SUMMARY

1. The fatty-acid compositions of the total phospholipids of the seed kernels of the cotton plant of variety Tashkent-2, after the elimination of carbohydrates, and of their main components have been studied.

2. It has been found that at 37-38°C the time of enzymatic hydrolysis is considerably shortened.

3. A study of the products of acid and enzymatic hydrolysis has shown the structure of the individual phospholipids.

LITERATURE CITED

1. M. U. Babaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 417 (1974).
2. M. U. Babaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 328 (1975).
3. J. Folch, M. Lees, and J. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
4. M. E. McKillican and J. A. G. Larose, J. Amer. Oil Chemists' Soc., 47, 256 (1970).
5. D. Tevekelov, Izvestiya na Instituta po Khranene [Bulgaria], 7, 21 (1968).
6. G. V. Novitskaya, Methodical Handbook on the Thin-Layer Chromatography of the Phospholipids [in Russian], Moscow (1972).
7. L. Fieser, in: Modern Methods of Experimentation in Organic Chemistry [Russian translation], Moscow (1960), p. 94.
8. G. J. Nelson, Lipids, 3, 104 (1968).
9. Handbook of Methods of Investigation, of Technological Control, and of the Accounting of Production in the Oils and Fats Industry, Vol. 1, Book 1 [in Russian], Leningrad (1967).
10. K. Kadyrov, T. V. Chernenko, and A. U. Umarov, Maslozhir. Prom., No. 11, 15 (1971).
11. E. Stahl, ed., Thin-Layer Chromatography, Springer-Verlag, New York (1969).
12. A. L. Markman, T. V. Chernenko, and A. U. Umarov, Prikl. Biokhim. Mikrobiol., 5, No. 5, 616 (1969).
13. H. E. Carter et al., J. Amer. Oil Chemists' Soc., 35, 335 (1958).
14. N. Z. Stanacey et al., Biochim. Biophys. Acta, 176, 650 (1969).

COMPOSITION OF THE SEED OILS OF *Origanum tyttanthum* AND *Mentha asiatica*

S. D. Gusakova, Kh. K. Kholmatov, and A. U. Umarov

UDC 547.915:665.3

Continuing a study of the oils of various species of the family Labiatae [1, 2], we have investigated the compositions of the fatty oils of *Origanum tyttanthum* and *Mentha asiatica*, growing in the Uzbek SSR. These oils have not been studied previously.

The oils isolated from the purified comminuted seeds of the plants mentioned by extraction with petroleum ether were greenish-yellow mobile liquids with a weak sage-like odor which were similar to one another in their main indices (Table 1).

The UV spectra of the oils investigated and the IR spectrum of the *O. tyttanthum* oil showed no specific absorptions or bands. The IR spectrum of the *M. asiatica* oil had weak bands of a hydroxy group at 3450 and 1070 cm^{-1} which were retained in the spectrum of the methyl esters of the fatty acids (MEs). In addition, in the UV spectrum of the MEs of the *M. asiatica* weak absorption appeared at λ_{max}^{hexane} 280 nm.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 149-155, March-April, 1976. Original article submitted April 24, 1975.

The characteristics of the mixtures of fatty acids isolated after the previous separation of the combined unsaponifiables are given in Table 1. The fatty-acid compositions of the oils (GLC) were as follows (%):

Acid	Origanum tyttanthum	Mentha asiatica
$C_{14:0}$	0.2	—
$C_{16:0}$	5.7	5.3
$C_{18:0}$	1.8	1.6
$C_{18:1}$	8.0	7.7
$C_{18:2}$	20.0	29.8
$C_{18:3}$	64.3	55.5
C_x	—	0.1

The results of the gas-chromatographic analysis of the acids were basically confirmed by the results of the chromatography of the combined acids on paper (PC) and of the combined MEs on silica gel with the addition of 20% of $AgNO_3$ (TLC + $AgNO_3$). By the PC method, the mixture of acids from *M. asiatica* was found to contain $C_{20:0}$ (R_f 0.12) and hydroxy acids (R_f 0.87).

The glyceride composition (%) of the *Mentha asiatica* oil was characterized mainly by triunsaturated glycerides and that of the *Origanum tyttanthum* oil by di- and triunsaturated glycerides:

Oil	SSS	SSU	SUS	SUU	USU	UUU
Origanum tyttanthum	0.01	0.17	1.22	19.56	0.69	78.35
Mentha asiatica	0.01	0.68	0.10	9.86	5.72	83.63

Of 11 species of mint investigated [3-5], the oil of *M. asiatica* is very close in its fatty-acid composition to the oil of *M. sylvestris* [*M. longifolia*], which grows in America [3]. According to these results, the mixed acids of *M. sylvestris* contained eight unidentified components (GLC) amounting to 1.2% of the combined acids.

To determine the structure of the unsaturated acids, the mixtures of MEs of the *O. tyttanthum* and *M. asiatica* were separated by preparative TLC in system 1 into fractions corresponding to saturated (R_f 0.84), monoenoic (R_f 0.62), dienoic (R_f 0.48), and trienoic (R_f 0.36) acids. The fractions isolated were then investigated by chromatographic (GLC, PC, TLC) and spectroscopic (UV, IR) methods.

The positions of the double bonds in the acids were determined by destructive oxidation with the periodate–permanganate reagent. The degradation products were identified by GLC and TLC on cellulose. On the basis of the results obtained, it was concluded that in the oils of both the plants investigated the $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ acids are mainly oleic, linoleic, and linolenic acids, respectively.

TABLE 1. Physicochemical Indices of the Oils and of the Mixtures of Fatty Acids

Index	Origanum tyttanthum			Mentha asiatica		
	seeds	oil	acids	seeds	oil	acids
Weight of 1000 seeds, g	0,07	—	—	0,09	—	—
Oil content, %	26,7	—	—	22,2	—	—
Refractive index, n_D^{20}	—	1,4869	1,4728	—	1,4827	1,4723
Density, d_{20}^4, g/ml	—	0,9553	—	—	0,9245	—
Acid No., mg KOH	—	5,3	—	—	2,4	—
Hehner No., %	—	95,6	—	—	96,9	—
Saponification No., mg KOH/g	—	201,7	—	—	203,2	—
Amount of unsaponifiables, %		3,8	—	—	2,3	—
Iodine No., % I_2 (Kaufmann)	—	196,6	206,5	—	202,3	208,1
Calculated iodine No., % I_2	—	—	209	—	—	203
Neutralization No., mg KOH/g	—	—	201,3	—	—	201,7
Mean molecular weight	—	—	278,7	—	—	278,1

The trienoic fraction of the MEs of *M. asiatica* consisted of two not completely separated bands. The UV spectrum of this fraction had weak absorption in the λ_{max}^{hexane} 280-nm region, and the IR spectrum contained the bands of a hydroxy group (3450, 1075 cm^{-1}) and the band of an ester carbonyl at 1740 cm^{-1} which was broadened and had a complex shape.

To isolate the unusual components, the combined acids of the *M. asiatica* were fractionated with the aid of urea to form six fractions which were checked by TLC in system 1, with respect to their degree of unsaturation, and in system 3, which separates the MEs of the hydroxy acids and the ordinary acids. This yielded six fractions, the compositions of which are given below (%):

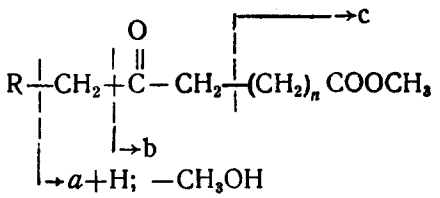
Fraction	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	17	5.6	19.8	22.3	35.3
2	—	—	12.6	31.7	51.7
3	—	—	—	36.2	63.8
4	—	—	—	24.6	74.4
5	—	—	—	15.8	84.2
6	—	—	—	—	Tr.

Fraction 6, enriched in hydroxy acids, was separated further by preparative TLC in system 2 into three zones with R_f 0.43, 0.14, and 0.09, respectively.

The zone with R_f 0.43 was identified on the basis of GLC (100% $C_{18:3}$) and the formation of a hexabromide with mp 176-177°C as normal linolenic acid. The acids of the other two zones did not appear in the polar phase under the conditions of GLC analysis.

The zone with R_f 0.14 in system 3 gave a single spot with R_f 0.17. The UV spectrum of the fractions showed the absorption of an isolated carbonyl in the λ_{max} 280-nm region. In the IR spectrum of the MEs of the zone, the band of an ester carbonyl was observed at 1750 cm^{-1} and that of an isolated carbonyl group at 1725 cm^{-1}, and the remainder of the spectrum was typical for the methyl esters of unsaturated fatty acids.

According to its mass spectrum, the fraction was the sum of MEs with molecular weights of 322, 320, 316, 308, 306, and 304. The main peaks in the spectrum were those of ions with m/e 43 (78%), 55 (100), 57 (74), 59 (38), 67 (31), 69 (57), 71 (40), 74 (78), 81 (43), 83 (65), 85 (26), 87 (46), 95 (82), 97 (38), 111 (37), 125 (16), 143 (23), 155 (14). In the region of high masses there was a series of ions with m/e $M^+ - 31$, $M^+ - 32$, $M^+ - 49$, $M^+ - 60$, $M^+ - 73$, and $M^+ - 74$. Fragmentation of this nature corresponds to acids containing double bonds [6] and a keto group not less than eight carbon atoms distant from the $-COOCH_3$ group [7]:



The presence of an oxo group was confirmed by the preparation of a mixture of the 2,4-dinitrophenylhydrazones of the MEs (2,4-DNPHs) in the form of a red–brown oil, in the UV spectrum of which there was intense absorption at $\lambda_{max}^{CHCl_3}$ 364 nm due to the 2,4-DNPH derivatives of aliphatic ketones [8].

When the fraction was oxidized with the periodate–permanganate reagent, among the degradation fragments we identified the following monocarboxylic acids: $C_{3:0}$ (TLC), $C_{9:0}$ (25.4%), $C_{7:0}$ (26.7%), and $C_{6:0}$ (33%) (GLC); 14.9% of the components were unidentified. The following dicarboxylic acids were present in the mixture: C_9 (85.6%), C_8 (10.7%), C_7 (4.4%), C_3 (1.2%), and C_4 (1.1%).

It was concluded from the IR and UV spectra that the unsaturated ketonic acids lacked any type of conjugation and, on the basis of the degradation fragments, that the double bonds and the carbonyl groups were located in the acids between the eighth and 16th carbon atoms. Because of its small amount and complex composition, the fraction was not separated into the individual acids.

The zone with R_f 0.09 had R_f 0.10 in system 3. The UV spectrum of the fraction showed no absorption, and the IR spectrum had the bands of double bonds at 3015 and 1630 cm^{-1} and of a hydroxy group at 3460 and 1070 cm^{-1} (Fig. 1).

According to the neutralization number, 190.7 mg KOH/g, the molecular weight of the acid was 294.2. The mass spectrum of the ME had no peak of the M^+ ion, but there were peaks of the ions $M^+ - 18$ (292; 3%), $M^+ - OCH_3$ (279; 3%), and $M^+ - 49$ (264; 2%), which is characteristic for the fragmentation of hydroxy acids [6, 7]. On the basis of these facts, the molecular weight of the hydroxy acid (I) is 296 and of its methyl ester (II) 310.

The NMR spectrum of (II) (Fig. 2) showed: the signal of olefinic protons of an isolated double bond (τ 4.78 ppm, multiplet, 4 H, J = 10.3 Hz); a combined four-proton signal of the methine proton of a CH—OH group (6.35-6.8 ppm, weak multiplet) and of a methoxy group —OCH_3 (6.5 ppm, singlet, 3 H); a three-proton signal of a diallylmethylene group =$CHCH_2CH$=, and of the proton of a hydroxy group (7.35 ppm, triplet); the signal of the methylene protons of allyl CH_2CH= and CH_2COO— groups (7.7-8.2 ppm, multiplet, 6 H); the signal of equivalent protons of methylene groups of an alkyl chain (8.72 ppm, broad singlet, 14 H), and the signal of the protons of the methyl group in a CH_3CH_2CH= grouping (9.07 ppm, resolved triplet, J = 7.2 Hz) [9].

The following structure of the acid was established on the basis of its spectral characteristics:

$$CH_3CH_2CH{=}CHCH_2CH{=}CH(CH_2)_n\underset{\underset{OH}{|}}{CH}(CH_2)_mCOOCH_3.$$

The position of the hydroxy group was determined from the mass spectrum and from the fragments obtained by destructive oxidation.

The mass spectrum of (II) (Fig. 3) contained, in addition to the ions mentioned above, the peaks of ions with m/e 187 (16%), 155 (44%), and 158 (16%), formed by α cleavage with respect to the hydroxy group [6, 7]:

$$R-CH_2-\overset{9}{\underset{\underset{OH}{|}}{CH}}-(CH_2)_7\overset{1}{C}OOCH_3$$

$\rightarrow m/e\ 157 + H \rightarrow m/e\ 158$

$\rightarrow m/e\ 187 - CH_3OH \rightarrow m/e\ 155$

The mass numbers of the fragments correspond to a hydroxyl located on the ninth carbon atom. In the region of low masses, ions with m/e 41 (56%), 55 (100), 57 (52), 59 (38), 67 (30), 69 (32), 74 (65), 81 (21), 87 (23), 95 (30) correspond to olefinic, hydrocarbon, and oxygen-containing fragments.

In the oxidation products of (II) the following dicarboxylic acids were detected: azelaic (C_9; 86.1%), suberic (C_8; 4.9%), adipic (C_7; 1.3%), succinic (C_4; 1.3%), and 6.4% of unidentified products; among the monocarboxylic acids, propionic acid, $C_{3:0}$, was identified. As has been shown previously [10], a similar mixture of dicarboxylic acids is formed in the periodate—permanganate oxidation of hydroxy acids with cleavage of the carbon bonds close to the hydroxy group:

$$R-CH_2-\underset{\underset{OH}{|}}{CH}-CH_2-(CH_2)_5COOCH_3$$

$\rightarrow C_{9\ di}$ $\rightarrow C_{8\ di}$ $\rightarrow C_{7\ di}$

and the position of the hydroxyl is determined by the fragment predominating in the combined dicarboxylic acids. In this case, the formation of 83% of azelaic acid confirmed the position of the hydroxyl at C_9.

On the basis of the results obtained, the structure of the acid was determined as 9-hydroxyoctadeca-cis-12,cis-15-dienoic acid:

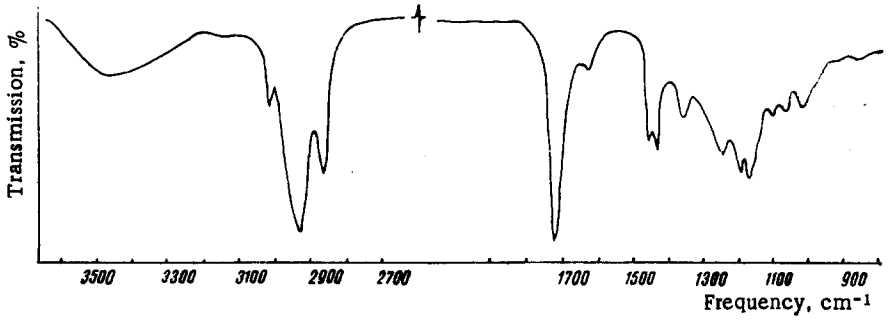


Fig. 1. IR spectrum of the methyl ester of the hydroxydienoic acid.

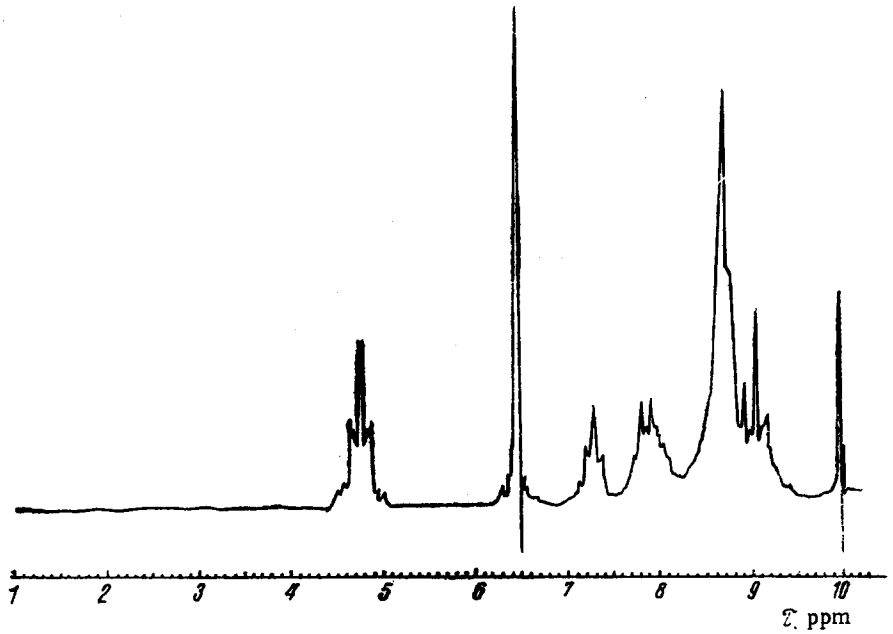


Fig. 2. NMR spectrum of the methyl ester of the hydroxydienoic acid.

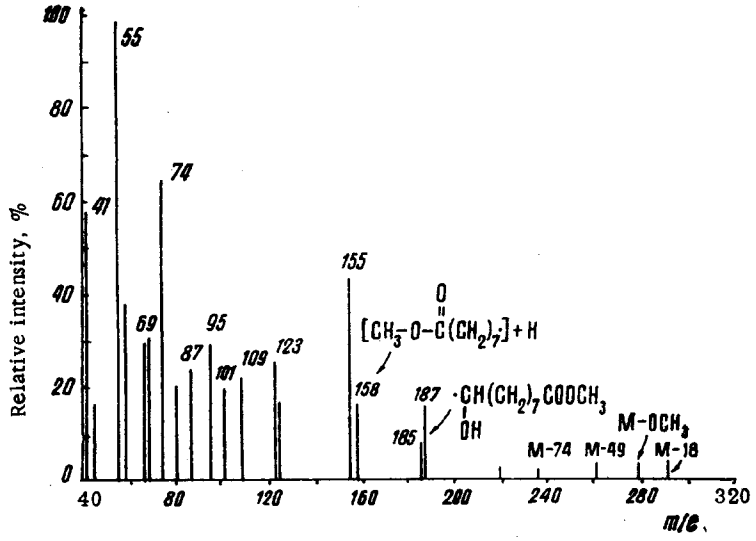


Fig. 3. Mass spectrum of the methyl ester of the hydroxydienoic acid.

$$CH_3CH_2CH{=}CH\cdot CH_2CH{=}CHCH_2CH_2\underset{\underset{OH}{|}}{CH}(CH_2)_7COOCH_3.$$

The cis configuration of the double bonds was established from the absence of the band of a trans bond in the IR spectrum in the 960-cm^{-1} region and from the value of the coupling constants of the olefinic protons in the NMR spectrum of (II) (J_{cis} = 10.3 Hz).

The new hydroxydienoic acid is an isomer of densipolic acid, 12-hydroxyoctadeca-cis-9, cis-15-dienoic acid [11], and this is the first time that it has been detected in the oils of the family Labiatae.

EXPERIMENTAL METHOD

The main methods of isolation and investigating the oils and fatty acids have been described previously [2].

The mass spectra were taken on an MKh-1303 instrument at 120°C with an ionization energy of 40 eV, the UV spectra on a Hitachi instrument, the IR spectra on a UR-10 spectrophotometer in a film, and the NMR spectrum on an M-4H-100 MHz instrument (CCl_4; internal standard TMS).

The urea fractionation of the acids was performed as described by Iverson et al. [12] using 5 g of acids at a ratio of acids to urea to absolute MeOH of 1:1:3.

Analytical TLC with respect to the degree of unsaturation (system 1) was performed on silica gel with the addition of 10% of gypsum and 20% of $AgNO_3$ in benzene; the separation of the trienoic from the unsaturated hydroxy acids in the form of the MEs was performed on silica gel + 10% of gypsum with the addition of 30% of $AgNO_3$ in the petroleum ether (40-60°C)—benzene (7:3, v/v) system (system 2).

Conditions of TLC in system 3: "Silufol" plates, petroleum ether (40-60°C)—diethyl ether (9:1, v/v).

The mixture of unsaturated ketonic acids was separated in an amount of 1.1% from the total acids in the form of a dark yellow oil, and the hydroxydienoic acid in an amount of 1.2% from the total acids also in the form of a dark yellow oil.

The 2,4-DNPH derivatives of the oxo acids were obtained by Allen's method [13].

SUMMARY

The fatty oils of the seeds of *Origanum tyttanthum* and *Mentha asiatica* (family Labiatae) have been investigated. The glyceride and fatty-acid compositions of the two oils have been determined. From the oil of *M. asiatica* have been isolated the new acid 9-hydroxyoctadeca-12-cis,15-cis-dienoic acid and a mixture of six unsaturated oxo acids.

LITERATURE CITED

1. S. D. Gusakova, A. U. Umarov, and A. L. Markman, Khim. Prirodn. Soedin., 315 (1968); Khim. Prirodn. Soedin., 324 (1975).
2. S. D. Gusakova and A. U. Umarov, Khim. Prirodn. Soedin., 27 (1972).
3. J. M. Hagemann, F. R. Earle, A. A. Wolff, and A. S. Barclay, Lipids, 2, 371 (1967).
4. F. R. Earle, C. A. Glass, G. C. Geisinger, and A. A. Wolff, J. Amer. Oil Chemists' Soc., 37, 440 (1960).
5. G. V. Novitskaya and V. I. Krishtopa, Rast. Res., No. 1, III, 32 (1971).
6. A. Zeman and H. Scharman, Fette, Seifen, Anstrichm., No. 9, 509 (1972); No. 1, 32 (1973).
7. R. Ryhage and E. Stenhagen, Arkiv für Kemi, B 15, 545 (1960).
8. L. A. Jones, J. C. Holmes, and R. B. Seligman, Anal. Chem., 28, 191 (1956).
9. Progress in the Chemistry of Fats and Other Lipids, 8, Part 2, 215 (1965); R. Kannan, M. R. Subbaram, and K. T. Achaya, Fette, Seifen, Anstrichm., No. 8, 344 (1974).
10. S. D. Gusakova, É. I. Gigienova, A. U. Umarov, and A. L. Markman, Maslob.-Zhir. Prom., 10, 9 (1969).
11. C. R. Smith, T. L. Wilson, R. B. Bates, and C. R. Scholfield, J. Org. Chem., 27, 3112 (1962).
12. J. L. Iverson, J. Eisner, and D. Firestone, J. Amer. Oil Chemists' Soc., 42, 1063 (1955).
13. M. Goryaev and I. Pliva, Methods of Investigating Essential Oils [in Russian], Alma-Ata (1962), p. 408.