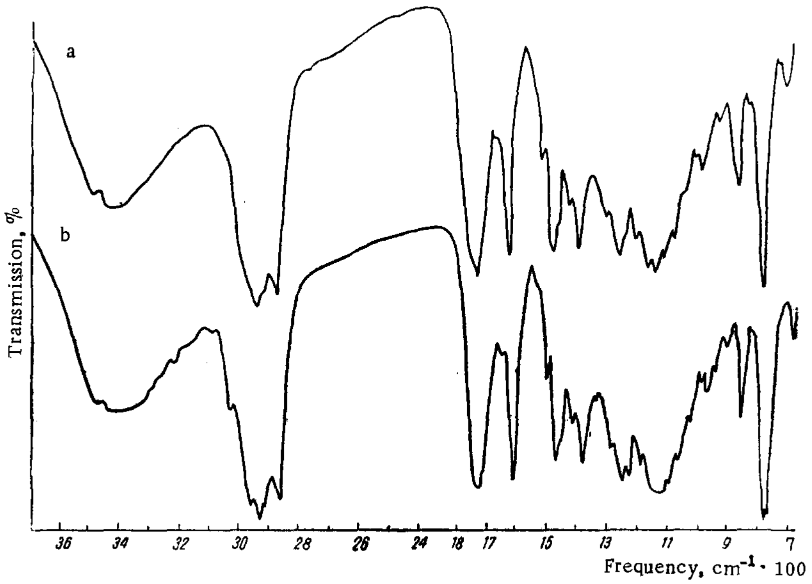

Fig. 2. IR spectra of the lactones C-2 (a) and C-3 (b).

lactone with an acyl residue in position 3'. The singlets at 1.75 and 1.85 ppm (12 H) are due to the protons of α- and β-methyl groups, and the multiplet at 6.05 ppm (2 H) to the olefinic protons of two angelic acid residues.

Thus, according to their NMR spectra, both coumarins are esters of khellactone and angelic acid.

The mass spectrum of the binary mixture showed a peak with M^+ 344, which could correspond only to monoesters of khellactone and the acid mentioned. The fact that the substances are monoesters was confirmed by the presence in the IR spectrum of the absorption band of hydroxy groups (3400-3500 cm^{-1}). To determine which of the structures corresponds to the C-2 and C-3 components, we used the stepwise hydrolysis [5] of the individual substances eluted in micro amounts from thin-layer chromatograms.

Acyl residues in khellactone esters are distinguished by different capacities for being saponified according to their positions [5-7]. When they are located on the benzyl carbon atom in position 4', they can be split off under mild conditions (0.25 N solution of alkali, 2 h), while in position 3' more severe conditions are required (time of hydrolysis 14 h).

In the substance with R_f 0.65 (C-3) the angelic acid split off after mild hydrolysis, and in substance with R_f 0.70 (C-2) after severe hydrolysis.

These facts show that the C-2 lactone has the structure of 3'-angeloyloxy-4'-hydroxy- and lactone C-3 that of 4'-angeloyloxy-3'-hydroxy-3',4'-dihydroseselin. This is confirmed by the passage from the C-3 lactone to the known coumarin pteryxin, which we performed by the acetylation of the substance with acetic anhydride. The product obtained was identical, from its IR spectrum and R_f value, with pteryxin (3'-acetoxy-4'-angeloyloxy-3',4'-dihydroseselin).

The monoesters of khellactone described above have been detected in the herb Seseli libanotis [2], but they were not isolated in the individual state and characterized, and the positions of the acid residues were not shown.

By thin-layer chromatography on silica gel (with the elution of 2800 spots) we obtained about 16 mg of the individual components, the IR spectra of which are given in Fig. 2, while their structures are shown in Fig. 1. The mother liquor from the separation of anomalin contains two components: anomalin (M^+426) and an unknown substance (M^+428) with the same R_f value.

The alkaline methanolysis of this mixture gave only methylkhellactone. Consequently, both coumarins are khellactone esters, and in fact the hydrolyzate contained isovaleric acid, in addition to angelic acid. The latter appeared only after severe hydrolysis, which shows that it is located at position 3'. According to what has been said above, unlike anomalin, the C-4 lactone is a mixed diester of khellactone and has the structure of 4'-angeloyloxy-3'-isovaleryloxy-3',4'-dihydroseselin. A substance with such a structure has been found in the herb Seseli libanotis [2], but it was not characterized, and the positions of the acid residues were not proved by chemical methods.

EXPERIMENTAL

The NMR spectrum was taken on a JNM-4-H-100/100 MHz instrument (in carbon tetrachloride). The IR spectra were obtained on a UR-10 instrument (paraffin oil), and the mass spectra on an MKh-1303 instrument.

Chromatography was performed by the descending method on paper impregnated with a 10% solution of formamide in methanol. The solvent used was hexane—benzene—methanol (5 : 4 : 1). The spots were revealed with diazotized sulfanilamide.

In the chromatography of the acids, the ascending method was used in the ethanol—ammonia—water (95 : 5 : 5) system, the spots being revealed with Bromophenol Blue.

Isolation of the Lactones C-2 and C-3. The dried and comminuted raw material (3 kg) was extracted with methanol (20, 15, 15 liters). The extract was concentrated in vacuum to 1 liter, and 2 liters of water was added. This mixture was treated with ether (3 × 500 ml), and the solvent was distilled off to dryness. The residue (30 g) was mixed with an equal volume of alumina and transferred to a column of the same adsorbent (20 × 9 cm, Brockmann activity II). The column was washed with 5 liters of petroleum ether and then with 15 liters of benzene. Concentration of the benzene eluate gave anomalin; on further washing of the adsorbent with benzene a fraction was obtained which on paper chromatography gave spots which fluoresced violet in UV light, were stained red on treatment with diazotized sulfanilamide, and had R_f 0.65 and 0.70.

Alkaline Cleavage of C-2 and C-3. A. Preparation of Methylkhellactone. A solution of 0.1 g of the mixture of C-2 and C-3 in 10 ml of 10% KOH in methanol was heated in the water bath for 2 h. The liquid was diluted with water and acidified with sulfuric acid solution. Then it was treated with ether (3 × 15 ml), and the solvent was distilled off. This gave an oily residue which, on a chromatogram, showed a spot with R_f 0.3 and 0.5; the IR spectrum of the latter corresponded to cis,trans-methylkhellactone.

B. Khellactone. A solution of 0.1 g of the mixture of C-2 and C-3 in 10 ml of dioxane was treated with 5 ml of a 10% aqueous solution of KOH and the mixture was heated for 2 h and was then acidified and extracted with ether. The solvent was driven off to give a residue which, on the basis of its IR spectrum, was identified as khellactone.

C. Angelic Acid. The saponification of 0.1 g of the mixture was performed by the method described above, and the alkaline solution was acidified, diluted with water (two volumes) and distilled with steam. The distillate was saturated with sodium chloride and treated with ether. The ethereal extracts were distilled, and the residue was chromatographed on paper in system 2.

D. Stepwise Alkaline Methanolysis of C-2 and C-3. 1. Under Mild Conditions. A mixture of the lactones C-2 and C-3 was chromatographed on paper (system 1). The fluorescing spots (60 spots) corresponding to the individual substances were cut out and eluted with 50 ml of methanol with heating for 15 min. The solvent was distilled off, the residue was dissolved in a 0.25 N solution of KOH in methanol, and this solution was left at room temperature for 2 h. Then the liquid was treated by the method used for methylkhellactone, whereupon the spot of the lactone with R_f 0.60 disappeared, and substances were formed with R_f 0.3 and 0.5 (cis- and trans-methylkhellactones). On paper chromatography in system 2, the hydrolyzate gave a spot with R_f 0.60, which corresponds to the R_f value of angelic acid. On saponification under the same conditions, the C-2 lactone underwent no change.

2. Under Severe Conditions. The methanolic eluate containing the C-2 lactone was treated with 5 ml of a 0.25 N solution of KOH in methanol, and the mixture was left for 14 h and worked up as described above.

A paper chromatogram in system 1 showed the presence of cis- and trans-methylkhellactones (spots with R_f 0.3 and 0.5), and a chromatogram in system 2 showed the presence of angelic acid (R_f 0.60).

Alkaline Methanolysis of the C-4 Lactone and Isolation of Isovaleric Acid. The mother liquor after the separation of the anomalin was evaporated to dryness, and the residue was dissolved in a 10% solution of KOH in methanol and treated in the same way as for angelic acid. On paper chromatography in system 2, a spot with R_f 0.78 was obtained, corresponding to isovaleric acid (marker).

Separation of the Lactones C-2 and C-3 by Thin-Layer Chromatography in Silica Gel. The mixture of the lactones C-2 and C-3 (20 drops) was deposited on a plate (13 × 18 cm) with a fixed layer of silica gel, and chromatography was performed in the diethyl ether—petroleum ether (3 : 1) system. In UV light, violet spots appeared with R_f 0.65 and 0.70 corresponding to the components C-2 and C-3. The adsorbent was removed from the plate and eluted with methanol, and the solvent was distilled off. The lactones were obtained from

140 such plates (13 mg with R_f 0.65 and 16 mg with R_f 0.70) in the form of viscous amorphous masses. The IR spectra of the substances were taken in paraffin oil.

The lactone C-3 was acetylated by the usual method of heating it with acetic anhydride in the presence of sodium acetate.

SUMMARY

The herb Seseli tenuisectum has been found to contain – in addition to anomalin – another three coumarins (C-2, C-3, and C-4) which are derivatives of khellactone.

On the basis of NMR, mass, and IR spectroscopy, the products of stepwise alkaline methanolysis and hydrolysis, and conversion into pteryxin, it has been established that two of them are isomeric monoesters of khellactone and angelic acid with the structures 3'-angeloyloxy-4'-hydroxy- and 4'-angeloyloxy-3'-hydroxy-3',4'-dihydroxyseselin, respectively. They have been obtained in the individual state for the first time by thin-layer chromatography, which has enabled their IR spectra to be recorded.

The lactone C-4 is a diester of khellactone with the structure 4'-angeloyloxy-3'-isovaleryloxy-3',4'-dihydroseselin.

We are the first to have found these substances in plants of the flora of the USSR.

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