THE OIL OF THALICTRUM SIMPLEX

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Slimtop meadowrue (Thalictrum simplex) belongs to the Ranunculaceae family. The seeds of some plants of this family have been studied with respect to their oil content [1]; however, we have found no information on a thorough study of the nature of these oils in the literature.

Slimtop meadowrue is a perennial plant widely distributed in Europe and Asia and, in particular, in almost all regeions of the U.S.S.R. It is found particularly in Central Asia (Tarbagatai, the Dzungarian Ala-Tau, Tien Shan, and the Pamir-Alai). The plant contains the alkaloids thalictrinine $C_{38}H_{46}N_2O_7$ and other alkaloids [2, 3].

TABLE 1

Characteristic	Unit of measurement	Oil	Fatty Acids
Specific gravity, d_4^{20}	g/ml	0.9181	–
Absolute viscosity, $[\eta]^{20}$	poise	0.7314	–
Refractive index, n_D^{20}	—	1.4728	–
Saponification number	mg KOH/g	177.75	–
Hehner number	%	96.29	–
Neutralization number	mg KOH/g	–	204.49
Mean molecular weight	—	–	274.39
Reichert-Meissl number	ml KOH	0.71	–
Polenske number	ml KOH	2.78	–
Iodine number	% I_2	167.46	182.71
Thiocyanogen number	% I_2	89.04	84.47
Content of saturated acids by Bertram's method	%	–	11.77
Content of solid acids by Grossfeld's method	%	–	10.17
Iodine number of the solid acids	% I_2	–	32.61
Content of unsaponifiables	%	4.29	–
Content of phosphatides	%	1.29	–

The seeds of meadowrue are glabrous, ovate, and have six long ribs. The length of the seed is 2-2.5 mm, and the breadth 0.75-1mm [4, 5]. We have determined the bulk density of these seeds (307 g/liter) and the oil content, which proved to be 21.62% on extraction with petroleum ether and 24.0% on extraction with diethyl ether.

The oil was extracted from the previously ground seeds with petroleum ether in the cold; it has a dark green coloration with a cherry-red fluorescence, and a grassy odor, and is characterized by a high acidity (60.54 mg of KOH/g) which rapidly rises on storage: after a month it had become 74.76, and after 4 months 114.73. This indicates that the oil contains a highly active lipolytic enzyme. It was impossible to refine the oil because of its high acidity, and therefore all the characteristics were determined on the crude oil.

The fatty acids were isolated from the oil by the usual method; the physical and chemical characteristics of the oil and the fatty acids are given in Table 1.

To characterize the fatty-acid composition of the oil, we had recourse to the paper-chromatographic method in the variant developed by Kaufmann and Nitsch [6] and improved by Alimova [7]. For this purpose, a mixture of paraffin wax and vaseline oil (1:1) was dissolved in a mixture of benzene and chloroform (1:4) in a ratio of 1:9. The paper was impregnated with this solution. The mobile solvent used was a mixture of 85% formic and 98% acetic acids and water (25:75:2.5). The chromatograms were shown up by solutions of copper acetate and rubeanic acid.

The following materials were subjected to chromatography (Fig. 1):

1) A mixture of fatty acids isolated from the seeds of meadowrue;

2) A mixture of the saturated fatty acids isolated by Bertram's method (iodine No. 2.06; neutralization number 219.42; mean M 255.72);

3) A mixture of the solid fatty acids isolated by Grossfeld's method (iodine No. 32.61; neutralization number 182.93; mean M 306.73); and

4) A mixture of solid fatty acids isolated from the oil and hydrogenated with platinum catalyst at 70° (mp 46°; iodine No. 17.99).

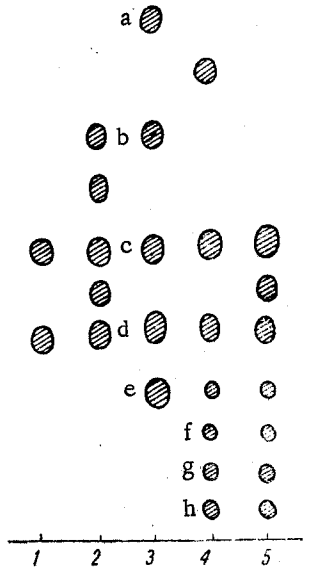

Fig. 1. Chromatograms of the fatty acids of the oil of meadowrue and its individual fractions: 1) Hydrogenated fatty acids; 2) Mixture of fatty acids of Thalictrum simplex; 3) Reference substances; 4) Saturated acids; 5) Solid acids. a) Lauric acid; b) Myristic acid; c) Palmitic acid; d) Stearic acid; e) Arachidic acid; f) Behenic acid; g) Lignoceric acid; h) Cerotic acid.

The initial mixture of fatty acids of the oil gave 5 spots, of which 3 were clearly identified (on the basis of a comparison with the spots of "reference substances") with the acids stearic, palmitic + oleic, and linoleic. Of the remaining spots, one was located between the stearic and palmitic acid spots and the other between the oleic and linoleic acid spots.

In the chromatogram of the saturated acids, the stearic and palmitic acid spots were retained and the linoleic spot disappeared; this convinced us that we were in fact dealing with linoleic acid and not with myristic acid, which is a critical partner of linoleic acid. The disappearance of the spot located between stearic and palmitic acids justifies the assumption that it belongs to an unsaturated acid, most probably some octadecenic acid, i.e., an isomer of oleic acid. And, finally, the fact that the spot between oleic and linoleic acids disappears indicates the presence of an unsaturated acid belonging, from the position of the spot, to the group of hexadecenic (palmitoleic) or octadecadienic (isolinoleic) acids.

On the other hand, the chromatogram of the acids obtained by Bertram's method showed weakly expressed spots of C_{20}—C_{26} acids (arachidic, behenic, lignoceric, and cerotic) which are not visible on the chromatogram of the initial acids because of their extremely small amount. It is possible that these acids belong not so much to meadowrue oil itself as to the accompanying wax.

The same chromatogram of saturated acids shows a clearly expressed spot between the positions appropriate to myristic and lauric acids. This spot must be assigned to n-tridecanoic acid $C_{17}H_{26}O_2$, which can be formed during the process of oxidation by Bertram's method through the destruction of octadec-5-enoic acid:

$$CH_3(CH_2)_{11}CH = CH\,(CH_2)_3COOH \rightarrow CH_3\,(CH_2)_{11}COOH + HOOC\,(CH_2)_3COOH.$$

This acid has not previously been found in any of the natural oils.

On the chromatogram of the solid acids isolated by Grossfeld's method, we again found palmitic, stearic, and C_{20}—C_{26} acids and, in addition, an unsaturated acid the spot of which was located between the spots of palmitic and stearic acids. This confirmed our assumption that we were dealing with some solid position isomer of oleic acid, the presence of which explains the relatively high iodine number of this fraction of the acids. So far as concerns the acid giving the spot located on the chromatogram of the initial acids between the spots for oleic and linoleic acids, it evidently is one of the liquid acids since it no longer appears in the acid fraction isolated by Grossfeld's method.

The chromatogram of the hydrogenated acid shows only two spots, corresponding to stearic and palmitic acids. We did not detect the spots of the C_{20}—C_{26} acids because of their small amount; however, all the unsaturated acids belong to the C_{16} and C_{18} types and therefore, after hydrogenation, fuse on the chromatogram into two spots located at the levels of palmitic and stearic acids.

Finally, it must be noted that we found no spot of linolenic acid, which should have been located at the level of lauric acid, on the chromatogram of the initial acids. This can be explained by its very small amount. However, the presence of linolenic acid was detected by the results of spectrophotometric analysis and the formation on bromination of a bromide insoluble in ether (hexabromide number of the oil 2.36%). We may mention that this bromide had mp 135-139°, while the hexabromide of α-linolenic acid has mp 176-178°.

Consequently, two assumptions may be made: either the hexabromide which we obtained was not sufficiently pure or the fatty acids contain not α-linolenic acid but some position or geometrical isomer of it.

The investigations described above thus indicate that meadowrue oil contains palmitic, stearic, arachidic, behenic, lignoceric, cerotic, oleic, octadec-5-enoic, linoleic, isolinoleic, (or one of the n-hexadecenoic acids), and one of the linolenic acids.

With such a complex fatty-acid composition of the oil, the method of iodine and thiocyanogen numbers is unsuitable for the quantitative evaluation of the content of each of the acids or even of each group of acids. For this purpose, we used the spectrophotometric method [8]. Spectra were taken of the acids in their native state and after they had been isomerized by heating for 45 min at 180° in alkaline glycerol solution. The characteristics of the composition of the fatty-acid mixture and (taking into account the content of unsaponifiables and the glycerol residue) of the oil have been calculated on the basis of the results so obtained (Table 2).

TABLE 2

Component	Composition, %	
	Mixture of Fatty acids	Oil
Saturated acids	8.95	8.29
Oleic acid	18.48	17.12
Isooleic acid	3.68	3.40
Linoleic acid	65.59	60.76
Linolenic acid	3.30	3.06
Unsaponifiables	—	4.29
Glycerol residue	—	3.08

The glyceride composition of the oil was calculated from the rule of random probability of the distribution of the fatty-acid radicals. It was as follows: GlS_3 – 0.07%; GlS_2U – – 2.28%; $GlSU_2$ – 22.68%; GlU_3 – 74.97% (Gl – glycerol residue, S – saturated fatty acids, U – unsaturated acids).

In order to study the pigment complex, the oil was diluted with a five-fold amount of petroleum ether and was filtered through a column filled with alumina. The lowest band, No. 1, was colored orange; at a distance 2 mm above it band No. 2 was also orange. Adjacent to it above was band No. 3– yellow; still higher was band No. 4 – olive-yellow, containing several thin ill-defined bands (yellow, olive, yellow, green-yellow). The highest band, No. 5, was green and consisted of alternating thin bands (green-yellow, yellow, bright green, dark green, light olive, and dark olive).

The pigments were eluted in the first place with petroleum ether. The spectrum of the eluates was studied by means of a SF-4 spectrophotometer. Band No. 1 gave a spectrum in which maxima at 445 and 475 mμ were clearly outlined with a less distinct one at 420 mμ, which correspond to α-carotene. Band No. 2, with maxima at 420, 448, and 472 mμ, and band No. 3, with maxima at 420, 445, and 472 mμ, were provisionally assigned, like band No. 1, to pigments of the carotenoid group, since the eluates corresponding to them gave the qualitative reaction with sulfuric characteristic for this group [9].

The over-all content of total carotenoids was determined by the photocolorimetric method by comparing the color of the eluate with the color of a standard solution of potassium dichromate. It was 22.1 mg-%.

After the petroleum ether elution, we washed the column with diethyl ether. This solvent eluted bands Nos. 4 and 5, although not completely. The green ring remaining after elution could not be washed out with anything but chloroform. Judging from their spectra, bands Nos. 4 and 5 correspond respectively to chlorophyll-a and chlorophyll-b. The nature of the pigment eluted with chloroform was not established [10].

The colorless fraction of the eluates obtained on column chromatography yielded a substance of paraffin-wax-like consistency with mp 53-54° and M 458.2 (Rast).

Found %: C 85.0, 85.2; H 14.4, 14.4. $C_{32}H_{66}$. Calculated %: C 85.33; H 14.67; M 450.85.

Judging from the infrared spectrum of this compound (2 mg of substance in 150 mg of KBr; an NaCl prism was used between 800 and 1800 cm^{-1} and an LiF prism between 1800 and 3600 cm^{-1}) (Fig. 2) and, in particular, the absence from it of a band at 1340 cm^{-1} characteristic for a tertiary carbon atom, it is a hydrocarbon of normal structure. However, n-dotriacontane $C_{32}H_{66}$ has mp 70°. We had apparently isolated a mixture of several paraffinic hydrocarbons of similar composition.

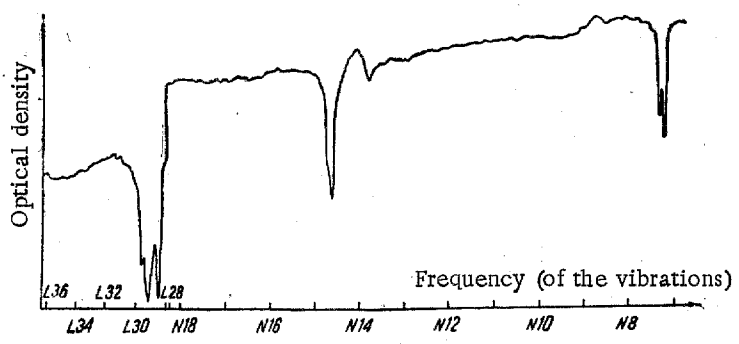

Fig. 2. IR spectrum of the $C_{32}H_{66}$ hydrocarbon.

The enzyme from the seeds of meadowrue was isolated in the same way as the lipase from castor beans. The activity of the enzyme was determined on refined cottonseed oil. For this purpose, two flasks were each filled with 25 g of the oil, 300 mg of the enzyme was added to one of them, and after predetermined intervals of time the acid number of the oil in the main and the blank experiments was determined (Table 3). The results of the determinations indicate the presence of an active lipolytic enzyme in meadowrue seeds.

TABLE 3

Date of the determination	Acid number		Date of the determination	Acid number	
	With enzyme	Without enzyme		With enzyme	Without enzyme
27. I	0.57	0.57	12. II	4.89	—
29. I	2.32	—	14. II	5.93	0.62
30. I	2.48	—	16. II	6.21	—
31. I	2.74	—	19. II	6.62	—
1. II	2.86	—	22. II	7.09	0.66
5. II	4.33	—	18. IV	17.52	1.18
10. II	4.67	0.59			

Meadowrue oil mixed with iron oxide and deposited on a metal plate was tested for drying properties (linseed oil was used for comparison). Meadowrue oil dried after 120 hr, giving a matt film, while linseed oil dried in 48 hr under the same conditions. With red lead, meadowrue oil and linseed oil dried in 68 and 20 hr, respectively.

When meadowrue oil was used in the formulation of pentaphthalic lacquers (20, 30, and 50% of the total oil content) they dried at 20° dust-free in 30 min and completely in 8 hr; at 80° they dried completely in 2 hr. The hardness of the film was 0.5. The properties of the lacquers satisfied the requirements of the standard.*

SUMMARY

Octadec-5-enoic acid, not found previously in other plant or animal oils and fats, has been found in the oil of slimtop meadowrue. The hydrocarbon fraction isolated contains n-dotriacontane and, apparently, other paraffinic hydrocarbons of similar composition. From its fatty-acid composition and its drying properties, the oil must be classed with the Papaveraceae group.

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*The experiments on the preparation of the lacquers and the determination of their properties were carried out by I. F. Zamesova (Tashkent paintworks).