OILS OF THE SEEDS OF Ziziphora pedicellata AND Eremostachys molluceloides

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We have investigated the triglyceride oils of the seeds of Ziziphora pedicellata Pazij. et Vved. and Eremostachys molluceloides Bgl., family Labiatae, collected in the Tashkent oblast. The oil contents of the seeds of the Ziziphora and Eremostachys were 17.6 and 22.3%, respectively. The Ziziphora oil was light yellow and the Eremostachys oil green. Both oils were transparent with the sage-like odor characteristic of the Labiatae. The main indices of the oils are given below:

Index	Ziziphora oil	E. molluceloides oil
Density, d_{20}^4, g ml	0.9288	0.9115
Refractice index, n_D^{20}	1.4831	1.4732
Acid No., mg KOH	8.9	2.5
Saponification No. mg KOH/g	194.3	185.9
Content of unsaponifiables, %	1.9	2.2
Iodine No., % I_2	192.6	107.1

The glyceride types of the oils calculated by Coleman's method on the basis of the results of enzymatic hydrolysis are combined in six monotypical groups (%):

Oil	S_3	SSU	USU	SUU	SUS	U_3
Ziziphora	0.01	0.1	0.49	17.98	1	80.42
Eremostachys*	None	0.3	4.4	6.5	0.1	88.7

Thus, the Ziziphora oil consists mainly of diunsaturated of the SUU type and triunsaturateds, and the oil of E. molluceloides of diunsaturateds of types USU and SUU and triunsaturated glycerides.

By alkaline hydrolysis under mild conditions we isolated the mixtures of acids and unsaponifiable components from the oils, and these were investigated separately. The indices of the combined acids of these oils are as follows:

Index	Acids of Ziziphora	Acids of Eremostachys
Refractive index, n_D^{20}	1.4815	1.4652
Iodine No., % I_2	220.1	122.5
Theoretical iodine No. †, % I_2	221.6	111.4‡
Neutralization No., mg KOH/g	199.2	191.8
Mean molecular weight of the acids	281.7	292.5

The gas–liquid chromatography of the methyl esters of the acids (MEs) was performed on the polar phase PEGS. The acids were identified by the graphical method [1] taking separating factors into account

*Without taking unusual components into account.

†Calculated from the GLC results.

‡Taking 8.5 % of laballenic acid into account.

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[1, 2] and also by comparison with model samples. The results of GLC are given below (r represents the relative retention time $C_x/C_{18:0}$ [1]):

Methyl esters of the acids	Ziziphora Area, %	r	Eremostachys Area, %	r
$C_{16:0}$	5.7	0.56	2.1	0.56
$C_{18:0}$	1.2	1.0	0.3	1.0
$C_{18:1}$	9.3	1.12	70.8	1.16
$C_{18:2}$	16.2	1.29	26.3	1.35
$C_{18:3}$	67.6	1.64		
C_x			0.5	1.91

The paper chromatography of the acids confirms the GLC results and, in addition, this method shows the presence in the Ziziphora acids of traces of $C_{20:0}$.

To determine the structure of the unsaturated acids we used thin-layer chromatography according to the degree of unsaturation ($AgNO_3$–TLC). In the mixture of ziziphor MEs four spots were found corresponding to $C_{18:3}$ (R_f 0.13), $C_{18:2}$ (R_f 0.39), $C_{18:1}$ (R_f 0.75) and the combined saturated acids (R_f 0.95). By the same method in the mixture of MEs from E. molluceloides we found six spots with R_f 0.05, 0.14, 0.39, 0.75, 0.86, and 0.95, of which only the three spots with R_f 0.75, 0.86, and 0.95 were identified as the $C_{18:1}$, $C_{18:2}$-allenic, and the combined saturated acids, respectively. The identification of the spot with R_f 0.39 as only the normal $C_{18:2}$ acid and that with R_f 0.14 as the $C_{18:3}$ acid was not confirmed by PC and GLC.

In the UV and IR spectra of the oils and acids of Ziziphora there were no specific absorptions relating to any unusual structural groups present in the native acids. These results confirmed those of oxidative degradation by the periodate–permanganate reagent at the double bonds of the individual MEs of Ziziphora isolated by preparative $AgNO_3$–TLC. After the degradation of the ME with R_f 0.13 ($C_{18:3}$) we obtained as fragments azelaic and propionic acids, from that with R_f 0.39 ($C_{18:2}$) azelaic and caproic acids, and from that from R_f 0.75 ($C_{18:1}$) azelaic and pelargonic acid. On the basis of the absence from the IR spectrum of the MEs of the absorption bands of a trans bond it was concluded that all the double bonds have the cis configuration, and the acids themselves are ordinary oleic, linolenic acids.

The increased index of the mean molecular weight of the acids of Eremostachys and the discrepancy (11 units) between the experimental iodine number of its combined acids and the theoretical figure calculated from the GLC results shows the presence of unsaturated acids of unusual structure not revealed in the separation of the combined acids under the conditions used for GLC.

The UV spectrum of the Eremostachys oil had weak but distinct absorption mazima at λ_{max} 233, 243, 249, 255, and 261 nm and a weak broad maximum at 301 nm (hexane); the mixture of acids had λ_{max} 234, 256, 261, 280, and 305 nm (hexane), and the MEs λ_{max} 234, 243, 249, 255, 261, 271.5, and 285 nm (C_2H_5OH).

The disappearance of absorption in the 300-nm region in the UV spectrum of an ethanolic solution of the MEs and the presence of a relatively sharp maximum in this region in the spectrum of a hexane solution is characteristic for carbonyl groups [3].

The IR spectrum of the Eremostachys oil had, in addition to the usual bands, absorption bands at 870 and 1975 cm^{-1} (C=C=C), 980 cm^{-1} (trans-CH=CH), and 1550-1830 cm^{-1} with a maximum at 1765 (ester and conjugated carbonyl) and one at 3470 cm^{-1} (–OH).

The IR spectrum of the MEs showed the same absorption bands but a more distinct band of a secondary OH group (1100 cm^{-1}) masked in the triglycerides by the strong absorption of the C–O of an ester group (1110 cm^{-1}), and the C=O band appeared in the 1675-1790 cm^{-1} region with a maximum at 1735 (C=O of an ester).

The oil and MEs of Eremostachys gave a positive qualitative reaction with $FeCl_3$ for α,β-diketones or α-ketols [3].

Thin-layer chromatography of silica gel in the hexane–diethyl ether–CH_3COOH (7 : 3 : 0.1) system of the mixture of acids showed a spot with R_f 0.3 corresponding to acids with oxygen-containing groups. Consequently, the Eremostachys oil contains in addition to the usual acids, an allenic acid and saturated keto or hydroxyketo compounds.

To determine the structure of the allenic acid, the MEs of Eremostachys were separated by preparative $AgNO_3$–TLC. Four fractions of unsaturated acids were obtained with R_f 0.14, 0.4, 0.7, and 0.86, the

first three of which contained keto compounds according to IR spectroscopy (1550-1760, 1250 cm^{-1}). The allenic fraction with R_f 0.86 (IR spectrum: 870, 1950 cm^{-1}) consisted of 3.2% of $C_{16:0}$, 83.4% of $C_{18:1}$, and 13.4% of $C_{18:2}$ acids (GLC).

The MEs from the zone with R_f 0.86 were rechromatographed by $AgNO_3$–TLC. This gave monoenic (98% $C_{18:1}$) and allenic (81% $C_{18:2}$) fractions consisting, according to chromatography and spectroscopy, of oleic acid and a $C_{18:2}$-allenic acid. This was confirmed by the formation of azelaic and pelargonic acids when the $C_{18:1}$ acid was oxidized with the periodate–permangante reagent. The degradation of the methyl ester of the $C_{18:2}$-allenic acid with $KMnO_4$ in acetone [4] gave lauric and glutaric acids, which shows the structure of the dienic acid as laballenic, $\Delta^{5,6}$-$C_{18:2}$. The MEs contained a 8.5% of the esters of this acid (TLC, gravimetrically).

Thus, both in the oleic-acid-containing oil (71% of $C_{18:1}$) of Eremostachys and in the linoleic-acid-containing oils of the Labiatae investigated previously [5] (60-70% of $C_{18:2}$) laballenic acid is present although it is practically absent from the linolenic-acid-containing oils (60-70% of $C_{18:3}$) of this family [6].

The structures of the other acids will be described later. The unsaponifiable fractions of the oils were studied by column chromatography (CC). The hydrocarbon fraction was isolated by CC on silica gel and recyrstallized twice from acetone. This gave white tabular crystals with mp 60° C (Ziziphora oil) and 59-60° C (Eremostachys oil).

By IR spectroscopy (2800-2970, 1470, 1380, 720 cm^{-1}) and melting point, the hydrocarbon isolated from the Ziziphora oil was identified as octacosane. The mass spectrum of the hydrocarbon fractions of the Eremostachys oil showed that it consisted of a mixture of C_{27}-C_{35} hydrocarbons (M^+ with m/e 380, 394, 408, 422, 436, 450, 464, 478, and 492) and C_{32}-C_{34} alcohols ($M^+ - 1$ with m/e 466, 480, and 494). The pigments were isolated by CC on Al_2O_3 using solvents of increasing polarity. The pigments isolated were identified by comparison with the spectra of known samples from tables of the carotenoids [7].

The following were found in Ziziphora oil:

α-Carotene – a yellow pigment with λ_{max} 421, 445, and 475 nm (petroleum ether);
β-Carotene – a yellow pigment with λ_{max} 427, 451, and 478 nm (petroleum ether).

In the Eremostachys oil we found:

1) β-Carotene – a yellow pigment with λ_{max} 472, 451, 479 nm (petroluem ether);

2) a mixture of two hydroxycarotenes – a yellow pigment with λ_{max} 380, 404, and 430 nm and λ_{max} 430, 457, and 486 nm (chloroform); IR spectrum (cm^{-1}): 3400-(–OH), 1070 (–OH) in a ring, 3030, 1660, 960, 975 (CH=CH), 2890-2980 (CH_2, CH_3–), 1840 (CH_2), 1380 [$(CH_3)_2$–C], 830 (–CH=C<);

3) an unidentified yellow pigment with λ_{max} 377, 400, and 424 nm (acetone);

4) 3,3'-dihydroxy-α-carotene (lutein) – an orange pigment with λ_{max} 420, 445, and 473 (methanol); and

5) a ketocarotene – a yellow pigment with λ_{max} 376, 398, and 422 nm (methanol); IR spectrum (cm^{-1}): 3030, 1660, 970 (CH=CH), 2890-2980 (CH_2, CH_3), 1720, 1590, 1575, 1560, 1530 [CO(CH=CH)$_2$], 1450 (CH_2), 1380 [$(CH_3)_2$–C], 840 (CH=C<).

Thus, the pigments of Eremostachys also contain keto and hydroxy groups as structural elements.

EXPERIMENTAL METHOD

The main methods of isolating and investigating oils and fatty acids have been described previously [5].

The mixture of hydrocarbons and high-molecular-weight alcohols was isolated by passing 2 g of a 10% solution of the oil in petroleum ether through a column (d 19 mm) containing 30 g of silica gel (KSK, passing 0.2 mm). The mixture was eluted with 150 ml of a 1% solution of diethyl ether in petroleum ether (40-60°C).

The mass spectra were taken on an MKh-1303 instrument at a temperature of 175° C with an ionizing voltage of 70 V.

The pigments were isolated from the combined unsaponifiable components of the oil by passing 0.3 g of a 20% solution of the combined components in petroleum ether (40-60° C) through a column (d 10 mm) containing 4 g of Al_2O_3 followed by elution with petroleum ether (8 ml), chloroform (10 ml), acetone (13 ml), and methanol (10 ml).

The UV spectra of the pigments were taken on a Hitachi instrument in the same solvents and the IR spectra of the substances in the form of films after the solvents had been driven off in vaccum in a current of N_2 were taken on a UR-10 spectrometer.

SUMMARY

The fatty oils of the seeds of the wild species Ziziphora pedicellata and Eremostachys molluceloides (Labiatae) have been investigated. The glyceride, pigment, and hydrocarbon compositions of these oils and the fatty-acid composition of the oil of Z. pedicellata have been determined.

The oil of E. molluceloides contains laballenic acid.

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