LIBANOTIN: A NEW FUROCOUMARIN FROM LIBANOTIS TRANSCAUCASICA SCHISCHK

A. P. Prokopenko

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Libanotis transcaucasica Schischk. (Transcaucasian Libanotis), family Umbelliferae, grows in the wild state primarily in the Caucasus [1] but is relatively easily cultivated under the conditions of Leningrad province, in the Ukraine (Khar'kov province), and in other regions of the Soviet Union [2-4].

The fruit of this plant contains about 4% of essential oil, including geraniol (in the form of esters), phellandrene ($C_{10}H_{16}$), a sesquiterpene ($C_{15}H_{24}$), and other substances [5-9].

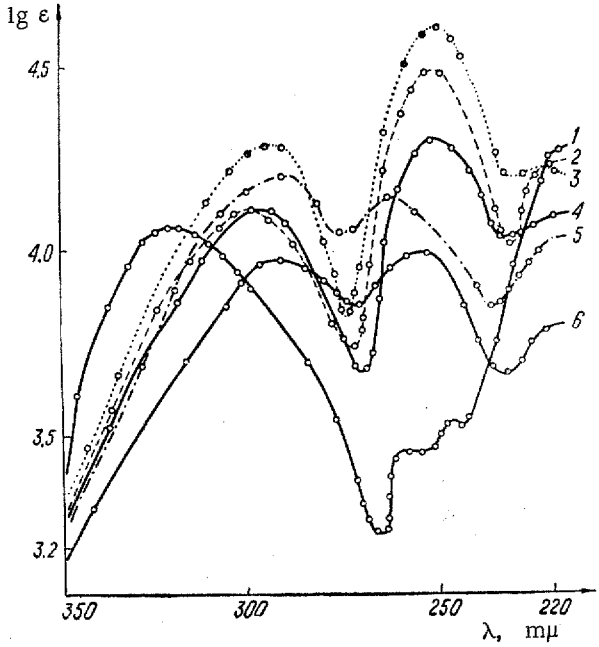
We have investigated the chemical composition of the fruit of the Transcaucasian Libanotis gathered in an experimental field of the Khar'kov Chemical and Pharmaceutical Research Institute, and have found not less than five substances which are derivatives of benzo-α-pyrone. The total amount of coumarins in the fruit of Transcaucasian Libanotis, according to our results, is 0.88% [10].

The present paper gives results of the isolation and chemical study of a new furocoumarin, which has been named libanotin. Libanotin – $C_{21}H_{22}O_7$, $[\alpha]_D^{20}$ + 75° (c 1; chloroform), mp 160-161° (from ethanol) – forms fine needle-like crystals readily soluble in chloroform, dichloroethane, and methylene chloride, less soluble in ethyl and methyl alcohols, and practically insoluble in petroleum ether and water.

The IR spectrum of libanotin has absorption bands at 1735, 1623, 1580, 1489, and 888 cm^{-1} and the UV spectrum has absorption maxima at 335, 260, and 245 mμ (log ε 4.04; 3.45; and 3.52, respectively), which are characteristic for furocoumarin derivatives.

Libanotin is readily hydrolyzed by alcoholic solutions of alkalis and acids. The hydrolysis of libanotin with 8% methanolic caustic soda forms five benzo-α-pyrone derivatives, three of which are present in considerable amounts and the others as traces; in addition, two acids have been detected.

Chromatography of the products of alkaline hydrolysis on alumina has given three substances in the pure state: the first $C_{11}H_6O_3$ (mp 139-140°); the second $C_{14}H_{10}O_3$ (mp 187-189°); and the third $C_{15}H_{14}O_4$ (mp 120-122°). From their physicochemical properties (melting points, empirical formulae, UV spectra) and also by paper chromatography with reference samples, the substances isolated have been identified, respectively, as angelicin, oroselone, and the methyl ether of oroselol (see figure).



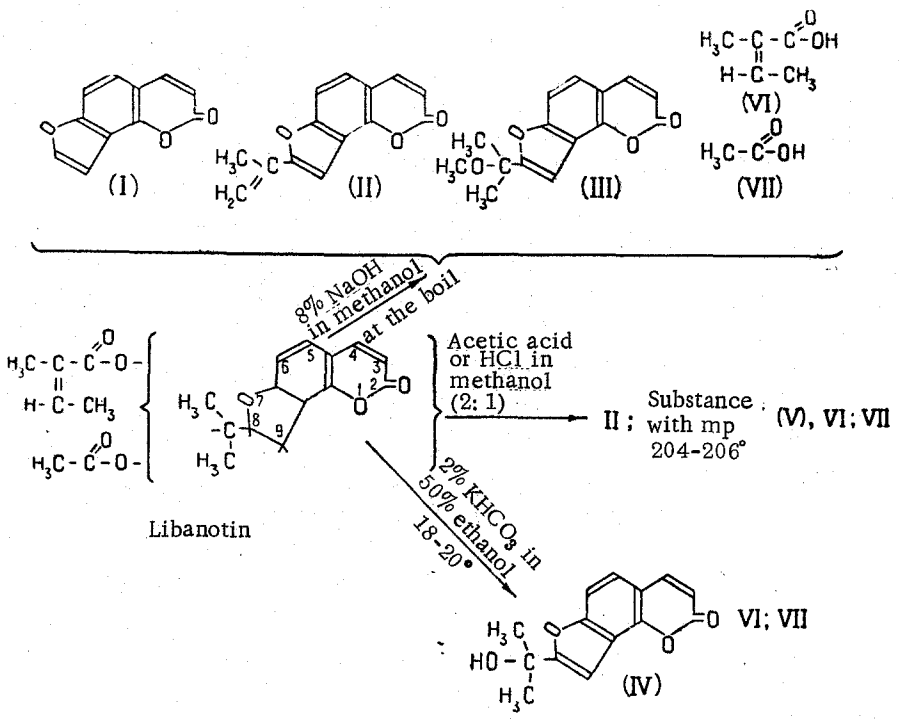
UV spectra of libanotin and its derivatives: 1) libanotin; 2) angelicin; 3) methyl ether of oroselol; 4) oroselol; 5) oroselone; 6) substance with mp 204-206°.

The alkaline hydrolysis of libanotin was expected to give rise to a compound with an alcoholic group, in this case oroselol. However, several compounds, including the methyl ether of oroselol, were found simultaneously among the hydrolysis products. It appears that during the hydrolysis of the esters with alcoholic solutions of caustic alkalis substituent interchange takes place in the derivatives of coumarin with angelicin and seselin nuclei (athamantin, edultin, peucenidin, visnadin, etc.), under which conditions alkoxy (methoxy or ethoxy) groups are added in place of the acid residues split off [11-15].

Oroselol $C_{14}H_{12}O_4$ (IV), mp 154-156°, was isolated by treating libanotin with a 2% solution of potassium bicarbonate in 50% ethanol at room temperature, while the saponification of libanotin with 0.4% methanolic caustic soda gave not only oroselol but also its methyl ether.

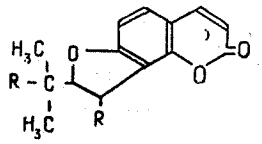
Under all the conditions mentioned above, the acidified hydrolyzates were found by paper chromatography to contain, in addition to the benzo-α-pyrone derivative mentioned, two organic acids which were identified as acetic and 2-methyl-cis-crotonic (angelic) acids.

The conversion of libanotin takes place in accordance with the following scheme:



A study of the products of alkaline hydrolysis and their identification showed that libanotin is very similar to athamantin [14], peucenidin [15], and edultin [13].

Compound	*R*	R_1
Athamantin	$(CH_3)_2CHCH_2COO-$	$(CH_3)_2CH-CH_2COO-$
Edultin	CH_3COO-	$CH_3-C(=CH-CH_3)-COO-$
Peucenidin	CH_3COO-	$CH_3-C(=CH-CH_3)-COO-$



Moreover, libanotin has exactly the same substituents as edultin, but differs from it, and also from peucenidin, in that saponification with 0.4% methanolic caustic potash does not give oroselol acetate. Nevertheless, the formation of oroselol and its methyl ether confirms that one of the acid residues in the libanotin molecule is attached to the tertiary carbon atom of the isopropyl group.

The investigation showed that there is no acetyl at the tertiary carbon atom, and therefore it may be assumed that the angelic acid residue is attached at this position and the most probable point of attachment of the acetyl group is the secondary carbon atom of the furan ring (position 9). In this case, the hydrolysis of libanotin with 0.4% methanolic caustic soda would be expected to form libanotin angelate; however, this could not be obtained. When the hydrolysis was carefully followed by paper chromatography with samples taken every 5 min for several hours no angelate could be detected, although all the compounds characterized above were found.

On considering the mechanism of the alkaline hydrolysis of compounds of the edultin or peucenidin type, which have different substituents, it can be seen that initially the secondary ester group is saponified and a monoester is formed (in both cases oroselol acetate), after which saponification of the tertiary ester group takes place with the formation of oroselol or, with substituent interchange, the methyl ether of oroselol. In view of the fact that the more voluminous substituent is present on the tertiary carbon atom and the acetic acid residue on the secondary carbon atom (on the basis of the assumptions expressed on the order of addition of substituents in the libanotin molecule), the velocities of the hydrolysis of these residues probably approach one another and therefore the two residues that are found in the hydrolyzate are split off almost simultaneously.

The acid hydrolysis of libanotin, like that of athamantin, edultin, and peucenidin, gives a substance with mp 204-206° (from ethanol) of undetermined structure.

Experimental

Isolation of libanotin. Eight kilograms of comminuted fruits of Transcaucasian Libanotis were extracted with 50 liters of 96% alcohol. The extract was evaporated in vacuum until the solvent had been completely eliminated and was then treated in the hot state with 2.5 liters of petroleum ether. The petroleum ether extracts were cooled, filtered, evaporated to 1 liter, and chromatographed on alumina of activity grade III (height of the column of adsorbent 75 cm, diameter 7 cm).

The column was developed first with petroleum ether until the fatty and essential oils and also some colored impurities had been eluted completely, and then with a mixture of petroleum ether and chloroform in a ratio of 19:1. The eluates containing the libanotin were evaporated in vacuum until the solvent had been removed completely, and the residue was dissolved in a small amount of ethanol with heating and left in the refrigerator for crystallization. The crystals which separated out were filtered off and recrystallized from ethanol. This gave 4 g of libanotin, mp 160-161°, $[\alpha]_D^{20}$ + 75 (c 1; chloroform); R_f 0.45 (in the petroleum ether-formamide system).

Found: C 65.87; H 5.80%; molecular weight 381, 374 (Rast). Calculated for $C_{21}H_{22}O_7$: C 65.27; H 5.74%; molecular weight 386.

Hydrolysis of libanotin. With heating, 2 g of libanotin was dissolved in 50 ml of 8% methanolic caustic soda, and the mixture was boiled under reflux for 30 min. It was then diluted with 50 ml of water and the methanol was distilled off in vacuum. The cooled aqueous solution was acidified with sulfuric acid to pH 5 and was treated with six 50-ml portions of ether. The combined ethereal extracts were washed with 5% aqueous sodium bicarbonate, dried with anhydrous sodium sulfate, and evaporated, to give 0.7 g of dry residue (fraction A).

The sodium bicarbonate extracts were acidified with sulfuric acid and treated with three 20-ml portions of diethyl ether. The ethereal extracts were washed with 15 ml of water, dried with anhydrous sodium sulfate, and evaporated to give an oily residue with a sharp acidic odor (fraction B).

Fraction B. The chromatography of fraction B in the butanol-ammonia (19:1) system showed the presence of two acids: acetic (R_f 0.1) and angelic (R_f 0.48). In a retort, 0.2 g of fraction B was sublimed in a vacuum of 0.1 mm at 100° in the water bath. The crystals formed melted at 43-43.5° and gave no depression of the melting point with an authentic sample of angelic acid.

Fraction A. The mixture of substances of fraction A was separated by means of partition chromatography on alumina. For this purpose, 50 g of alumina was mixed with 50 ml of a 5% solution of formamide in acetone and, with periodic stirring, was dried at 60° for several hours. The prepared adsorbent was used to make a column on which the mixture of substances of fraction A was chromatographed. The column was washed with a mixture of benzene and petroleum ether (1:2) saturated with formamide. Ten-milliliter fractions were collected.

The first 12 fractions (beginning from the time of appearance of coumarin derivatives in the eluate) gave oroselone, fractions 18-30 gave the methyl ether of oroselol, and fractions 40-64 gave angelicin.

Oroselol – white needle-like crystals, mp 187-189° (from methanol).

Found: C 73.74; H 4.19%; molecular weight 221 (Rast). Calculated for $C_{14}H_{10}O_3$: C 74.07; H 4.42%; molecular weight 226.

Methyl ether of oroselol – white silky needles, mp 119-120° (from methanol).

Found: C 69.66; H 5.51%; molecular weight 253 (Rast). Calculated for $C_{15}H_{14}O_4$: C 69.75; H 5.42%; molecular weight 258.

Angelicin – white lustrous plates, mp 138-140° (from methanol).

Found: C 70.90; H 3.30%; molecular weight 182 (Rast). Calculated for $C_{11}H_6O_3$: C 70.96; H 3.23%; molecular weight 186.

Production of oroselol. A solution of 2 g of libanotin in 250 ml of ethyl alcohol was treated with 250 ml of 4% potassium bicarbonate solution and left at room temperature. The course of the hydrolysis was followed by paper chromatography; it was found that the libanotin was completely hydrolyzed after 8.5 days.

After the completion of this process, the reaction mixture was acidified with sulfuric acid, evaporated to half its initial volume, cooled, and treated with diethyl ether. The ethereal extracts were washed with sodium bicarbonate solution, dried with sodium sulfate, and crystallized from ethanol. The substance obtained melted at 154-155° and gave no depression of the melting point with an authentic sample of oroselol. R_f 0.65 (in 25% methanol).

Found: C 68.98; H 5.05%. Calculated for $C_{14}H_{12}O_4$: C 68.85; H 4.92.

The IR spectrum had an absorption band at 3461 cm^{-1} (OH group). The UV spectrum had maxima at 250 mμ (log ε 4.35) and 300 mμ (log ε 4.05).

Hydrolysis of libanotin. A solution of 1.5 g of the substance in 100 ml of 0.4% methanolic caustic soda was heated under reflux at 40-50° for 5 hr. The reaction mixture was acidified with sulfuric acid and diluted with 100 ml of water, and the methanol was distilled off in vacuum. The residue was treated with diethyl ether, and the extracts were washed with sodium bicarbonate solution, dried with sodium sulfate and evaporated. The dry residue obtained was chromatographed on alumina, giving two substances with mp's 153-155° and 120-121°, which were identified, respectively, as oroselol and its methyl ether.

Decomposition of libanotin with glacial acetic acid. A solution of 0.5 g of libanotin in 5 ml of glacial acetic acid was treated with stirring with five drops of concentrated sulfuric acid. The reaction mixture was left at room temperature for 18-20 hr. The crystals which precipitated were filtered off and recrystallized from a mixture of ethanol and chloroform (1:2). The substance obtained melted at 204-206°. The UV spectrum had maxima at 255 mμ (log ε 4.02) and 295 mμ (log ε 3.97). The IR spectrum contained absorption bands at 1730, 1621, 1580, 1558, and 1445 cm^{-1}.

Summary

A new furocoumarin of composition $C_{21}H_{22}O_7$ has been isolated from the fruit of Libanotis transcaucasica Schischk. It has been established that libanotin is a derivative of 8-isopropyloxy-8,9-dihydroangelicin. The side groups of libanotin – acetic and 2-methyl-cis-crotonic acids – are attached to the main part of the molecule by ester bonds.

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