A STUDY OF THE OILS OF THE FAMILY UMBELLIFERAE

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In 1909, an unusual fatty acid, cis-octadec-6-enoic, was found for the first time in the oil of the seeds of Petroselinum sativum (parsley), and from the generic name of the plant it was given the name of petroselinic acid [1]. This acid was subsequently found in many seed oils of other plants of the same family [2]. In the majority of these oils the amount of petroselinic acid is extremely large, amounting to 75% in individual cases. According to not very convincing literature information, the same acid is present in the oils of some species of the family Araliaceae [3], in one of the species of the family Simarubaceae [4], and even in the fat of human hair [5].

Starting from the idea that petroselinic acid is a peculiar chemicophysiological-classification characteristic of the family Umbelliferae, we have continued an investigation of the oils of plants of this family begun previously [6].

On studying petroselinic acid, Markley [2] observed that its physical properties vary within wide limits according to the nature of the initial raw material and the method of extracting the oil and do not coincide with the figures for synthetic octadec-6-enoic acid (specific gravity, refractive index, and melting point of 29-34°C). This fact has induced us to investigate the nature of this acid in more detail using modern techniques.

We have studied the oil of four representatives of the family Umbelliferae (annuals growing widely in Central Asia) which are distributed on the rocky slopes of mountains: Athamanta macrophylla, Libanotis marginata, Archangelica tschimganica, and Bunium hissaricum [7].

The petroleum ether extracts from seeds of the plants formed mobile liquids, dark green in the case of Bunium hissaricum, reddish green for Athamanta macrophylla and Archangelica tschimganica and light yellow for Libanotis marginata. The extract from L. marginata possessed a weak herbaceous odor and the other three extracts specific pungent odors. It is obvious that this is due to the presence in them not only of fatty oils but also of essential oils, which is characteristic for many of the Umbelliferae (coriander, celery, caraway, anise, dill, etc.).

Table 1 gives some physical and chemical indices characterizing the properties of the extracts after the solvent had been distilled off. The fatty-acid compositions of the oils determined by gas-liquid chromatography (GLC) are shown in Table 2. Under our conditions of recording the chromatograms, petroselinic acid and oleic acid, which have the same retention time, give a common peak (Fig. 1a). To detect these acids separately, we had recourse to the oxidation of the methyl esters of the mixture of acids with permanganate in dry acetone by Hilditch's method [8]. The fragments from destructive oxidation – esters of monocarboxylic and dicarboxylic acids – were analyzed by the GLC method. From the GLC indices of the mixture of monocarboxylic acids we calculated the amounts of oleic and petroselinic acids in the oil separately. We show the technique of the calculation using L. marginata oil as an example.

Among the monocarboxylic acids obtained from the degradation of this oil were found 19.7% of pelargonic and 80.3% of lauric acids. The molar ratio of these acids is

$$\frac{19.7}{158.23} : \frac{80.3}{200.31} = 12.45:40.08.$$

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TABLE 1. Characteristics of the Oils Investigated

Index	Units of measurement	Athamanta macrophylla		Archangelica tschimganica		Libanotis marginata		Bunium hissaricum	
		oil	fatty acids	oil	fatty acids	oil	fatty acids	oil	fatty acids
Total extractive substances	% on the wt. of the seeds	6,15	—	11,50	—	6,25	—	6,60	—
fatty oil	% on the wt. of the seeds	4,00	—	5,10	—	6,00	—	4,90	—
essential oil	% on the wt. of the seeds	—	—	0,17	—	0,08	—	0,10	—
unsaponifiables	% on the wt. of the extract	3,15	—	8,10	—	2,60	—	1,70	—
Refractive index	n_D^{20}	1,4788	—	1,4675	—	1,4890	—	1,4841	—
Acid No.*	mg KOH/g	5,70	—	9,0	—	2,70	—	3,12	—
Hehner No.	%	95,07	—	95,67	—	95,60	—	95,15	—
Saponification No.	mg KOH/g	191,0	—	193,80	—	189,70	—	190,70	—
Iodine No.	% I_2	115,20	120,18	113,40	117,80	110,30	120,00	105,10	108,30
Neutralization No.	mg KOH/g	—	192,72	—	199,70	—	200,93	—	201,00
		—	282,35	—	280,97	—	279,25	—	279,15

* After purification of the extract on a column of alumina, the acid Nos. of all the oils did not exceed 0.01 mg KOH/g.

TABLE 2. Fatty-Acid Compositions of the Oils according to GLC

Acid	Athamanta macrophylla	Archangelica tschimganica	Libanotis marginata	Bunium hissaricum
Capric	—	—	2,84	1,47
Lauric	—	—	0,84	1,91
Myristic	1,10	1,10	0,67	9,90
Palmitic	5,04	9,50	4,73	7,07
Stearic	—	1,80	1,82	5,89
Octadecenoics	62,83	60,20	59,15	46,00
Linoleic	31,03	26,90	29,95	27,76
Linolenic	—	0,50	—	—

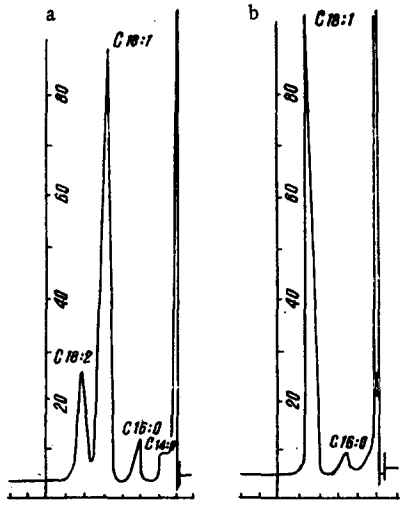

Fig. 1. Chromatograms of the mixture of acids from the oil of Athamanta macrophylla (a) and of the monoenic fraction (b).

Since oleic and petroselinic acids have the same molecular weight, their weight ratio is also 12.45 : 40.08. If we divide the total amount of octadecenoic acids in the oil (59.15%) (see Table 2) in this ratio, we obtain the amounts of oleic acid (14.01%) and petroselinic acid (45.14%).

The very curious fact was that after the oxidation of the oils of A. macrophylla, A. tschimganica, and B. hissaricum the monocarboxylic acid fractions of the degradation products contained, besides pelargonic (C_9) and lauric (C_{12}) acids, also capric (C_{10}) and undecylic (C_{11}) acids, and, correspondingly, the dicarboxylic acid fractions contained not only azelaic (C_9) and adipic (C_6) acids but also suberic (C_8) and pimelic (C_7) acids. Consequently, these oils contain not only oleic and petroselinic acids but also acids isomeric with oleic – octadec-7-enoic and octadec-8-enoic.

Before us, Kartha et al. [9] had found octadec-7-enoic acid in ten oils of the family Umbelliferae of Indian origin and octadec-5-enoic acid in two oils of plants of the same family. The latter acid was identified by the presence of glutaric acid among the degradation products. However, until the present time, no one has reliably established the presence of octadec-8-enoic acid in natural oils. Only unconfirmed hypotheses of its presence in human-hair fat [5] and in olive oil [10] have been put forward.

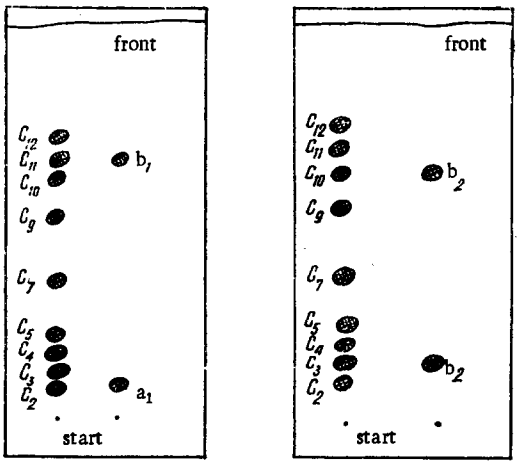

Fig. 2. Thin-layer chromatograms of the products of the oxidation of substance 1 (a_1, b_1) and 2 (a_2, b_2). C_2 – acetic acid; C_3 – propionic acid; C_4 – butyric acid.

TABLE 4. Glyceride Compositions of the Oils

Types of glycerides	Athamanta macrophylla	Archangelica tschimganica	Libanotis marginata	Bunium hissaricum
Gl SSS	0,17	0,26	0,16	0,80
Gl SSU	1,60	2,73	1,13	3,80
Gl SUS	2,20	2,30	2,20	5,00
Gl SUU	22,70	24,45	24,10	31,06
Gl USU	5,17	7,07	2,39	6,46
Gl UUU	66,16	63,19	70,02	52,88

TABLE 3. Octadecenoic Acids of the Oils of Plants of the Family Umbelliferae

Octadecenoic acids	Athamanta macrophylla	Archangelica tschimganica	Libanotis marginata	Bunium hissaricum
Petroselinic	42,22	39,90	45,14	29,90
Oleic	14,20	10,60	14,01	10,20
Octadec-7-enoic	3,70	6,93	—	2,80
Octadec-8-enoic	2,71	2,77	—	3,10
$\sum C_{18}H_{34}O_2$	62,83	60,20	59,15	46,00

We shall consider how a more far-reaching study of the fatty-acid composition of an oil was performed using as example the Archangelica tschimganica oil. The methyl esters of the acids of this oil were separated according to their degree of unsaturation in the form of acetoxymercurimethoxy derivatives in a thin layer of adsorbent as described below.

The monoenoic fraction obtained in this way was completely free from acids with different degrees of unsaturation (Fig. 1b). The subsequent separation of the monoenes in a thin layer of silica gel impregnated with silver nitrate in the diethyl ether–petroleum ether (28-40°C) (3 : 7) system yielded three zones, with R_f 0.86, 0.55, and 0.40. In this system, the petroselinic and oleic acids appeared in the form of a common spot (R_f 0.86).

When the petroleum ether–benzene (1 : 4) system was used, it was possible to separate the petroselinic acid (R_f 0.68) from the oleic (R_f 0.81) and to obtain yet another two zones with R_f 0.16 (substance 1) and 0.28 (substance 2). Substances 1 and 2 were the subject of further investigation.

Each of these substances was removed with a layer of sorbent and was then quantitatively extracted with diethyl ether. Their mass spectra were recorded; however, these gave us no information on the positions of the double bonds in the molecules of the unsaturated acids. The presence of a molecular ion M^+ 296 confirmed that we were actually dealing with methyl esters of octadecenoic acids. To determine the positions of the double bonds, substances 1 and 2 were oxidized by the periodate–permanganate method [11]. The products of oxidative degradation were separated in a thin layer of cellulose in the hexane–diethyl ether–dimethylformamide (40 : 20 : 1) system. The monocarboxylic acids in the oxidation products (undecylic for substance 1 and capric for substance 2) were identified by comparison with authentic samples. No authentic samples were available for the monomethyl esters of the dicarboxylic acids. Literature information shows that the R_f value of monomethyl suberate coincides with that for methyl propionate, and R_f for monomethyl pimelate with that for methyl acetate, as was also observed in our experiments (Fig. 2).

In order finally to convince ourselves of the correctness of the conclusions deduced, we isolated the acids from the monomethyl esters of the dicarboxylic acids and showed their identity with pimelic and suberic acids by comparison with authentic samples in the n-propanol–ammonia–water (9 : 1 : 2) system [15].

What has been said shows that substance 1 is methyl octadec-7-enoate and substance 2 is methyl octadec-8-enoate. Both acids are liquid at room temperature, their lead salts are soluble in ethanol, and their IR spectra have no absorption bands at 970 cm^{-1}, from which it follows that they are the cis acids.

Table 3 gives the indices of the breakdown of the group of octadecenoic acids into its individual components.

The glyceride compositions of the oils were determined by enzymatic hydrolysis, using the lipase of the pancreatic gland of large-horned cattle [12]. The results of a calculation of the glyceride composition by Coleman's method [13] are given in Table 4.

The pigments from the oils of A. macrophylla, A. tschimganica, and P. hissaricum yielded β-chlorophyll, showing in the UV and visible spectrum in diethyl ether absorption bands at 420, 470, and 668 nm; the oil of L. marginata contained α-carotene, showing in petroleum ether solution absorption maxima at 420 and 470 nm [14].

EXPERIMENTAL

The oils were extracted from the seeds with petroleum ether by steeping at room temperature for 4-6 h. The extracts were evaporated, dried in vacuum, and purified on a column of alumina; the purified oils were saponified with ethanolic alkali in the cold, and the fatty acids were liberated. The procedure for methylation and permanganate oxidation has been described in the preceding paper [6].

Mercuration of the Fatty Acids and Their Separation according to Degree of Unsaturation. As reagent we used a solution of 14 g of mercuric acetate in 250 ml of methanol containing 2.5 ml of water and 1 ml of glacial acetic acid.

A mixture of 3.6 g of the methyl esters of the mixture of acids and 144 ml of the reagent solution was left for two days, after which the methanol was evaporated in vacuum at 30°C and the residue was dissolved in 50 ml of chloroform; the solution was washed several times with water to eliminate the excess of mercuric acetate, dried with anhydrous sodium sulfate, concentrated to a volume of 10-15 ml, and deposited on 16 glass plates (18 × 24 cm) coated with silica gel–gypsum (10 : 1) adsorbent.

For the more complete separation of the saturated acid from the unsaturated, the chromatograms were run twice, first in the petroleum ether (40-70°C)–diethyl ether (4 : 1) system for 30-40 min, and then in a n-propanol–glacial acetic acid (100 : 1) system for 6-6.5 h. The plates were dried, and a section 2.5 cm wide over the whole height of each plate was sprayed with a 1% solution of diphenylcarbazone in 96% ethanol. Two well-defined violet spots with R_f 0.87 and 0.66 on a pink background were obtained.

Each of the two zones was removed from each plate and was treated twice with 25 ml of methanol containing 1.2 ml of hydrochloric acid. The adsorbent was separated off by filtration, the filtrate was diluted with an equal volume of water, and the methyl esters of the unsaturated acids were extracted with diethyl ether. Gas-liquid chromatography showed that the zone with R_f 0.87 contained 98.9% of monoenoic acids and 1.1% of palmitic acid and the zone with R_f 0.66 contained dienoic acids.

Separation of the Monoenoic Fraction into Position Isomers. The zone with R_f 0.87 (1.26 g) was dissolved in 7 ml of benzene and deposited on glass plates (18 × 24 cm) coated with a mixture of silica gel and gypsum which had been impregnated with a 20% aqueous solution of silver nitrate. Separation was performed in the petroleum ether (40-70°C)–benzene (1 : 4) system for 40-45 min, after which, by calcining the edges of the plates, four zones were revealed, of which the zones with R_f 0.16 (substance 1) and 0.28 (substance 2) were subjected to oxidative degradation.

Oxidation. At 40°C, 32 mg of substance 1 and 40 mg of substance 2 were each, separately, treated with a mixture of 0.001 mole of potassium carbonate and 0.001 mole of potassium permanganate in 10 ml of water and 0.001 mole of sodium periodate in 10 ml of water (1 : 2). The oxidation mixture was added dropwise to the weighed sample to be analyzed, and the reaction mixture was heated after each addition of oxidizing agents until the color of the permanganate no longer disappeared. After the end of the reaction (30-40 min), a 2 N solution of hydrochloric acid was added and the excess of permanganate was destroyed in the medium with bisulfite. The degradation products were extracted with diethyl ether.

SUMMARY

1. The oils of four representatives of the family Umbelliferae have been investigated. Their physical and chemical indices have been characterized, and their fatty-acid and glyceride compositions have been determined. In all the oils the acid present in largest amount is petroselinic acid (30-45%), which may be considered as a chemicophysiological-classification characteristic of plants of the family Umbelliferae.

2. Unusual acids – octadec-7-enoic (2.8-6.9%) and octadec-8-enoic (2.7-3.1%) – have been found in the oils of Athamanta macrophylla, Archangelica tschimganica, and Bunium hissaricum. Octadec-8-enoic acid had never previously been found in natural oils.

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