THE OILS OF SOME PLANTS OF THE FAMILY LABIATAE

S. D. Gusakova and A. U. Umarov

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We have studied the seed oils of five species of the family Labiatae growing in the Uzbek SSR: Leonurus turcestanicus, Lagochilus occultiflorus, Salvia glabricaulis, Salvia virgata, and Dracocephalum integrifolium Bgl. The indices of the oils and of the mixtures of fatty acids are given in Table 1.

The IR spectra of the initial oils, excluding the usual spectrum, showed the following absorption bands, cm^{-1}: 860, 890 (C–H of an epoxide ring) (all five oils); 950, 975 (Lagochilus occultiflorus) or 955, 980 (Salvia glabricaulis), or 955, 975 (Dracocephalum integrifolium) (C=C in cis-trans or trans-cis conjugation); 950, 990 (Salvia virgata); 955, 990 (Dracocephalum integrifolium) (C=C in the all-trans or the cis-trans-trans conjugation); and 1070, 1975 cm^{-1} (C=C=C) (Lagochilus occultiflorus, Salvia glabricaulis) [1, 7].

The same bands are present in the IR spectra of the corresponding mixtures of methyl esters of fatty acids, but with greater intensities. In the spectra of the methyl esters of the acids, the absorption of the allene group (1070, 1975 cm^{-1}) has shifted into the 1050, 1950 cm^{-1} regions.

The compositions of the mixtures of fatty acid methyl esters determined and calculated on the basis of gas-liquid chromatography (GLC) for three of the oils are shown in Table 2. The acids were methylated with diazomethane, except that part of the mixture of acids from Lagochilus occultiflorus was subjected to acid methanolysis. The equivalent length of the chains of the molecules, according to GLC, was 20.8 for the $C_{20:1}$ acids and 21.8 for the $C_{20:2}$ acids (in a polar phase) [2].

The difference between the experimental iodine No. (Kaufmann's method) and the theoretical figure calculated on the basis of the GLC analysis for the mixture of esters of Lagochilus occultiflorus was 11.2 and for Salvia glabricaulis 7.6 units.

TABLE 1

Index	Leonurus turcestanicus	Lagochilus occultiflorus	Salvia glabricaulis	Salvia virgata	Dracocephalum integrifolium
Moisture content of the seeds, %	5,2 34,4	4,35 30,25	4,53 21,6	6,68 22,31	7,98 3,72
			Oils		
Density, d_{20}^4, g/ml	0,9207	0,9262	0,9258	—	—
Refractive index, n_D^{20}	1,4775	1,4771	1,4753	—	—
Acid No., mg KOH	1,1	1,19	17,4	2,1	—
Iodine No., % I_2	127,7	122,3	123,5	164,4	156,2
Iodine No. of the fatty acids, % I_2	128,5	128,2	125,6	166,4	161,4
Neutralization No., mg KOH/g	—	200,61	201,81	—	—
Mean mol. wt. of the acids	—	279,7	279	—	—
Amount of conjugated dienes in the oil (λ_{max} 233 nm), %	1,23 ($E_1^{1\%}$=15,5)	1,75 ($E_1^{1\%}$=21,8)	1,39 ($E_1^{1\%}$=17,4)	1,37 ($E_1^{1\%}$=17,1)	0,21 ($E_1^{1\%}$=4,37)

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TABLE 2

Acids, %*	Leonurus turcestanicus	Salvia glabricaulis	Lagochilus occultiflorus	
			CH_3OH + 1% H_2SO_4	CH_2N_2
$C_{12:0}$	—	0,39	—	—
$C_{14:0}$	—	—	Traces	—
$C_{16:0}$	4,53	4,49	4,34	3,69
$C_{18:0}$	1,58	2,15	1,84	1,49
$C_{18:1}$	24,09	30,45	25,21	24,04
$C_{18:2}$	68,35	61,26	63,33	66,11
$C_{20:1}$	1,44	1,25	3,32	2,92
$C_{20:2}$	—	—	1,94	1,75

*Abbreviated symbols for the acids: the figure in the index before the colon gives the number of carbon atoms in the chain, and the figure after the colon the number of double bonds in the molecule.

According to paper chromatography (PC) all five of the mixtures of acids studied contained (in order of decreasing R_f) $C_{18:2}$, $C_{18:1}+C_{16:0}$ (critical pair), $C_{18:0}$, and $C_{20:1}$, and four of the oils – all except that of Salvia virgata – contained traces of $C_{20:0}$, $C_{22:0}$, and $C_{24:0}$, while only Salvia virgata and Dracocephalum int. contained $C_{18:3}$.

The glyceride compositions determined and calculated for Lagochilus occultiflorus and Leonurus turcestanicus are given below:

Type of glycerides, %	*Lagochilus occultiflorus*	*Leonurus turcestanicus*
Gl S_3	0.01	0.04
Gl SSU	0.5	1.08
Gl SUS	0.27	0.49
Gl SUU	9.48	12.46
Gl USU	4.49	6.83
Gl U_3	85.25	79.10

The mixture of fatty acids from the seed oil of Lagochilus occultiflorus which, according to its chromatographic and spectral characteristics, contained allenic, conjugated dienic, and epoxide groups and eicosanoic and eicosadienoic acids, was investigated in more detail.

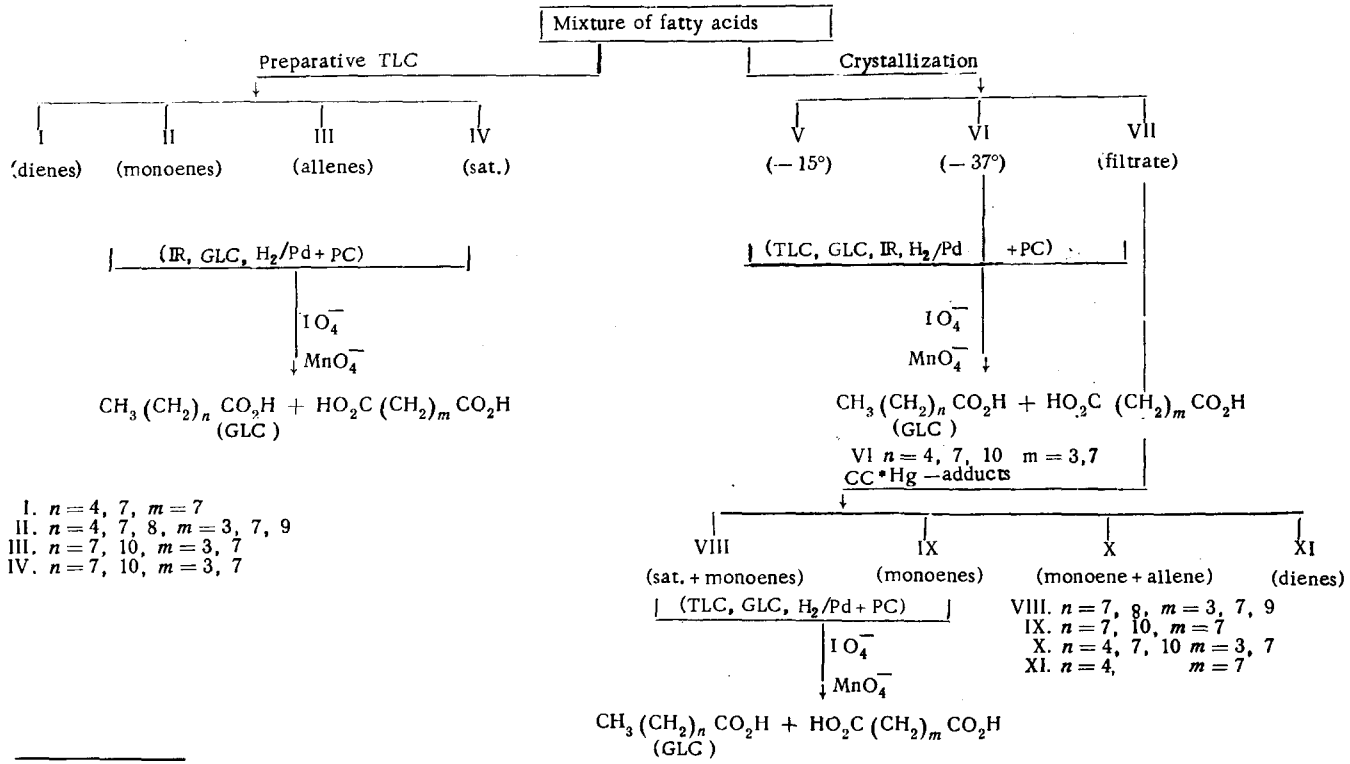
When the mixture of acids was subjected to microhydrogenation with Pd on paper with subsequent separation of the acids by paper chromatography, the $C_{16:0}$, $C_{18:0}$, and $C_{20:0}$ acids were obtained.

Analytical thin-layer chromatography (TLC) of the mixture of methyl esters of the acids on silica gel with the addition of $AgNO_3$ yielded four fractions corresponding in the degree of increase in R_f to dienes (I, 0.31), monoenes (II, 0.47), allenes (III, 0.65), and saturated esters (IV, 0.81). The allenes were poorly separated from the saturated esters. The results of the GLC of these fractions are shown in Table 3.

The IR spectra of the fractions obtained (with the exclusion of the ordinary spectrum) showed the following absorption bands, cm^{-1}: 810, 850 (C–H of an epoxy group) (fraction I); 870, 860 (C–H of an epoxy group); 965 (trans-C=C) (II); 850, 880 (C–H of an epoxy group); 960, 975 (cis,trans- or trans,cis-C=C); 1090, 1950 (C=C=C) (III); 870 (C–H of an epoxy group); 925, 965 (cis,trans- or trans,cis-C=C); 1050, 1950 (C=C=C) (IV). On GLC, the allenic ester gave a single peak with the normal ester of a dienic acid (polar phase). On PC, the allene, like the $C_{20:2}$ acid, was not separated from $C_{18:1}+C_{16:0}$. These acids migrate as a single spot. The microhydrogenation of the fraction on Pd with subsequent PC analysis led to the disappearance of the spots of the unsaturated acids and also to the appearance of intense spots of the $C_{18:0}$ acid (fractions I-IV) and the $C_{20:0}$ acid (fractions (II-IV).

Periodate–permanganate oxidation with subsequent GLC of the degradation products in the form of esters gave the following fragments: fraction I – azelaic, pelargonic, and caprylic acids; fraction II – azelaic, undecanedicarboxylic, traces of glutaric, pelargonic, caprylic, and a small amount of caproic acids; fraction III – glutaric, azelaic, lauric, and pelargonic acids; and fraction IV – lauric, pelargonic, azelaic, and glutaric acids.

Scheme



I. $n = 4, 7, m = 7$
II. $n = 4, 7, 8, m = 3, 7, 9$
III. $n = 7, 10, m = 3, 7$
IV. $n = 7, 10, m = 3, 7$

*Column chromatography.

TABLE 3

Acids, %	Fractions			
	I (dienes)	II (monoenes)	III (allenes)	IV (saturateds)
$C_{12:0}$	—	0,39	1,52	1,71
$C_{14:0}$	—	—	1,02	2,67
$C_{16:0}$	—	0,69	14,26	45,02
$C_{18:0}$	—	0,47	5,16	22,30
$C_{18:1}$	10,28	81,93	4,12	—
$C_{18:2}$	89,72	10,03	64,65	16,01
$C_{20:0}$	—	—	—	7,14
$C_{20:1}$	—	6,49	1,78	—
$C_{20:2}$	—	—	7,49	2,34
$C_{22:0}$	—	—	—	2,81
$C_{24:0}$	—	—	—	Traces

TABLE 4

Acids (%) (GLC)	Fractions			
	VIII (eluent benz.)	IX (elu. benz. + diethyl ether)	X (elu. diethyl ether)	XI (eluent methanol + acetic acid)
$C_{12:0}$	1,1	—	—	—
$C_{14:0}$	1,58	—	—	—
$C_{16:0}$	21,98	—	—	—
Substance	—	—	0,89	—
$C_{18:0}$	4,07	—	—	—
$C_{18:1}$	60,96	80,90	56,55	—
$C_{18:2}$	1,92	3,7	26,45	100
$C_{20:0}$	0,3	—	—	—
$C_{20:1}$	7,42	—	—	—
Substance B	—	3,37	5,39	—
Substance C	—	12,12	11,5	—

By comparing the quantitative ratios of the fragments obtained in each fraction it is possible to conclude that fraction I contains normal oleic (cis-9-$C_{18:1}$) and linoleic (cis-9-cis-12-$C_{18:2}$) acids; II oleic, eicosanoic (cis-11-$C_{20:1}$), traces of linoleic, and very small amounts of isolinoleic (cis-5-trans-8- or trans-5-cis-8-$C_{18:2}$) acids; III laballenic ($\Delta^{5,6}$-$C_{18:2}$-allenic) [3] and traces of oleic and an eicosadienoic (presumably cis-9-trans-11- or trans-9-cis-11-$C_{20:2}$) acids; and IV laballenic and traces of eicosadienoic acids.

On periodate–permanganate oxidation of the acids with a double bond at the fifth carbon atom reckoning from the COOH group, no glutaric acid was found among the degradation fragments [3]. In our experiments in the oxidation of fractions containing such acids (10-12%) [4], we found glutaric acid, but in far less than the stoichiometric amounts.

$$CH_3(CH_2)_{10}CH{=}C{=}CH(CH_2)_3CO_2H \xrightarrow{IO_4^-/MnO_4^-} CH_3(CH_2)_{10}CO_2H + HO_2C(CH_2)_3CO_2H.$$

Part of the initial mixture of fatty acids from Lagochilus occultiflorus was subjected to low-temperature crystallization from acetone at −15°C (fraction V) and at −37°C (fraction VI). Fraction V consisted of the following acids: $C_{16:0}$ (25.01%), $C_{18:0}$ (44.55%), $C_{20:0}$ (14.65%), $C_{22:0}$ (15.77%), and $C_{24:0}$ (traces) (GLC, PC, and TLC).

According to GLC, fraction VI contained the following acids: $C_{12:0}$ (0.35%), $C_{16:0}$ (33.65%), $C_{18:1}$ (9.32%), $C_{18:2}$(44.51%), $C_{20:0}$ (1.35%), and $C_{20:2}$ (10.82%). IR spectrum, cm^{-1}: 1060, 1975 (C=C=C), 955, 975 (C=C in cis-trans or trans-cis conjugation). Analytical TLC and PC showed that the main octadecadienoic acid in this fraction is laballenic acid. Microhydrogenation with Pd gave the $C_{16:0}$, $C_{18:0}$, and $C_{20:0}$ acids. Among the products of $IO_4^- - MnO_4^-$ oxidation we found lauric, pelargonic, glutaric, azelaic, and very small amounts of capric acids.

The concentration of the eicosadienoic acid (~11%) in the low-temperature fraction supported the assumption that it contains a conjugated system of bonds [5].

The mixture of fatty acids from the filtrate (fraction VII) consisted of the following acids: $C_{16:0}$ (1.04%), $C_{18:1}$ (25.12%), $C_{18:2}$ (69.21%), $C_{20:1}$ (3.38%), and $C_{20:2}$ (1.25%) (GLC). The octadecadienoic acids consisted mainly of linoleic with a small amount of laballenic acid (PC, TLC). The methyl esters from the filtrate were subjected to further fractionation by the column chromatography of the mercury adducts. The compositions of the four fractions isolated are given in Table 4.

According to TLC, PC, and destructive oxidation, the laballenic acid was concentrated in fraction X (monoenes eluted by diethyl ether) and the eicos-11-enoic and the $\Delta^{5,8}$-isolinoleic acid in fraction VIII (saturateds + monoenes eluted by benzene).

The unsatisfactory separation of the monoenoic acids from the saturated acids is apparently due to the incomplete mercuration of the initial mixture containing laballenic acid under the standard conditions of the method.

Substances A, B, and C appearing on the chromatograms (GLC) of fractions IX and X in the $C_{16:1}$-$C_{17:1}$ (A) and $C_{20:2}$-$C_{22:0}$ (B, C) regions are hydroxy compounds. This is shown by the behavior of these substances under the conditions of the paper chromatography of the acids, where they appear as a spot at the solvent front.

Under the conditions of the TLC of the lactones and hydroxy acids in fractions IX and X two spots were found which fluoresced in UV light (R_f 0.1 and 0.21).

In the GLC of products of the oxidative degradation of these fractions, the peaks corresponding to A, B, and C had disappeared, but in the dicarboxylic fraction two peaks appeared in the $C_{13:0}$ and $C_{15:0}$ region which were not identified. The TLC of the dicarboxylic acid fractions showed that they contained hydroxy compounds (R_f 0.24).

The formation of such substances has also been observed in the isolation of ricinoleic acid from the mixture of fatty acids of castor oil by the mercury adduct method with subsequent destructive oxidation by $IO_4^- - MnO_4^-$. We have not studied the structures of these substances or of the epoxy compounds present in the acids of Lagochilus occultiflorus according to the IR spectra.

Thus, the oil of the seeds of Lagochilus occultiflorus contains, in addition to the usual acids, 12% of laballenic acid (the $\Delta^{5,6}$-$C_{18:2}$ allenic acid) (gravimetrically, TLC) 3% of eicos-cis-11-enoic acid, 1.75% of an eicosadienoic acid (presumably either cis-9-trans-11 or trans-9-cis-11), and traces of cis,trans- or trans,cis-$\Delta^{5,8}$-icolinoleic acid.

According to GLC and TLC, IR spectroscopy, and destructive oxidation of these species studied, laballenic acid is present in the seed oil of Salvia glabricaulis and eicos-11-enoic acid in the seed oils of Leonurus turcestanicus (1.44%) and Salvia glabricaulis (1.25%).

Acids containing an allene group at the fifth carbon atom have recently been found in two oils of the family Labiatae and in one oil of the family Euphorbiaceae [3, 12]; this is the first time that eicos-11-enoic acid, which is present in a number of plants of the families Ranunculaceae and Ephedraceae [6], has been found in the oils of the family Labiatae. Eicosadienoic acids with a conjugated system of double bonds have not been detected previously.

EXPERIMENTAL

The oils were isolated by cold extraction with petroleum ether (40-50°C). The bulk of the solvent was driven off in vacuum in a current of N_2, and the remainder of it in a vacuum-drying chest at 30-40°C (740 mm Hg).

The mixtures of fatty acids were obtained by alkaline hydrolysis at room temperature [9] with the preliminary separation of the unsaponifiable substances [7]. The methyl esters of the acids were prepared by esterification with diazomethane or by acid methanolysis ($CH_3OH + 1\% H_2SO_4$).

The main indices of the oils and of the mixtures of fatty acids were determined by standard methods [7]. The iodine nos. were found by Kaufmann's method [7].

The IR spectra were taken on a Hitachi instrument (in hexane) and the IR spectra of the oils and of the methyl esters on a UR-10 spectrometer (as thin films).

The amount of conjugated dienes was calculated from the UV absorption at λ_{max} 233 nm [7].

Gas-liquid chromatography was performed on a UKh-2 chromatograph with a 2.5×0.04 m column filled with TND-TS-M (passing through a 0.3 mm sieve) with 17% of poly(ethylene succinate) using helium as the carrier gas. The compositions of the methyl esters of the initial mixtures and of the dicarboxylic acids were determined at a column temperature of 198-200°C with a rate of flow of helium of 80-100 cm^3/min, and the esters of the low-molecular-weight monocarboxylic acids (up to $C_{13:0}$) at a column temperature of 124-125°C with a rate of flow of helium of 40-50 cm^3/min. The acids were identified by comparison with model substances, by the internal-standard method, and by mixed melting points, and also by the graphical dependence of the logarithm of the retention volume on the number of carbon atoms. The quantitative ratios of the esters in the mixtures were calculated as the sum of the areas [8].

The paper chromatography of the acids [9] was performed in the $CH_3COOH - HCOOH - H_2O$ (3:1:0.1) system on type "M" ["slow"] paper (Leningrad No. 2 mill) impregnated with a 10% solution of paraffin oil in benzene. The spots were revealed with a 1% solution of rubeanic acid, and also with a 1% solution of $KMnO_4$ (to determine the unsaturated acids). The spots were identified by comparing their R_f values with markers.

Microhydrogenation with Pd on paper with subsequent paper chromatography was performed by Kaufmann's method [10] under analytical conditions [9].

The triglyceride compositions of the oils were determined by enzymatic hydrolysis with lipase [11] and the determination of the products by TLC and GLC.

The analytical TLC of the fatty acid methyl esters [12] was carried out on plates of the "sandwich" type [13] (18×18 cm) with a thin layer of type KSK silica gel (passing a 0.1 mm sieve) with the addition of 5% of gypsum and 15-10% of $AgNO_3$ using benzene as the mobile phase.

Preparative TLC was performed on plates (24×18 cm) bearing a 1-mm layer of KSK silica gel with the addition of 5% of gypsum and 30% of $AgNO_3$, again using benzene as the mobile phase. The spots were revealed by treatment with 50% H_2SO_4 followed by heating to 150°C. They were identified by means of literature data [12] and by comparison with markers. The substances were eluted from the respective zones with diethyl ether and then with chloroform–methanol (9:1).

The conditions for the TLC of the lactones and hydroxy acids were as follows [14]: plates (10×18 cm) bearing a thin layer of KSK silica gel with the addition of 5% of gypsum and 20% of $AgNO_3$ in the isooctane–diethyl ether (1:1) system. The spots were revealed in UV light.

The mixture of fatty acids was crystallized from a 10% solution of them in acetone [5]. The mixtures of mercury derivatives of the methyl esters were fractionated by column chromatography [15].

Periodate–permanganate oxidation was performed by von Rudloff's method [16] as modified by us. After the destruction of the excess of oxidizing agent, the reaction mixture was made alkaline with KOH, the solvent (tert-butanol) was eliminated in vacuum, the potassium salts were decomposed with 15% HCl, and the degradation fragments were isolated from the solution, previously saturated with NaCl, by extraction with diethyl ether.

SUMMARY

The seed oils of five species of plants of the family Labiatae – Leonurus turcestanicus, Lagochilus occultiflorus, Salvia glabricaulis, Salvia virgata, and Dracocephalum integrifolium Bgl. – have been studied. The oil of Lagochilus occultiflorus was found to contain laballenic, octadeca-5,8-dienoic, eicos-11-enoic, and, presumably, eicosa-9,11-dienoic acids; the oil of Salvia glabricaulis contained laballenic and eicos-11-enoic acid; and the oil of Leonurus turcestanicus eicos-11-enoic acid.

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