THE SEED OIL OF *Nepeta cataria*

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Nepeta cataria (catnip, catmint) is one of the representatives of the 201 species of the family Labiatae growing in the territory of the Uzbek SSR [1]. The literature contains information on the total fatty-acid composition of the seed oil of *N. cataria* growing in the USA [2]. We have investigated the seed oil of this plant collected in the Tashkent Botanical Garden.

The seeds contained 24.8% of fatty oil, which was isolated by extraction with hexane. It consisted of a yellow liquid with a pleasant smell and was characterized by the following indices: n_D^{20} 1.4821; d_4^{20} 0.9208 g/ml; acid No. 3.3 mg KOH; Hehner number 95.4%; iodine No. (Kaufmann) 212.3% I_2; amount of unsaponifiables 2.9%.

Rough chromatography of the oil on a column of silica gel followed by rechromatography of combined fractions of similar polarity led to the isolation of the following classes of lipids: hydrocarbons, sterol esters, fatty acid methyl esters (FAMES), triglycerides (TGS), di-2-ethylhexyl phthalate, free fatty acids (FFAS), free sterols, diglycerides (DGS), monoglycerides (MGS), and phospholipids (traces).

The fatty acid methyl esters were partially separated from the sterol esters by crystallization and were finally freed from impurities by preparative TLC (PTLC) on silica gel in system 1. The GLC analysis of the methyl esters of the acids was confirmed by their mass spectrum.

The combined α,α'- and α,β - isomers of the DGS were isolated after rechromatography on a column by the PTLC method. The assignment of the fraction to the class of DGS was made from the results of analytical TLC (ATLC) and IR and mass spectra [3]. According to the results of ATLC, the α,α'- isomer predominated in the combined DG isomers.

The MG fraction was freed from phospholipids by rechromatography on a column and was identified from the results of ATLC.

The compositions of the fatty acids of the natural FAMES (GLC), the SFA fraction, and the acids isolated from the TGS, DGS, and MGS are given below (%)

Acid	FAME's	TG's	FFA's	DG's	MG's
12:0	1	—	—	0,3	0,9
Iso- 13:0	1	—	—	—	—
14:0	1,1	—	—	0,5	—
15:0	0,6	—	—	—	—
16:0	22,3	6,3	3,0	6,9	9,8
Iso- 17:0	0,9	Tr.	0,3	1,0	2,6
18:0	12,4	0,7	0,5	2 4	3,2
18:1	23,9	11,0	4,2	11,8	14,3
18:2	20,6	24,5	25,6	19,4	22,5
18:3	16,2	57,5	66,4	57,7	46,7

The oil was enriched in linolenic acid (TG, 18:3 57%). The greatest amount of this acid was present in the FFA fraction (18:3, 66%). The DG fraction, unlike the TG fraction, also showed the presence of such acids as lauric and myristic. The relative amount of saturated acids in the DG fraction was somewhat higher and in the MG fraction much higher than in the TGS. A comparison of the composition of the acids of the TGS and DGS also confirmed the predominance of the α,α'- isomer in the total DGS.

Attention is attracted by the enrichment of the natural FAMES with saturated acids (39.3%), among which myristic, lauric, and others were identified. The iso structures of the 17:0 and 13:0 acids identified by GLC was confirmed by the $(M^+ - 15)$ ions in the mass spectrum of the fraction.

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According to the literature, the oil of *N. cataria* contains laballenic acid [2]. There was no band of an allene group in the IR spectrum of the TGS of the oil that we investigated. Analytical TLC with the addition of 30% of $AgNO_3$ (in benzene) of the methyl esters of the acids of the TGS showed the presence of unsaturated acids, which were identified by GLC, and the absence of laballenic acid [4].

The glyceride composition of the TGS was determined from the results of enzymatic hydrolysis and on the basis of the fatty-acid compositions of the TGS (see below) and the β-MGS having the composition: 16:0, 1.4%; 18:1, 21.8%; 18:2, 47.7%; and 18:3, 29.1% (GLC). According to these figures, the position-type composition of the TGS is determined by such types as (%): G1SSU, 0.2; G1SUS, 0.9; G1USU, 1.1; G1SUU, 18.6; $G1U_3$, 79.2.

The position-type composition of the TGS of the seed oil of *N. cataria* was as follows (mole %: P, 16:0; O, 18:1; L, 18:2; Le, 18:3):

Glycerides with P in the β position	Content	Glycerides with O in the β position	Content	Glycerides with L in the β position	Content	Glycerides with Le in the β position	Content
PPLe	0.2	OOO	0.1	LLL	0.8	LeLeLe	14.9
OPLe	0.2	POO	0.2	PLO	0.6	PLeO	0.2
LPLe	0.2	POL	0.6	PLL	1.2	PLeL	0.8
LePLe	0.7	POLe	3.0	PLLe	6.6	PLeLe	4.0
		SOLe	0.2	SLLe	0.2	SLeLe	0.4
		POP	0.2	SLLe	0.6	OLeO	0.1
		OOL	0.4	OLO	0.1	OLeL	0.4
		OOLe	1.6	OLL	0.6	OLeLe	2.2
		LOL	0.4	OLLe	3.6	LLeL	0.5
		LOLe	4.0	PLP	0.4	LLeLe	5.2
		LeOLe	11.2	LLL	8.6	PLeP	0.3
				LeLLe	24.5		

Of the 60 possible types of TGS, 22 are realized in the oil in amounts of less than 0.1% and therefore are not given, while 12 types amount to 0.1-0.2%. Thus, the position-type composition of the TGS is determined by 26 types, the main ones being LeLLe (24.5%), LeLeLe (14.9%), LeOLe (11.2%), and LLLe (8.6%). Consequently, the predominating most unsaturated 18:3 acid is distributed in the TGS mainly in the α,α' positions. Those types of TGS predominate in which the biologically important 18:2 acid is localized in the β position in spite of its smaller amount relative to the 18:3 acid.

Di-2-ethylhexyl phthalate was identified by TLC and NMR and mass spectroscopy [5]. The free sterols after recrystallization from methanol had mp 127-128°C, M^+ 414 [6], which corresponds to β-sitosterol.

Thus, by comparing our results on the fatty-acid composition of the seed oil of *N. cataria* with literature information it is possible to conclude that these compositions are qualitative and quantitatively close, but the Central Asian species contains 6.5% more linolenic acid and contains no laballenic acid, which is present in the oil of the American species in an amount of 1.2%. In the species that we investigated we found an iso-17:0 acid which has been identified previously in trace amounts in the seed oil of *Salvia nilotica*, belonging to the same family [7].

Unusual is the presence in the oil of natural FAMES, this being the first time they have been isolated from the seed oils of higher plants. Natural methyl and ethyl esters of fatty acids have been detected in lipid extracts of lower fungi [8, 9], animal and human livers, maize pollen, insects, the surface waxes of higher plants [9], fresh-water algae [10], and photosynthetic bacteria [11]. Their biological role in the organism is not clear, but it is assumed that these compounds can participate in a mechanism of cell detoxication and in trace amounts increase auxin and gibberellin activity [12].

EXPERIMENTAL

The conditions of taking the IR, NMR, and mass spectra were similar to those described previously [4, 5]. Gas–liquid chromatography was carried out on a Khrom-4 chromatograph with a flame-ionization detector and a stainless-steel column filled with Chromaton N-AW-DMCS bearing 15% of Reoplex 400 (Chemapol). The column was 2.5 m long and the temperature 205°C. The rate of flow of carrier gas (helium) was 62 ml/min, of H_2 60 ml/min, and of air 60 ml/min. Acetone was used as the solvent for the samples.

The basic methods of isolation, separation, and purification of the oil, the gylceride fractions, and the fatty acids have been described elsewhere [3].

The oil (26 g) was separated by eluents [3] on a column of silica gel (Woelm), which gave the combined hydrocarbons, the sterol esters and the FAMES (2.2% of the weight of the oil), the pure TGS (85%), the sum of the di-2-ethylhexyl phthalate, SFAS, sterols, and DGS (12.5%), and the MGS contaminated with phospholipids (0.3%).

The FAMES were freed from sterol esters by crystallization from chloroform–ethanol (1:1) at −5°C with subsequent preparative TLC on silica gel L 5/40 (Chemapol) with 10% of gypsum in system 1 [hexane–diethyl ether–acetic acid (90:10:1)]. The free sterols were crystallized three times from absolute methanol at −5°C.

The conditions of enzymatic hydrolysis are given in [3].

SUMMARY

In a study of the glyceride and fatty-acid compositions of the neutral lipids of *N. cataria* seeds the presence of di- and monoglycerides in the lipids has been established. It has been shown that the predominating acid in all the glycerides is the 18:3 acid and the main types of triglycerides are those in which the β position is esterified with the 18:2 acid and, to a smaller extent, the 18:3 acid.

Natural fatty acid methyl esters have been isolated from a seed oil of a higher plant for the first time.

LITERATURE CITED

1. Flora of the Uzbek SSR [in Russian], Vol. V, Tashkent, (1961), p. 263.
2. J. M. Hagemann, F. R. Earle, I. A. Wolff, and A. S. Barclay, Lipids, 5, 371 (1971).
3. T. V. Panekina, S. D. Gizakova, M. Ya. Tabak, and A. U. Umarov, Khim. Prirodn. Soedin., 44 (1978).
4. S. D. Gusakova and A. U. Umarova, Khim. Prirodn. Soedin., 324 (1975).
5. S. D. Gusadova and A. U. Umarova, Khim. Prirodn. Soedin., 511 (1975).
6. A. Z. Abyshev, P. P. Denisenko, D. Z. Abyshev, and Yu. B. Kerimov, Farmatsiya, No. 2, 42 (1977).
7. M. B. Bohannon and R. Kleiman, Lipids, 10, 703 (1975).
8. N. N. Stepanichenko, S. D. Gusakova, A. A. Tyshchenko, S. Z. Mukhamedzhanov, A. U. Umarov, and O. S. Otroshchenko, Khim. Prirodn. Soedin., 431 (1976).
9. I. M. Chu, M. A. Wheeler, and C. E. Holmlund, Biochim. Biophys. Acta, 270, 18 (1972).
10. V. M. Khasanova, S. D. Gusakova, and T. T. Taubaev, Khim. Prirodn. Soedin., 49 (1978).
11. B. Maudinar and J. J. Villoutreix, Phytochemistry, 16, 1299 (1977).
12. J. L. Laseter and J. D. Weete, Science, 172, 864 (1971).