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A N I N T E R N A T I O N A L J O U R N A L

Essential Oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay

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ABSTRACT

Essential oils obtained by hydrodistillation from leaves of Mentha pulegium *L. and* Mentha rotundifolia *(L.) Huds. from Uruguay were analysed by GC-FID and GC-MS. Oxygen-containing monoterpenes were the main group of constituents in both oils. Pulegone, isomenthone and menthone were the major components in the oil of* M. pulegium*, whereas piperitenone oxide and (Z)-sabinene hydrate were the major ones in* M. rotundifolia*. Enantiomerically pure (-)-menthone, (+)-isomenthone, (+)-isomenthol, (-)-menthol and (+)-pulegone were detected by multidimensional gas chromatography in the case of* M. pulegium *oil.*

Key Words: *Mentha* essential oil; multidimensional chiral gas chromatography

INTRODUCTION

The genus *Mentha* (Lamiaceae) includes aromatic herbs of difficult taxonomic classification due to a great variability in their morphological characters and frequent hybridisation. Previous investigations of their essential oils have revealed the existence of an important chemical polymorphism (Lawrence, 1978) and several varieties and chemotypes have been described for *M. spicata* (Kokkini and Vokou, 1989; Misra et al., 1989; Pino et al., 1998), *Mentha longifolia* (Maffei, 1988; Venskutonis, 1996), *M.* suaveolens (Hendriks and Van Os, 1976) and *M. diemenica* (Brophy et al., 1996) among others. *M. pulegium* L., commonly known as pennyroyal, is traditionally used in the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties (Newall et al., 1996). Previous reports (Lawrence, 1998; Hefendehl, 1970; Pino et al., 1996) on the composition of its essential oil showed that pulegone was the main constituent, and its percentage ranged from 25 to 92%.

M. rotundifolia (L.) Huds. is an hybrid between *M. longifolia* (L.) L. and *M. suaveolens* Ehrh., whose essential oil has been the object of several studies (Nagell and Hefendehl, 1974; Kokkini and Papageorgiou, 1988; Hendriks et al., 1976; Umemoto, 1998), and different chemotypes have been characterised. Some authors have considered

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M. rotundifolia (L.) Huds. as a synonym of *M. suaveolens* Ehrh (Hendriks and Van Os, 1976).
The present paper deals on the chemical composition of the essential oils of *M. pulegium* and *M. rotundifolia* grown wildly in Uruguay as well as on the enantiomeric distribution of the major chiral constituents of its oil.

MATERIAL AND METHODS

***Plant Material and Isolation of Volatile Constituents*:** Flowering aerial parts of *M. pulegium* and *M. rotundifolia* were collected from the North of Montevideo (Uruguay) during the end of January 1997. Voucher specimens were included in the Herbarium of the Faculty of Agronomy of the University of the Republic (Montevideo, Uruguay) with the numbers MVFA 26337 and MVFA 26356, respectively.
Air-dried plant material was submitted to hydrodistillation in a Clevenger–type apparatus, affording essential oil yields of 1.93 and 1.02 % (v/w), respectively.

GC Analysis: The analysis of the oil was carried out by GC on a Shimadzu 14 B gas chromatograph equipped with a FID and a Shimadzu data processor software EZ-Chrom, using two capillary columns. The first was a SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column coated with 5% phenyl-polymethylsiloxane (30 m x 0.32 mm i.d. x 0.40-0.45 μm film thickness); column temperature, 60ºC (8 min) to 180ºC at 3ºC/min, 180-250ºC at 20ºC/min, 250ºC (10 min). Injector temperature 250ºC; detector temperature 280ºC; injection mode, split; split ratio 1:30; volume injected, 0.2 μL of the oil. Carrier gas was Hydrogen, 55 kPa. The second was a Carbowax 20M (Ohio Valley, USA) bonded fused-silica capillary column coated with polyethylene glycol (25 m x 0.32 mm i.d. x 0.25 μm film thickness); column temperature, 40ºC (8 min) to 180ºC at 3ºC/min, to 230ºC at 20ºC/min. Injector temperature 250ºC; detector temperature 250ºC; injection mode, split; split ratio 1:30; volume injected, 0.2 μL of the oil. Carrier gas was Hydrogen, 30 kPa.

GC/MS Analysis: GC/MS analysis was conducted using a Shimadzu QP 5050 equipped with reference libraries (Adams, 1995; McLafferty and Stauffer, 1991) using two capillary columns. The first was a SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column coated with 5% phenyl-polymethylsiloxane (25 m x 0.25 mm i.d. x 0.25 μm film thickness); column temperature, 60ºC (8 min) to 180ºC at 3ºC/min, to 230ºC at 20ºC/min. Injector temperature 250ºC; injection mode, split; split ratio 1:40; volume injected, 0.2 μL of the oil. Helium was used as a carrier, using 122.2 kPa (51.6 cm/sec); interface temperature 250ºC; acquisition mass range 40-400. The second was a BP 20 (SGE, Australia) bonded fused-silica capillary column coated with polyethylene glycol (25 m x 0.25 mm i.d. x 0.25 μm film thickness); column temperature, 40ºC (8 min) to 180ºC at 3ºC/min, to 230ºC at 20ºC/min. Injector temperature 250ºC; injection mode, split; split ratio 1:40; volume injected, 0.2 μL of the oil. Carrier gas was He, 92.6 kPa (55.9 cm/sec); interface temperature 250ºC; acquisition mass range 40-400.

Identification and quantification: The components of the essential oil were identified by comparison of their Linear Retention Indices on the two columns, determined in relation to a homologous series of n-alkanes, with those from pure standards or reported in literature. Comparison of fragmentation patterns in the mass spectra with those stored on databases (Adams, 1995; McLafferty and Stauffer, 1991) was also performed. The quantification of the components was performed on the basis of their GC peak areas on SE-52, except those indicated in Tables 1 and 2, which were obtained on Carbowax 20M, without corrections for response factors.

Chiral Analysis: For *M. pulegium* essential oil, the enantiomeric ratios of menthone, isomenthone, isomenthol, menthol and pulegone were obtained by multidimensional gas chromatography, using a developmental model Mondello et al., 1998) set up with two GC ovens. The first was equipped with a column coated with SE-52 and the second with a chiral column coated with a derivatized β-cyclodextrin, a hot interface, a rotary switching valve and a system to maintain a constant flow during the transfer. With this system a heart-cut of the relevant fractions can be made and these fractions transferred from the non-chiral column to the chiral one in the following experimental conditions: precolumn, SE-52 (Mega, Legnano,

Italy) cross-linked fused-silica capillary column coated with 5% phenyl-polymethylsiloxane (30 m x 0.32 mm i.d. x 0.40-0.45 μm film thickness); column temperature; 45ºC (6 min) to 280ºC at 2ºC/min; 280ºC (15 min); analytical columns, fused-silica capillary columns coated with 2,3-di-*O*-ethyl 6-*O*-*t*-butyldimethylsilil-β-cyclodextrin (2,3-DiEtTBS-β-CDX) in PS 086 (13 % phenylmethyl-polysiloxane) (Mega, Legnano, Italy) (25 m x 0.25 mm i.d. x 0.25 μm film thickness), and 2,3-di-*O*-acetyl 6-*O*-*t*-butyldimethylsilil-β-cyclodextrin (2,3-DiAcTBS-β-CDX) in OV 1701 (14 % cyanopropyl phenyl-methylpolysiloxane) Mega, Legnano, Italy) (25 m x 0.25 mm i.d. x 0.25 μm film thickness); injection temperature, 250ºC; column temperature, 45ºC (6 min) to 90ºC at 2ºC/min, 90ºC (20 min); 90ºC to 180ºC at 2ºC/min, 180ºC (10 min); interface temperature, 200ºC; detector FID, 280ºC (for both chromatographs). Volume injected, 1 μL of an oil dilution 1:10 in n-hexane; injection mode, split; split ratio 1:15. Carrier gas was Helium, 90 kPa (precolumn) , 110 kPa (analytical column).

Table 1 - Composition of the essential oil of *M. pulegium* L

		LRI***	
Constituents*	% **	SE-52	CW-20M
α-Pinene	0.5	928	1037
Camphene	tr	941	1012
Sabinene	0.1	965	1083
β-Pinene	0.4	968	1083
Myrcene	0.3	986	1124
3-Octanone	tr	978	
3-Octanol	1.5	994	1380
Limonene	0.9	1021	1155
1,8-Cineole	0.1	1021	1186
(1R,4S)-(-)-Menthone	3.6	1146	1461
(1R,4R)-(+)-Isomenthone	12.9	1159	1420
neo-Menthol	0.3	1161	1599
Isopulegone	1.4	1167	1533
(1R,3R,4S)-(-)-Menthol	0.6	1178	1599
(1R,3S,4R)-(+)-Isomenthol	0.1		1686
neo-Isomenthol	0.8	1180	1622
α-Terpineol	0.1	1189	1652
(1R)-(+)-Pulegone	73.4	1241	1637
Piperitone	0.1	1247	1697
Piperitenone	0.9	1329	1851
(E)-Caryophyllene	0.1	1410	1563
α-Humulene	0.9	1445	1642
Caryophyllene oxide	0.3	1572	
Monoterpene hydrocarbons	2.2		
Oxygen-containing monoterpenes	94.3		
Sesquiterpene hydrocarbons	1.0		
Oxygen-containing sesquiterpenes	0.3		
Others	1.5		
Total identified (%)	99.3		

* Components are reported according to their elution order on SE-52.

** Percentages were obtained on SE-52, except for limonene, 1,8-cineole and isomenthol, which were obtained on Carbowax 20M.

*** LRI: Linear Retention Indices in relation to n-alkanes.

RESULTS AND DISCUSSION

The results obtained in the analysis of the essential oils of *M. pulegium* and *M. rotundifolia* are shown in Tables 1 and 2, respectively. Twenty two different components were identified in the essential oil of *M. pulegium*, meaning 99.3% of the total sample (Table 1). Oxygenated monoterpenes (94.3%) were found to be the major group of

constituents, the main one being pulegone (73.4%) followed by isomenthone (12.9%). The enantiomeric ratio of the five main components (menthone, isomenthone, isomenthol, menthol and pulegone) was established for *M. pulegium* oil by subsequent transfers during different analysis using two chiral stationary phases. Under the experimental conditions, menthol was best resolved on 2,3-DiAcTBS-β-CDX, while menthone, pulegone, isomenthol and isomenthone were best resolved on 2,3-DiEtTBS-β-CDX. To improve the separation on the second chiral phase (2,3-DiEtTBS-β-CDX) menthone and pulegone were analysed in one chromatographic run and isomenthol and isomenthone in a second one.

Table 2 - Composition of the essential oil of *M. rotundifolia* (L.) Huds.

		LRI***	
Constituents*	% **	SE-52	CW-20M
α-Pinene	0.5	927	1000
Sabinene	0.7	962	1076
β-Pinene	0.7	970	1065
1-Octen-3-ol	0.9	977	1406
Myrcene	0.8	989	1120
α-Terpinene	0.1		1138
p-Cymene	0.7	1024	1208
Limonene	0.8	1024	1155
β-Phellandrene	tr		1163
1,8-Cineole	0.1		1168
(Z)-b-Ocimene	0.8	1040	1182
(E)-b-Ocimene	0.1	1048	1200
(Z)-Sabinene hydrate	2.0	1068	
Octen-3-yl acetate	0.2	1115	1323
4-Terpineol	1.5	1177	1551
α-Terpineol	0.2		1655
Piperitenone oxide	80.8	1386	1940
β-Longipinene	0.2	1410	
(E)-Caryophyllene	0.4	1415	1516
β-Farnesene	0.8	1457	1583
Germacrene D	0.6	1479	1613
(E)-Nerolidol	0.1	1572	
Globulol	0.4	1590	
Monoterpene hydrocarbons	5.3		
Oxygen-containing monoterpenes	84.6		
Sesquiterpene hydrocarbons	2.0		
Oxygen-containing sesquiterpenes	0.5		
Others	1.1		
Total identified (%)	93.5		

* Components listed according to their elution order on SE-52.

**Percentages were obtained on SE-52, except for α-terpinene, limonene, ß-phellandrene, 1,8-cineole and α-terpineol, which were obtained on Carbowax 20M.

*** LRI: Linear Retention Indices in relation to n-alkanes.

Enantiomerically pure (1*R*,4*S*)-(-)-menthone, (1*R*,4*R*)-(+)-isomenthone, (1*R*,3*S*,4*R*)-(+)-isomenthol, (1*R*,3*R*,4*S*)-(-)-menthol and (1*R*)-(+)-pulegone were detected in the essential oil of *M. pulegium* (Table 1). This result agrees with that biosynthetically expected, since (-)-menthone and (+)-isomenthone are the precursors of (-)-menthol and (+)-isomenthol, respectively. All five chiral monoterpenoids occurred as pure (1*R*)-configurated enantiomers, resulting in an useful tool for the identification and genuineness of this oil, particularly in the case of the optically pure

(+)-pulegone, where previous results (Ravid, 1998) indicated lower values for *M. pulegium* oils.

In conclusion, the basic composition of the oil of *M. pulegium* from Uruguay was similar to that reported previously (Bigo and Moyna, 1985), although higher percentages of neo-menthol and pulegone and lower ones for isomenthone were found in the sample investigated. As (+)-pulegone is the precursor of (-)-menthone and (+)-isomenthone in the biosynthesis of monoterpenes in the genus *Mentha*, the results could indicate possible variations in the biosynthetic behaviour for the population studied. Furthermore, the role of (+)-pulegone as a chiral starting material for enantioselective synthesis of natural products, supports the potential application of the essential oil from the selected population of *Mentha pulegium* from Uruguay as a source of enantiomerically pure (+)-pulegone.

The analysis of the oil of *M. rotundifolia* allowed the identification of 23 components, which corresponded to a 93.5% of the total (Table 2). The major constituent was piperitenone oxide (80.8%), an oxigenated monoterpene which has been reported to characterise the volatile oil of some chemotypes of *Mentha* sp., such as *M. spicata* (Pino et al., 1998), *M. longifolia* (Venskutonis, 1996), *M. x villosa* (Abreu Matos et al., 1999) and *M. rotundifolia* (Nagell and Hefendehl, 1974). Previous studies on the essential oil of *M. rotundifolia* revealed the existence of chemotypes with different major components, as for example piperitone oxide (Kokkini and Papageorgiou, 1988; Hendriks and Van Os, 1976), menthyl acetate (Kokkini and Papageorgiou, 1988), dihydrocarvone (Hendriks and Van Os, 1976) and also piperitenone oxide (Nagell and Hefendehl, 1974), indicating that the Uruguayan population could be a representant of the chemotype reported for Nagell et al. (Nagell and Hefendehl, 1974).

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RESUMO

Óleos essenciais obtidos por hidrodestilação das folhas de *Mentha pulegium* L. e *Mentha rotundifolia* (L.) Huds. do Uruguay foram analisados por GC-FID e GC-MS. O grupo de monoterpenes oxigenados foi o mais importante em ambos os óleos, sendo que a pulegona, isomenthona e menthona foram os constituintes maioritarios no óleo de *Mentha pulegium*, no entanto, o ôxido de piperitenona e (Z)-hidrato de sabineno foram os maioritarios na *Mentha rotundifolia*. (-)-Mentone, (+)-isomentone, (+)-isomenthol, (-)-menthol e (+)-pulegone enantioméricamente puras foram detectadas por cromatografía gasosa multidimensional no caso do óleo de *Mentha pulegium*.

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