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Composition of the Essential Oil of *Mentha microphylla* from the Gennargentu Mountains (Sardinia, Italy)

PAOLO EMILIO TOMEI AND RITA ELISABETTA UNCINI MANGANELLI

Dipartimento di Agronomia e Gestione dell'Agroecosistema, Via S. Michele degli Scalzi 2, 56124 Pisa, Italy

GUIDO FLAMINI,* PIER LUIGI CIONI, AND IVANO MORELLI

Dipartimento di Chimica Bioorganica e Biofarmacia, Via Bonanno 33, 56126 Pisa, Italy

The essential oil obtained by hydrodistillation of the fresh aerial parts of *Mentha microphylla* C. Kock (Lamiaceae) collected on the Gennargentu Mountains (Sardinia, Italy) has been investigated by gas chromatography (GC) and GC/mass spectrometry (MS). The main constituents that resulted were pulegone (34.1%), piperitenone oxide (32.9%), and piperitenone (11.3%). The presence of small amounts of compounds such as ethyl hexanoate, 1-octen-3-ol, nonanal, and ethyl 2-methylbutanoate could justify the particular odorous profile of the plant, resembling the aroma of milk and other dairy products such as mozzarella.

KEYWORDS: ***Mentha microphylla* C. Kock; Lamiaceae; essential oil composition; dairy products aroma; pulegone; piperitenone oxide; piperitenone**

INTRODUCTION

Several *Mentha* oils are used as flavors, for example, in confectioneries, chewing gums, candies, and liqueurs. Also, these are utilized in medical supplies such as toothpaste, facial lotions, and gastrointestinal spasmolitics. The *Mentha* oils using flavors and medical supplies have an exceedingly small toxicity for the human body. Peppermint oil, one of the constituents of chewing gum, has shown antiallergic effects (*1*).

The essential oil of *Mentha microphylla* is active against insects attacking stored products, in particular against *Acantoscelides obtectus* (Say) (*2*), one of the most destructive pests of *Phaseolus vulgaris* (kidney bean), causing 20−40% loss of stored seeds per annum (*3*).

M. microphylla C. Kock, apparently an allotetraploid derived from *Mentha suaveolens* Ehrh. and *Mentha longifolia* (L.) Hudson, belongs to the group of *Mentha spicata s. l.* (*4*, *5*). Its identification is a very difficult task, because it is very similar to *M. suaveolens*; the main differences consist in the length and the shape of its leaves, from 5 to 20 mm, ovate to ovate-lanceolate, usually acute; furthermore, its verticillaster is not compact (*6*). This species is distributed in the Balcan Peninsula, Aegean region, and Italy (*7*); in Italy, this plant grows in the southern part of the peninsula, in Sicily and Sardinia (*4*).

The composition of the essential oil of *M. microphylla* has been already investigated. The first paper analyzed the oil by means of thin-layer chromatography and reported carvone, mentyl acetate, menthol, and menthone among the main constituents (*8*). More recently, Halim et al. (*9*) characterized the essential oil of this species identifying piperitone oxide and piperitenone oxide as principal compounds (which formed more than 65% of the whole oil); other minor constituents were 3-octanol, limonene, β-caryophyllene, and β-cubebene.

This paper deals with the analysis of the essential oil obtained from *M. microphylla* C. Kock collected on the Gennargentu Mountains (Sardinia, Italy). The plant that grows here emits a particular odor, similar to dairy products.

MATERIALS AND METHODS

The aerial parts of *M. microphylla* C. Kock (Lamiaceae) were collected during October 2001 in Sardinia (Italy), on the Gennargentu Mountains, where it grows in damp environments, on siliceous soil, having a south aspect.

About 100 g of flowering aerial parts, without the wooden parts, were submitted, within a few hours from the harvest, to hydrodistillation in a Clevenger type apparatus for 2 h. A voucher specimen is deposited in the Herbarium of Agriculture Faculty of Pisa (PI AGR no. 66 3252).

The gas chromatography (GC) analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures, 250 °C; carrier gas nitrogen (2 mL/min); detector dual flame ionization detection (FID); split ratio, 1:30; injection of 0.5 μL). The identification of the components was performed, for both of the columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. The relative proportions of the essential oil constituents were percentages obtained by FID peak area normalization. The analyses were performed in triplicate.

* To whom correspondence should be addressed. Tel: +3905044074. Fax: +3905043321. E-mail: flamini@farm.unipi.it.

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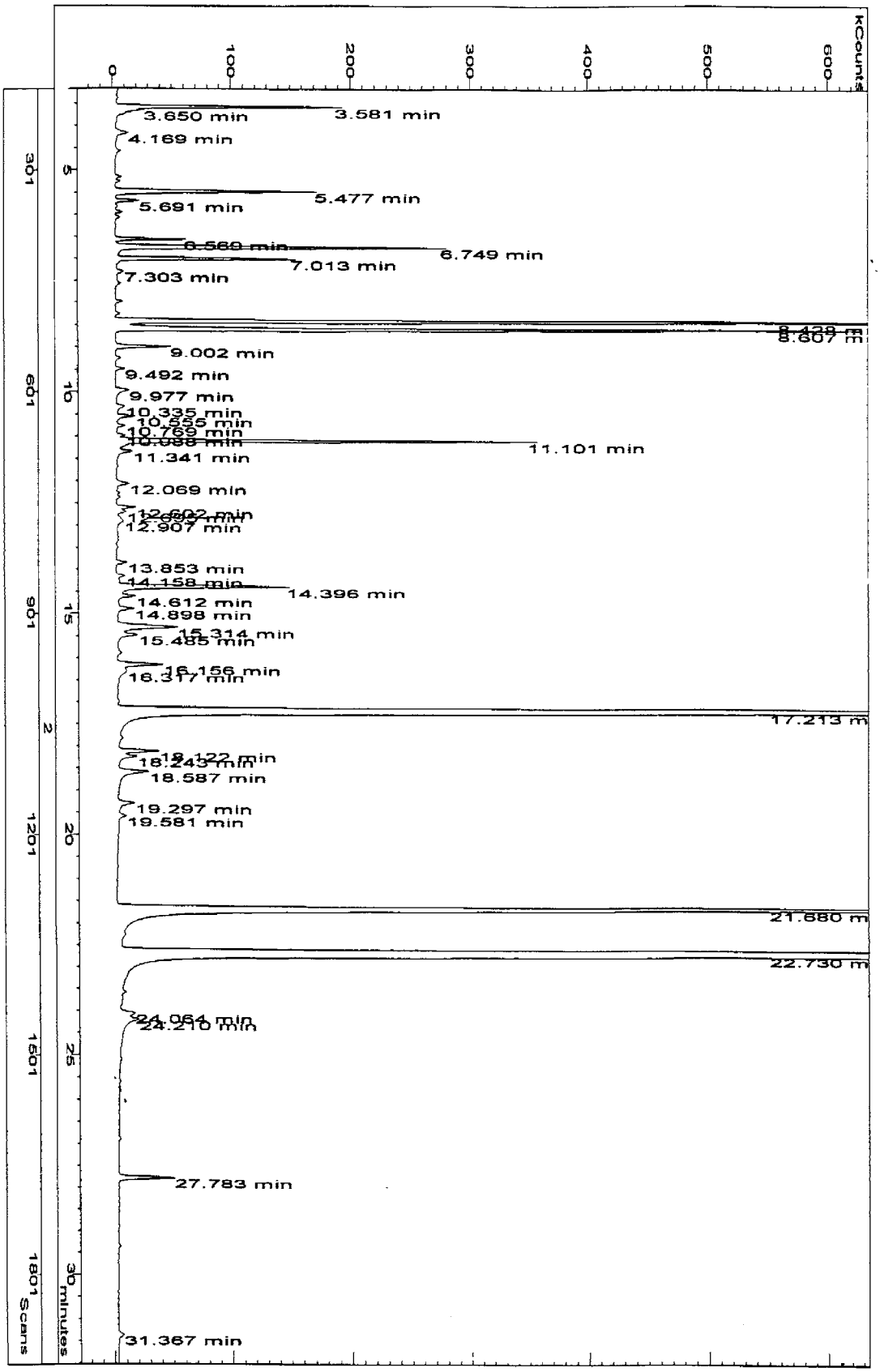

Figure 1. TIC chromatogram of *M. microphylla* oil (HP-5 column).

GC/EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness, 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. The analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their LRIs relative to the series of *n*-hydrocarbons, and on computer matching

Table 1. Composition[a] of the Essential Oil of *M. microphylla*

constituents	LRI[b]	LRI[c]	%	identification[d]
ethyl 2-methylbutanoate	841	1050	1.0	S
(*E*)-2-hexenal	856	1220	0.1	S
ethyl 3-methylbutanoate	859	1065	0.2	S
ethyl pentanoate	901	1131	0.1	S
ethyl 3-methylbutenoate	927		0.1	T
α-pinene	940	1029	1.1	S
sabinene	978	1126	0.3	S
1-octen-3-ol	980		0.7	S
β-pinene	981	1112	1.0	S
myrcene	992	1170	0.7	S
ethyl hexanoate	999	1229	0.1	S
limonene	1033	1198	3.9	S
(*Z*)-ocimene	1041	1199	3.9	S
(*E*)-ocimene	1051	1242	0.3	S
terpinolene	1089	1284	0.1	S
ethyl heptanoate	1099	1372	0.1	S
linalool	1101	1547	2.7	S
nonanal	1103	1385	0.2	S
trans-pinocarveol	1140		0.1	T
menthofurane	1165		0.2	T
ethyl benzoate	1175	1648	0.1	S
4-terpineol	1182	1607	0.1	S
myrtenal	1196	1596	0.5	S
verbenone	1206	1715	0.1	S
pulegone	1239		34.1	S
piperitenone	1343		11.3	H,N
piperitenone oxide	1368		32.9	H,N
(*Z*)-jasmone	1394		0.3	S
germacrene D	1483	1722	0.5	H,N
total identified			96.8	

[a] Percentages obtained by FID peak area normalization. [b] LRIs (HP-5 column). [c] LRIs (HP-WAX column). [d] S, comparison with standard component; H, homemade library; N, ^{13}C and DEPT NMR analysis of previous oils; and T, tentative identification.

against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*3*−*8*). Moreover, the molecular weights of all of the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

RESULTS AND DISCUSSION

Twenty-nine compounds were identified, accounting for 96.8% of the whole essential oil. The composition is reported in **Table 1**; the yield was 0.21% (w/w), calculated on the fresh plant material.

The main components were as follows: pulegone (34.1%), piperitenone oxide (32.9%), and piperitenone (11.3%), which constituted about 78% of the whole oil. Other important compounds were (*Z*)-ocimene (3.9%), limonene (3.9%), and linalool (2.7%) (**Figure 1**).

Besides other oxygenated mono- and sesquiterpenes commonly found in many *Mentha* oils, some nonterpene alcohol and aldehydes, as well as ethyl esters of aliphatic and aromatic acids with 4−7 carbons, were identified. Among these latter compounds, ethyl hexanoate is one of the most odorous constituents of cow, sheep, and goat milks, while 1-octen-3-ol and nonanal are responsible for the aroma of buffalo milk and the mozzarella obtained from it; ethyl isobutanoate is reported among the aromatic compounds of the mozzarella prepared from cow milk (*10*−*12*). The identification of these compounds in the essential oil of this species confirms the particular odor, similar to dairy products, of the living plant. Each of these odorants was present in the oil in amounts never higher than 1%. However, the concentration of aroma compounds in dairy products, especially in fresh milk, is very low (*13*−*15*). Nevertheless, these chemicals are very powerful odorants as demonstrated by some researchers by Charm analysis (*16*, *17*). Furthermore, the odor thresholds reported for these compounds are 0.01−0.001 ppm for 1-octen 3-ol in water or 0.084 ppm in the air or 0.034 ppm in oil, 0.85 ppm for ethyl hexanoate in oil, and 0.001−0.0025 ppm for nonanal in water (*18*). To the best of our knowledge, these chemicals, with the exception of 1-octen-3-ol and nonanal (ubiquitous volatiles), were not previously reported for other mint oils.

Because of these particularities, the plant investigated could represent a local chemical race of *M. microphylla*. The different qualitative and quantitative chemical compositions of this essential oil with respect to previous investigations (*8*, *9*) could be related first and foremost to the different environmental conditions. Additionally, the differences could be attributable to the different degree of hybridization that the populations of *M. microphylla* could have faced between various chemotypes of *M. longifolia* and *M. suaveolens* (*19*−*22*). In particular, piperitone oxide, one of the main constituents of the oil of *M. microphylla* from Egypt (*9*), was not identified in our analyses. Notable also was the absence of carvone, menthol, and menthyl acetate reported for the plant growing in Saudi Arabia (*8*).

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