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LIPIDS AND ESSENTIAL OIL OF *Origanum onites**

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Neutral lipids, glycolipids, phospholipids, pigments, and essential oil of the above-ground part of Origanum onites *L. (Lamiaceae) are isolated and characterized for the first time. The fatty-acid composition of the lipids and the components of the hydrocarbons, sterols, and essential oil are determined by GC/MS. Linolenic and palmitic acids dominate in the acids of the lipids. The main components of the hydrocarbons are nonacosane; of the sterols, β-sitosterol; of the pigments, chlorophylls a and b. A total of 54 components are identified in the essential oil.*

Key words: *Origanum onites*, lipids, fatty acids, sterols, hydrocarbons, essential oil.

"Oregano" is a general term applied to plants with a specific aroma arising from carvacrol in the essential oil. Turkey is the principal supplier of oregano. Species of *Origanum* (Lamiaceae) account for 90% of exports [1]. Of the 23 representatives, the species *O. onites*, *O. vulgare* subsp. *hirtum*, *O. majorana*, *O. minutiflorum* (endemic), and *O. syriacum* var. *bevanii* are exported in large quantities. They are widely used as an industrial raw material for producing essential oil, as tea, and as a culinary herb. Among them, *O. onites* is the most important economically. It is the principal cultivated plant with essential oil that is exported from Turkey. Green biomass of wild and cultivated *O. onites* is used [2, 3].

Data on the composition of essential and fatty oils of *O. vulgare* [4, 5], *O. majorana* [5, 6], and *O. tytthanthum* [7, 8] and the lipids of *O. dictamnus* [9] have been reported. The biological activity of the essential oil from *O. onites* has been studied [10-12]. The lipids from this species have not yet been investigated.

We investigated in detail lipids, pigments, and the essential oil of the above-ground part of *O. onites*. Data for their contents are given below:

Components	Content
Lipids, %:	4.68
neutral	2.44
glycolipids	2.11
phospholipids	0.13
unsaponified components	0.85
Pigments, mg/g	1.033
Essential oil, %	1.8

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TABLE 1. Fatty Acids of *O. onites* Lipids, % by GLC

Acids	Total lipids	Neutral lipids	Glycolipids	Phospholipids
12:0	Tr.	Tr.	Tr.	Tr.
14:0	0.1	3.4	0.6	Tr.
15:0	Tr.	Tr.	-	-
16:0	23.3	33.6	21.7	31.7
16:1(7Z)	Tr.	Tr.	-	0.2
16:1(9Z)	1.5*	1.0*	0.7	0.3
16:1(3E)	1.3	-	-	9.4
17:0	0.7	-	-	0.9
18:0	4.1	1.6	4.8	5.9
18:1	8.2	10.3	9.8	6.5
18:2	13.5	14.7	13.3	12.1
19:0	Tr.	-	-	0.1
18:3	44.5	35.4	49.1	30.5
20:0	2.2	-	-	1.1
22:0	0.6	-	-	1.3
Σ_{sat}	31.0	38.6	27.1	41.0
Σ_{unsat}	69.0	31.4	72.9	59.0

*Total 16:1(9Z) and 16:1(7Z) acids.

The yield in a $CHCl_3$—CH_3OH extract of *O. onites* was 7.2% of the dry weight. Purification from accompanying substances by column chromatography on molselect isolated total lipids as 65% of the extract. The lipids belonged to several classes: neutral lipids (NL), glycolipids (GL), phospholipids (PL), and unsaponified substances. The unsaponified substances represented 18% of the total lipids. Chromatography of the total lipids on a column packed with silica gel isolated NL (52.2%), GL (45.1%), and PL (2.7%). Components of the NL, GL, PL, and unsaponified substances were identified using specific qualitative reactions and mobilities in a thin layer of silica gel compared with those of standards.

The main components of the PL were phosphatidylethanolamines, phosphatidylglycerines, and phosphatidylinositols. The minor components were phosphatidylcholines, lyso-phosphatidylinositols, and phosphatidic acids. Impurities of two unidentified N-acylphospholipids were present.

The GL contained monogalactosyldiacylglycerines, digalactosyldiacylglycerines, sterylglycosides, and sterylglycoside esters of fatty acids. The monogalactosyldiacylglycerines were dominant.

The following NL were observed: hydrocarbons, esters of alkanols and sterols with fatty acids, free fatty acids (FFA), fatty alcohols, triterpenes, and sterols. Pigments and essential-oil components, which are lipophilic by nature, were concentrated in the NL. The principal components of the NL according to TLC were the FFA.

The fatty-acid content of the total lipids, NL, GL, and PL was found by GC/MS (Table 1). The dominant acids in both the total lipids and the separate groups from *O. onites* were linolenic (18:3) and palmitic (16:0). A large part of the fatty acids is unsaturated, principally the essential acids linoleic (18:2) and linolenic. The content of the 18:3 acid was largest in the GL; the smallest, in the PL. The fraction of the 18:2 acid in the acyl groups of the NL, GL, and PL was almost identical whereas the fraction of oleinic (18:1) was 1.5 times greater in the NL and GL compared with the PL.

Three homologs of hexadecenoic acid were identified. The acyl groups of all lipid classes contained (Z)-9-hexadecenoic [palmitoleinic, 16:1(9Z)] and (Z)-7-hexadecenoic [16:1(7Z)]. The PL acids included (E)-3-hexadecenoic [16:1(3E)]. The last acid is usually specifically found in the acyl groups of phosphatidylglycerines [13].

Even homologs consisted mainly of unsaturated acids 12:0-22:0. Minor components were acids with an uneven number of C atoms such as pentadecanoic acid (15:0), which was observed only in NL, and margarinic (17:0) and nonadecanoic acid (19:0), which were present in PL. It is noteworthy that the spectrum of saturated acids was more varied in the PL than in the NL and GL.

The unsaponified components of the lipids were obtained from the total lipids by hydrolysis with strong base. They included hydrocarbons, fatty alcohols, triterpenes, sterols, components of the essential oil, carotene, and an unidentified orange pigment from the xanthophyll group. The carotenoid content (total carotene and xanthophyll counted as β-carotene) in the unsaponified substances was 320 mg%. Column chromatography of the unsaponified lipid components isolated homogeneous fractions of carotenes, hydrocarbons, and sterols.

Eight components consisting mainly of uneven homologs $C_{23-33}H_{48-68}$ were observed in the hydrocarbons by GC/MS:

Components	Empirical formula	Content, mg/g dry wt.
Tricosane	$C_{23}H_{48}$	1.0
Pentacosane	$C_{25}H_{52}$	1.9
Hexacosane	$C_{26}H_{54}$	0.8
Heptacosane	$C_{27}H_{56}$	11.7
Octacosane	$C_{28}H_{58}$	4.1
Nonacosane	$C_{29}H_{60}$	55.1
Hentriacontane	$C_{31}H_{64}$	25.4
Tritriacontane	$C_{33}H_{68}$	Tr.

The hydrocarbons consisted mainly (>92%) of nonacosane, heptacosane, and hentriacontane. The sterol composition (% by GLC), in which the fraction of β-sitosterol is high, of *O. onites* is given below:

Components	Content
β-Sitosterol	83.2
Campesterol	7.4
Stigmasterol	7.1
Cholesterol	2.3

β-Carotene was identified in the carotene fraction by UV spectroscopy. The total pigments that were isolated from the above-ground part of *O. onites* were studied by UV spectroscopy and TLC. Chlorophylls, pheophytins, and carotenoids with predominantly blue-green *a* and yellow-green *b* chlorophylls were found:

Components	Content, mg/g dry wt.
Chlorophyll *a*	0.520
Chlorophyll *b*	0.220
Pheophytin *a*	0.003
Pheophytin *b*	0.140
Carotenoids	0.150

The physicochemical properties and composition of the essential oil of *O. onites* were determined. The specific gravity d^{25} is 0.92; index of refraction n_D^{25} 1.515; rotation angle $[\alpha]_D^{25}$ -0.2531°. According to GC/MS, 98% of the essential oil consists of 54 compounds (Table 2), among which carvacrol (71.2%) dominates. This is the component that is characteristic of the essential oil of "oregano".

Thus, lipids from the above-ground part of *O. onites* are studied for the first time. The data indicate that lipids from *O. onites* are qualitatively similar to those from the related species *O. dictamnus* that were studied earlier [9]. However, each species has a unique content of lipid components and fatty acids. *O. onites* contains biologically active lipidlike substances [14]. The lipophilic extract from the air-dried biomass is enriched in essential fatty acids (vitamin F), β-carotene (provitamin A), β-sitosterol, chlorophylls *a* and *b*, and essential-oil components. Therefore, *O. onites* is valuable as a source of essential oil and biologically active lipids and lipophilic substances. The lipophilic extract can be obtained from various wastes from essential-oil production.

TABLE 2. *O. onites* Essential Oil Composition

Component	Content, %	Component	Content, %
α-Pinene	0.11	*trans*-Pinocarveol	0.07
α-Thujene	0.03	α-Humulene	0.06
Camphene	0.05	α-Terpineol	0.32
β-Pinene	0.11	Borneol	1.69
δ-3-Carene	0.02	Carvenone	0.28
Myrcene	0.58	β-Bisabolene	0.70
α-Terpinene	0.55	Piperitone oxide I (*cis*-piperitone oxide)	0.31
Limonene	0.15	Carvone	0.30
1,8-Cineol	0.13	δ-Cadinene	0.06
β-Phellandrene	0.10	Mirtenol	0.06
γ-Terpinene	2.20	*trans*-Carveol	0.07
p-Cymene	4.18	*p*-Cymen-8-ol	0.19
Terpinolene	0.12	4-Isopropylsalicylaldehyde	0.23
3-Octanol	0.06	Piperitenone	0.83
α,*p*-Dimethylstyrene	0.03	Caryophyllene oxide	0.77
1-Octen-3-ol	0.37	Salvial-4(14)-en-1-one	0.04
trans-Sabinene hydrate	0.14	Globulol	0.03
α-Campholene aldehyde	0.04	Cuminic alcohol	0.10
Camphor	0.02	Spatulenol	0.47
Linalool	1.74	*cis-p*-Mentha-4-en-1,2-diol	0.23
cis-Sabinene hydrate	0.14	Isothymol (2-isopropyl-4-methylphenol)	0.08
trans-p-Menth-3-en-1-ol	0.10	Eugenol	0.06
Terpinen-4-ol	1.57	Thymol	5.97
β-Caryophyllene	0.85	Isocarvacrol (4-isopropyl-2-methylphenol)	0.06
cis-Dihydrocarvone	0.04	Carvacrol	71.22
Aromadendrene	0.09	Caryophylladienol II [caryophylla-2(12),6(13)-dien-5-α-ol]	0.06
cis-p-Menth-2-en-1-ol	0.07	Caryophyllenol II [caryophylla-2(12),6-dien-5-β-ol]	0.15

EXPERIMENTAL

UV spectra of pigments were recorded on a Perkin—Elmer Lambda-16 UV/VIS spectrometer.

GC/MS of fatty-acid methyl esters, hydrocarbons, and essential oil was performed in a Hewlett—Packard (USA) chromato-mass-spectrometer using a capillary column packed with Innowax (60 m × 0.25 mm, layer thickness 0.25 μm). The separation conditions were: initial temperature 60°C, isothermal for 10 min, programmed increase to 220°C at 4°C/min, isothermal for 10 min, and heating to 240°C at 1°C/min; injector temperature 250°C; carrier gas He; flow ratio 50:1.

Sterols were analyzed on a Hewlett—Packard 6890 GC/MS in a column (30 m × 0.32 mm) packed with HP-5 (layer thickness 0.25 μm); initial temperature 265°C for 30 min with subsequent increase to 280°C at 4°C/min and isothermal for 25 min; injector temperature 320°C; flow ratio 60:1.

Mass spectra of fatty-acid methyl esters, hydrocarbons, sterols, and essential oil were measured under the following conditions: 70 eV ionization potential; detection limits 35-425 *m/z*. Mass spectra of compounds were compared with standards from the Wiley GC/MS Library and the TBAM database of chromato-mass-spectra of essential oils.

Column chromatography of the $CHCl_3$—CH_3OH extract was performed on molselect G-25 Dextrantel (Hungary). Lipids were eluted by a $CHCl_3$—CH_3OH—H_2O mixture (90:10:1). Unsaponified components of lipids were separated on silica gel L 100/160 (Czech Republic). Hydrocarbons were eluted by hexane; β-carotene and sterols, by hexane—diethylether mixtures in the ratios 24:1 and 1:1, respectively.

TLC was carried out on Silufol UV-254 plates and silica gel 5/40 (Czech Republic) using C_6H_{14}—MEK—CH_3COOH

(43:7:0.1, NL), $CHCl_3$—$(CH_3)_2CO$—CH_3OH—CH_3CO_2H—H_2O (65:20:10:10:3, GL), $CHCl_3$—CH_3OH—NH_4OH (25%) (65:25:5, PL), and C_6H_{14}—$(CH_3)_2CO$—C_6H_6—$(CH_3)_2CHOH$ (69.5:25:4:1.5, pigments).

The above-ground part of *O. onites* was collected in southwestern Turkey (Mugla). Lipids from the air-dried and ground plant (8% moisture) were extracted by $CHCl_3$—CH_3OH (2:1 by vol.) by the Folch method [15]. Literature methods were used to purify lipids of extraneous substances, to identify lipids by class, to isolate unsaponified substances, to obtain fatty acids, and to methylate them using diazomethane [15, 16]. Lipids were identified using specific reagents: sterols, as a pink spot by spraying with 50% H_2SO_4 and heating for 10-15 min at 100°C; GL, as blue spots with α-naphthol and 50% H_2SO_4 with ignition; PL, as blue spots with Vaskovsky reagent; phosphatidylcholines, as orange spots with Dragendorff reagent; phosphatidylethanolamines, as a reddish-violet spot with ninhydrin and heating to 80°C.

The content of pigments was determined by spectrophotometry [17, 18] using the following analytical wavelengths: 664 (chlorophyll *a*), 645 (chlorophyll *b*), 668 (pheophytin *a*), 656 (pheophytin *b*), and 440.5 and 443 nm (carotenoids).

β-Carotene. UV spectrum (hexane, λ_{max}, nm): 425, 449, 476 [19].

Essential oil from *O. onites* was obtained on a Clevenger apparatus by steam distillation of the dried and ground above-ground part of the plant. The physicochemical properties were determined according to the literature [20].

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