STUDY OF THE OILS OF PLANTS OF THE FAMILY Umbelliferae. III

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UDC 547.915.665.3

In a determination of the fatty-acid composition of the oil of the seeds of Petroselinum sativum, family Umbelliferae, by gas–liquid chromatography (GLC) we found that it contained palmitic acid (2.30%), oleic acid (10.35%), petroselinic acid (73.75%) and linoleic acid (13.60%).

By thin-layer chromatography (TLC) of the methyl esters, in addition to the acids mentioned we detected the spot of another acid located on a chromatogram somewhat above ordinary linoleic acid. The unknown acid is apparently an isomer of linoleic acid.

To isolate the dienoic acid fraction we used low-temperature crystallization of the methyl esters of the acids with urea [1], and also the separation of the mercury derivatives of the acids on a column and in a thin layer of silica gel [2].

By crystallizing the esters with urea at lower temperatures than those given in the literature [1] (−20, −40, and −65°C, successively) we obtained a fraction I containing, according to GLC, 78% of dienoic acids. Similar results were achieved by isolating the dienoic acids in the form of the mercury compounds in a silica gel column.

Fraction I was chromatographed in a thin layer of silica gel–gypsum impregnated with a 12% solution of silver nitrate in the solvent systems petroleum ether (60-80°C)–diethyl ether (9 : 1) [3]; petroleum ether (40-60°C)–benzene (3 : 7); petroleum ether (40-60°C)–benzene (1 : 9) [4]; petroleum ether (40-60°C)–benzene (2 : 8); benzene–diethyl ether (9 : 1); and benzene–diethyl ether (8 : 2) [5]. In the last three systems on a fixed layer of silica gel impregnated with a 12% solution of silver nitrate a clear separation of the zones of fraction I was achieved.

For the preparative separation of the fraction into the individual acids the benzene–diethyl ether (9 : 1) system proved to be the best. In this system on silica gel impregnated with a 12% solution of $AgNO_3$, fraction I was separated into four acids: palmitic with R_f 0.91, oleic with R_f 0.78, linoleic with R_f 0.51, and a previously unidentified acid with R_f 0.60.

On a gas–liquid chromatogram of the zone with R_f 0.60 (removed from the plates) a single peak was found which did not differ in its retention time from the peak of ordinary linoleic (octadeca-9,12-dienoic) acid. According to its IR spectrum, the acid that we isolated has the cis configuration and is obviously a position isomer of linoleic acid.

To determine the position of the double bonds in this acid, we used periodate-permanganate oxidation [6] with the subsequent isolation of the fragments by extraction with diethyl ether. The fraction of the fragments extracted by ether, on separation by TLC on cellulose in the hexane–diethyl ether–dimethylformamide (40 : 20 : 1) system gave a spot which undoubtedly was due to pelargonic acid. Thus, one of the two double bonds of the dienoic acid occupies the Δ^9 position, and there is no other double bond between it and the methyl group.

Among the dicarboxylic acids both in the aqueous and in the ethereal layer, by TLC on cellulose in the n-propanol–ammonia–water (9 : 1 : 2) system we found malonic acid, which could arise if the second double

Order of the Red Banner of Labor Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 37-40, January-February, 1974. Original article submitted November 21, 1972.

TABLE 1. Distribution of the Fatty Acids in the Glycerides

Plants of the family Umbelliferae	Fatty-acid composition									
	triglycerides					monoglycerides				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$ Δ^9	$C_{18:1}$ Δ^6	$C_{18:2}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$ Δ^9	$C_{18:1}$ Δ^6	$C_{18:2}$
Coriandrum sativum	3,60	—	14,48	65,52	16,40	2,00	—	54,30	17,10	26,60
Petroselinum sativum	2,30	—	10,35	73,75	13,60	6,40	—	69,95	12,35	11,40
Eremodaucus Lehmanni	4,21	1,89	54,68	11,43	26,50	7,10	2,30	50,40	—	40,20

bond were located in the Δ^6 position, i.e., closer to the carboxyl. Consequently, to identify the third fragment of the acid under investigation (presumably the monomethyl ester of adipic acid, of which we had no reference sample), the ethereal extracts of the oxidation products were saponified, and adipic acid was identified by comparison with markers. On the basis of the results of the investigation, we assumed that the dienoic acid of the parsley oil has the structure of octadeca-cis-6-cis-9-dienoic acid:

$$CH_3\cdot(CH_2)_7\cdot\overset{10}{C}H=\overset{9}{C}H\cdot CH_2\cdot\overset{7}{C}H=\overset{6}{C}H\cdot(CH_2)_4\cdot COOH.$$

It can be seen from literature data that no one has previously detected this acid in a plant oil, but a homolog of it– $\Delta^{6,9}$ hexadeca-6,9-dienoic acid

$$CH_3(CH_2)_5\overset{10}{C}H=\overset{9}{C}HCH_2\overset{7}{C}H=\overset{6}{C}H\cdot(CH_2)_4\cdot COOH—$$

has been found in herring fat [7].

In studying the oils of plants of the family Umbelliferae, we were interested in the question of their glyceride structure, in particular, the position of the radical of the petroselenic acid in the triglyceride molecule. To give information on this question, we performed the enzymatic hydrolysis of the oils of Coriandrum sativum, Petroselinum sativum, each containing about 70% of petroselinic acid, and of Eremodaucus Lehmanni, containing 11% of this acid. The 2-monoglycerides isolated after hydrolysis were saponified in order to determine their fatty-acid composition, with subdivision of the monoenoic acids into oleic and petroselinic (Table 1).

It follows from Table 1 that the petroselenic acid, which is present in amounts of from 11 to 73% in the oils, is present in the monoglycerides in considerably smaller amounts or is completely absent. This shows that petroselinic acid, which is an unsaturated acid with mp 29-30°C, behaves as a saturated acid and occupies mainly the 1,3 positions in the triglycerides.

EXPERIMENTAL

The seeds (90 g) of Petroselinum sativum (oil content 22.6%) were extracted by shaking with 250 ml of methanol–acetone (4:1). Then they were dried, comminuted, and the fatty oil was extracted with petroleum ether. The methanolic acetone extract, after distillation of the solvent, yielded 1.6 g of dry residue, and the petroleum ether extract yielded 14.7 g of oil. By its saponification at room temperature the unsaponifiables were separated, and 11.8 g of fatty acids was isolated. The acids were converted into the metyl esters. To 11.5 g of the methyl esters was added a solution of 7.8 g of urea in 95 ml of hot methanol. Then the mixture was cooled and was kept at −5°C for 6 h. The precipitate was separated off and was washed with cooled methanol (35 ml) and the wash liquid was added to the filtrate, in which 6.34 g of urea had been dissolved.

After cooling at −5°C for 8 h, the precipitate was again separated off and washed, the wash liquid was added to the filtrate, 3.5 g of urea was added, and precipitates were separated twice more under the conditions given above.

After four crystallizations performed in this way, a filtrate (II) containing 58% of dienoic acids was obtained. Without the addition of urea, this filtrate was subsequently crystallized at −20, −40, and −65°C. The precipitate was separated off. Filtrate I was isolated. By treating it with hot water and extracting with diethyl ether (3 × 25 ml), 2.4 g of a residue containing, according to GLC, 78% of dienoic acids was obtained.

The residue (1.93 g) was deposited on 14 plates (18 × 24 cm) with fixed layers of silica gel impregnated with a 12% solution of $AgNO_3$. The plates were treated with benzene–diethyl ether (9:1), and then the edge of each plate was calcined to determine the boundaries of the zones; the zone with R_f 0.60 was removed from the plates, the esters were extracted with diethyl ether (5 × 30 ml), and the ether was distilled off. This gave 0.12 g of a dienoic acid the purity of which was checked by GLC.

The oxidation of the acid in order to determine the positions of the double bonds and the identification of the monocarboxylic acids among the oxidation products were performed by a published method [6]. The dicarboxylic acids were identified on a layer of cellulose in the n-propanol–ammonia–water (9:1:2) solvent system. The spots were revealed with a solution of Bromophenol Blue.

CONCLUSIONS

1. The oil of the seeds of Petroselinum sativum has been found for the first time to contain, in addition to linoleic acid, another dienoic acid, and its structure has been established as octadeca-cis-6-cis-9-dienoic acid. The acid has been called petroslinoleic.

2. For the case of three representatives of the family Umbelliferae (Petroselinum sativum, Coriandrum sativum, and Eremodaucus Lehmanni) it has been shown that petroselinic acid occupies predominantly the 1,3 positions in the triglyceride molecules, i.e., in this respect it behaves in the same way as saturated acids.

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