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LIPIDS OF *Origanum tytthanthum*

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The composition of lipids and fatty acids from the above-ground part of Origanum tytthanthum *Gontsch. (Lamiaceae) is studied. Lipids from the air-dried plant material are enriched in glycolipids, essential fatty acids, and biologically active lipophilic substances. The free fatty acids include 15-methylhexadecanoic acid (0.5% by GLC).*

Key words: *Origanum tytthanthum*, lipids, 15-methylhexadecanoic acid, pigments, fatty alcohols.

Origanum tytthanthum Gontsch. (small-flowered oregano, Lamiaceae) is endemic to Middle Asia and southern Kazakhstan. It is used as a spice and for the preparation of beverages in the fresh and dried state. The plant material is used in folk medicine as a gastric agent [1]. The composition of fatty acids and triacylglycerides in the seeds [2] and of essential oil in the above-ground part of this plant [3] have been reported.

We studied the lipids of the air-dried (8.0% moisture) above-ground part of *O. tytthanthum.*

The total lipids were extracted by a $CHCl_3$—CH_3OH mixture and separated into classes by column chromatography (CC) [4]. Components of the essential oil and pigments, which undergo definite changes during CC, accompanied the individual lipid classes. Therefore, the essential oil was extracted from the ground material by steam distillation [6]; the pigments, by acetone [5]. The composition and content of pigments were found using IR spectra and TLC. The results are listed in Table 1.

Pigments consisted of carotenes, xanthophylls, chlorophylls, and pheophytins. Chlorophyll *a* dominated. Carotene was identified as the β-isomer.

Glycolipids (GL, 48.8% with added altered chlorophylls) were predominant in the total lipids. The components in the GL were identified by TLC and placed in the order: monogalactosyldiacylglycerines > digalactosyldiacylglycerines > sterylglycosides > sterylglycoside esters.

The phospholipid (PL) content is 1.8%. The PL consisted of phosphatidylsterols, phosphatidylethanolamines, phosphatidylinositols, phosphatidic acids, and traces of N-acylphospholipids. It is noteworthy that phosphatidylcholines were not observed. These are essential PL components of higher plant cells [7]. The absence of phosphatidylcholines in the studied material can be explained by their lability and the potential enzymatic destruction of them [4, 7].

Fatty acids that were obtained from the total lipids, GL, PL, and fatty-acid esters of higher fatty and cyclic alcohols (FAE) in addition to free fatty acids (FFA) were separated by preparative TLC from the total lipids and converted to the methyl esters. The fatty-acid composition of GL, PL, and FAE was analyzed by GLC; of FFA and acids of total lipids, by GC/MS (Table 2).

The total lipids contain practically equal amounts of unsaturated (50.9%) and saturated acids (49.1%). The principal unsaturated acids were identified as 9Z-18:1, 9Z,12Z-18:2, and 9Z,12Z,15Z-18:3. They were concentrated mainly in the GL acids (65.4%). Furthermore, 9Z-hexadecenoic acid (9Z-16:1) was observed in the GL, PL, and FFA; 3E-hexadecenoic acid (3E-16:1), in the PL [8]. The last is known to be usually localized in phosphatidylglycerines of higher plants [7].

The FFA contained an octadecadienoic acid that has an equivalent chain length (ECL) of 18.72. It is an isomer of the known 9Z,12Z-18:2 acid with an ECL of 18.61 [8]. However, we did not locate the positions of the double bonds.

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TABLE 1. Essential Oils, Pigments, and Lipids in *O. tytthanthum*

Components	Content
Lipids, % of dry mass	4.4
Lipid composition, %:	
hydrocarbons and β-carotene	23.6
alkanol and sterol esters of fatty acids, triacylglycerines, and essential oils	9.7
free fatty acids	12.3
alkanols, native and altered pigments	3.0
sterols	0.8
glycolipids and altered pigment additive	48.8
phospholipids	1.8
Unsaponified lipid components, %	33.0
Pigments, mg/% of dry mass:	
β-carotene	1.3
xanthophylls	4.7
chlorophyll *a*	21.0
chlorophyll *b*	9.7
pheophytin *a* and *b*	3.2
Essential oil, ml% of dry mass	1.2

TABLE 2. Fatty-acid Composition of *O. tytthanthum* Lipids (%, GLC)

Acid	Total lipids	Esters of alcohols and sterols with fatty acids	Free fatty acids	Glycolipids	Phospholipids
9:0	0.1	-	0.1	-	-
10:0	0.1	-	Tr.	-	-
12:0	0.2	0.7	0.2	-	-
14:0	2.0	2.7	1.6	1.9	1.3
15:0	0.4	0.9	0.6	Tr.	Tr.
16:0	38.6	43.3	39.5	26.7	45.6
9Z-16:1	0.6	-	0.5	Tr.	4.3*
3E-16:1	0.8	-	-	-	-
iso-17:0	Tr.	-	0.5	-	-
17:0	0.4	0.9	0.6	-	
18:0	7.3	7.5	8.7	6.0	4.6
9Z-18:1	18.0	12.6	28.5	26.3	12.6
9Z, 12Z-18:2	12.7	8.6	12.2	17.7	14.9
18:2 (?, ?)	Tr.	-	0.4	-	-
9Z, 12Z, 15Z-18:3	18.8	2.2	6.1	21.4	16.7
20:0	Tr.	11.6	0.5	-	-
22:0	Tr.	9.0	Tr.	-	-
Σ_{sat}	49.1	76.6	52.3	34.6	51.5
Σ_{unsat}	50.9	23.4	47.7	65.4	48.5

*Total 9Z-16:1 and 3E-16:1 acids.

The dominant component of the saturated acids is palmitic acid. The acids 20:0 and 22:0 were observed mainly in the FFA and esters of fatty and cyclic alcohols. These acids were present mostly in the combined form.

The FFA contained 15-methylhexadecanoic acid (*iso*-17:0), which was identified in a GC/MS database and by ECL (16.64) [9]. This acid was previously observed in lipids of seeds from two representatives of the Lamiaceae family, *Nepeta cataria* and *Salvia nilotica* [10]. It was observed by us for the first time in lipids from the above-ground part of plants from the Lamiaceae family.

Lipophilic compounds of the extract, with the exception of chlorophylls and pheophytins, were concentrated in the unsaponified substances. They were obtained from the lipids by hydrolysis with strong base. The yield was greater than one quarter of the total lipid mass. The fraction of fatty alcohols and sterols isolated using CC was 28.7%. This value comprises the total content of both free and bound fatty alcohols and sterols that were liberated during hydrolysis of FAE, sterylglycosides, and sterylglycoside esters [4].

The alcohols observed by GLC were (% by GLC): $C_{14}H_{29}OH$ (0.4), $C_{16}H_{33}OH$ (0.2), $C_{18}H_{37}OH$ (0.1), $C_{20}H_{41}OH$ (0.3), $C_{22}H_{45}OH$ (2.0), $C_{24}H_{49}OH$ (0.1), $C_{26}H_{53}OH$ (91.7), $C_{28}H_{57}OH$ (1.0), $C_{30}H_{61}OH$ (1.6), β-sitosterol (1.0), and six unidentified components (total 1.6%). Ceryl alcohol ($C_{26}H_{53}OH$) was the main component of the alcohol fraction of *O. tytthanthum* lipids. Extracts that are enriched in higher alkanols, sterols, and esters of oils and pigments are known to exhibit biological activity [11]. The therapeutic effect of *O. tytthanthum* biomass may be due to the presence of these components.

Thus, the composition of the above-ground part of *O. tytthanthum* indicates that it has a high content of biologically active substances. These include glycolipids, linoleic and linolenoic acids (vitamin F), ceryl alcohol, β-carotene, chlorophyll *a* and *b*, and essential oil. Therefore, this plant, which is known to contain an essential oil, is interesting as a valuable source of medicinal substances.

EXPERIMENTAL

UV spectra of pigments were obtained on a Perkin—Elmer Lambda-16 spectrophotometer in acetone and hexane.

GC/MS of fatty-acid methyl esters were obtained using a Hewlett—Packard GCD and a capillary column (60 m × 0.25 mm) with a 0.25 μm coating of Innowax. The chromatography conditions were: 60°C for 1 min, programmed increase to 220°C at 4°C/min, isothermal for 10 min, programmed increase to 240°C at 1°C/min; sample-injection temperature 250°C.

Mass spectra were recorded at 70 eV in the range *m/z* 35-425. Methyl esters of fatty acids were identified using the Wiley GC/MS database and the ECL [7, 9].

GLC of fatty-acid methyl esters were run on a Chrom-4 (Czech Republic) chromatograph with a flame-ionization detector, a column (2.5 m × 4 mm) packed with Chromaton N-AW-DMCS with 15% Reoplex 400 at 198°C, and nitrogen carrier gas.

Alcohols were analyzed on a Hewlett—Packard GC-6890 chromatograph using a capillary column with HP-5 (30 m × 0.32 mm) at 265°C for 30 min followed by programmed increase to 280°C at 4°C/min, injector temperature 320°C, and helium carrier gas.

Silica gel 160/250 and the solvent mixtures hexane—ether (49:1, 9:1, 4:1, 1:1; neutral lipids), acetone (GL), and methanol (PL) were used for CC.

TLC was performed on silica gel 5/40 that was washed and activated at 110°C with 10% $CaSO_4$ (Czech Republic) and on Silufol UV-254 (Hungary) plates. The solvent systems were hexane—methylethylketone—acetic acid (43:7:0.1; neutral lipids), hexane—acetone—benzene—isopropanol (69.5:25:4:1.5; pigments), chloroform—acetone—methanol—acetic acid—water (65:20:10:10:3; GL), and chloroform—methanol—25% ammonia (13:5:1; PL). Spots were visualized with iodine vapor, 50% H_2SO_4 (neutral lipids), α-naphthol in 50% H_2SO_4 (GL), Vaskovsky reagent (PL), Dragendorff reagent (phosphatidylcholines), and ninhydrin (phosphatidylethanolamines) [4].

The above-ground part of *O. tytthanthum* Gontsch. was collected in the Chatkal mountains in Tashkent District in July, 1997.

The methods for isolating lipids, unsaponified substances, and fatty acids in addition to the methylation of acids with diazomethane have been described [4].

β-Carotene. R_f 0.97. UV spectrum (λ_{max}, nm): 425, 449, 476 (hexane) [12].

9Z-Hexadecenoic Acid Methyl Ester. ECL 16.23. GC/MS (*m/z*, %): 268 M^+ (6.0), 237 $[M - 31]^+$ (17.7), 236 $[M - 32]^+$ (20.6), 195 (5.0), 194 (19.1), 152 (20.6), 138 (11.4), 137 (11.4), 123 (18.4), 98 (43.3), 96 (48.2), 87 (54.5), 74 (81.6), 55 (100), 43 (44.7), 41 (68.1).

3E-Hexadecenoic Acid Methyl Ester. ECL 16.48. GC/MS (*m/z*, %): 268 M^+ (0.1), 237 $[M - 31]^+$ (0.1), 236 $[M - 32]^+$ (23.8), 194 (32.6), 152 (18.2), 137 (15.6), 123 (24.1), 98 (39.0), 96 (70.9), 87 (51.8), 74 (100), 55 (82.3), 43 (73.0), 41 (64.5).

Octadecadienoic Acid Methyl Ester. ECL 18.72. GC/MS (*m/z*, %): 294 M^+ (10.2), 263 $[M - 31]^+$ (7.4), 220 (2.6),

205 (0.8), 192 (1.3), 178 (4.8), 150 (11.3), 135 (11.3), 123 (14.4), 109 (28.4), 95 (57.3), 81 (88.4), 67 (100), 55 (68.4), 41 (55.0).

15-Methylhexadecanoic Acid Methyl Ester. ECL 16.64. GC/MS (*m/z*, %): 284 $[M]^+$ (11.1), 269 $[M - 15]^+$ (0.3), 253 $[M - 31]^+$ (0.3), 252 $[M - 32]^+$ (0.2), 241 (8.5), 199 (7.4), 185 (10.4), 164 (6.9), 143 (20.6), 111 (11.1), 87 (54.5), 74 (100), 57 (27.0), 55 (24.3), 43 (27.0).

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