

Enantiomeric Distribution of Oxygenated Monoterpenes in Some *Mentha* Essential Oils

By Uzi Ravid, Agricultural Research Organization, Neve Ya'ar Research Center, Ramat Yishay, Israel

In the last few years, the flavor and fragrance industry has shown a great interest in producing natural volatile compounds from plant materials. Many natural essential oil components are chiral; that is, they contain one or more asymmetric carbon atoms and exhibit optical activity. Natural chiral essential oil components generally have a characteristic enantiomeric distribution that is attributable to stereoselectivity-controlled biogenetic formation mechanisms. An excess of one or the other enantiomer occurs in a variety of oils and oleoresins. In many cases, enantiomers have different sensory qualities.¹

Many components of the commercial mint oils and other oils of the *Mentha* genus are chiral. Some of these components are present as optically pure enantiomers, while others are present in specific enantiomeric ratios. The enantiomeric ratio can be modified by the addition of synthetic racemic ingredients (adulteration). In many cases, enantiomers have different organoleptic properties and may have different metabolic pathways. Knowing the enantiomeric composition of an oil's components is of great importance for recognizing the oil's natural origin and—for a non-commercial oil—its chemotaxonomic implications.

The objectives of this research were as follows:

- To formulate effective modern agricultural practices to assure the quality of mints for the production of chiral monoterpenes.
- To isolate and purify optically pure building blocks for the synthesis of more complicated optically pure compounds.
- To determine the enantiomeric composition of the chiral monoterpenes in *Mentha* oils by chiral gas chromatography. This technique can be used to detect adulteration of natural *Mentha* and other essential oils by synthetic racemic components.
- To enhance the use of natural optically pure, enantiomers from *Mentha* oils, and mixtures of enantiomers from *Mentha* oils in improved food additives and fragrances.

This article is adapted from the author's presentation at the International Mint Symposium in Seattle, Washington, in 1997.

In this research we analyzed chiral oxygenated monoterpenes, which are the predominant and most valuable monoterpenes in *Mentha* oils. This technique enables us to determine the enantiomeric purities of other components in the oils, such as monoterpene hydrocarbons and sesquiterpenes.

Methods

At the Neve Ya'ar Research Center, growth trials were conducted to assess the yield possibilities and cultivation conditions for various mint plants. We subjected them to systematic selection and to modern agronomic development. The fresh foliage specimens were hydro- or steam-distilled for one hour in a modified Clevenger apparatus or in a 130-liter direct steam pilot plant apparatus.

We used chiral gas chromatography to determine the enantiomeric composition of oxygenated monoterpenes isolated from these mint oils. The chemically pure components or fractions were first collected from the oils by preparative gas chromatography on a packed column, using a 1:50 effluent splitter and a modified Brownlee-Silverstein collector. They were then injected into the fused silica chiral capillary column. We found that capillary gas chromatography on chiral-modified β - and γ -cyclodextrin phases, using hydrogen as a carrier gas, was a simple, accurate, fast and highly sensitive technique.

Table 1 shows the *Mentha* species and hybrids that we analyzed for the enantiomeric composition of their component oxygenated monoterpenes.

Oxygenated Monoterpenes

Piperitone: Piperitone, with its fresh, minty camphor-like odor, occurs in various mint oils. In the *Mentha* genus there are piperitone-rich species that are considered common sources of this natural monoterpene ketone. Piperitenone is the precursor of (+)- and (–)-piperitone in the biosynthetic reduction of monoterpenes in the *Mentha* genus. Piperitone is used to scent flavoring preparations and has been used as a starting material in the synthesis of menthone and menthol.

Table 1. *Mentha* species and hybrids analyzed for enantiomeric composition of oxygenated monoterpenes

Botanical name	Common name	Type or source
<i>M. longifolia</i> (L.) Huds.		Yizre'el Valley Negev Galilee
<i>M. x piperita</i> L.	peppermint	'Black' UK 'Prime' USA 'Mitcham' USA Romania, Var. 1 Romania, Var. 2 Romania, Var. 3
<i>M. arvensis</i> L.	cornmint	Brazil
<i>M. pulegium</i> L.	pennyroyal	UK
<i>M. sylvestris</i> L.	horsemint	UK
<i>M. requienii</i> Benth.	Corsican mint	UK
<i>M. citrata</i> Ehrh.	lemon mint	Portugal, England, Morocco, Israel
<i>M. spicata</i> L.	spearmint	USA, Romania, Switzerland, Morocco, Thailand, UK
<i>M. x aquatica</i>	Basil mint	UK

We found high enantiomeric purities (87-99%) of (+)-piperitone in four oils of *Mentha piperita*,² although its relative quantity in the oils was low (0.1-1.0%). Lower (79%) enantiomeric purity of (+)-piperitone oils was detected in the oil of *M. arvensis* (Table 2).

The presence of (-)-piperitone in high enantiomeric excesses in the Negev type of *M. longifolia* oil and in lower enantiomeric excesses in the other mint oils is contrary to the postulation that only (+)-piperitone occurs naturally in oils of the *Mentha* genus. The occurrence of (-)-piperitone in high enantiomeric ratios and in high relative quantity (50%) in the Negev-type oil of *M. longifolia* makes the plant a potential source of this chiral natural aroma compound.

Pulegone: (+)-Pulegone, with an odor reminiscent of peppermint and camphor, is the most abundant natural enantiomer. (+)-cis-Isopulegone is the only precursor of (+)-pulegone in the biosynthesis of monoterpenes in the *Mentha* genus. Very high enantiomeric purities of (+)-pulegone (95-99%) were detected in six oils of *M. piperita*, but the quantity was low (3-7%).³

Enantiomerically pure (+)-pulegone was isolated from the Yizre'el Valley and Galilee types of Israeli *M. longifolia*.

Table 2. Enantiomeric composition (%) and relative quantity (%) of piperitone in *Mentha longifolia* and *Mentha arvensis* oils

Oil, source	Relative quantity	(4S)(+)-piperitone	(4R)(-)-piperitone
<i>Mentha longifolia</i> (L.)			
Huds. oil, Yizre'el Valley	2.4	99	1
<i>Mentha longifolia</i> (L.)			
Huds. oil, Negev	49.7	6.5	93.5
<i>Mentha arvensis</i> L. oil, Brasil	1.2	79	21

We found high relative quantities (66-82%) of the optically pure (+)-pulegone in the oils of Galilee type of *M. longifolia*, *M. pulegium* and *M. sylvestris*.

(+)-Pulegone is an important "chiral pool" compound and has been used as a chiral starting material for many chiral auxiliaries in the enantioselective synthesis of natural products.

Menthone: (+)-Pulegone is the precursor of (-)-menthone and (+)-isomenthone in the biosynthesis of monoterpenes in the *Mentha* genus. Enantiomerically pure (-)-menthone and (+)-isomenthone were detected in the oils of *Mentha piperita* (Figure 1), *M. longifolia*, *M. pulegium*, *M. sylvestris* and *M. requienii*.⁴ The relative quantities of menthone and isomenthone in four oils of *M. piperita* were 37-47% and 2-6%, respectively.

In the three oils of *M. longifolia*, menthone was found in higher concentrations (6-61%) than isomenthone (0.4-3.0%). We found only low relative quantities of enantiomerically pure (-)-menthone and (+)-isomenthone in the oils of *M. pulegium* (5.9 and 6.2%), *M. sylvestris* (0.4 and 0.3%) and *M. requienii* (7.2 and 0.9%).

In commercial sample of geranium bourbon oil, (+)-menthone and (-)-isomenthone were detected in high enantiomeric purities (Figure 2). We know that different flavor, cooling and bitter threshold values of (-)- and (+)-menthone and of (-)- and (+)-isomenthone were determined by Emberger and Hopp.¹⁴ While optically pure

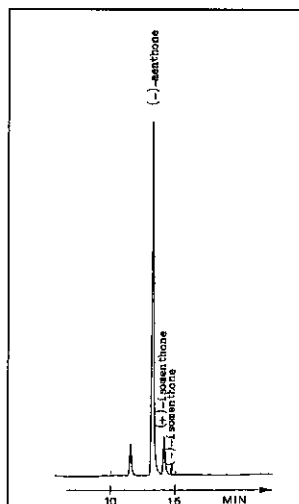


Figure 1. Enantiomer separation of menthone and isomenthone from *Mentha piperita* L. oil, 'Peppermint Black', using a modified γ -cyclodextrin column (Lipodex E)⁴

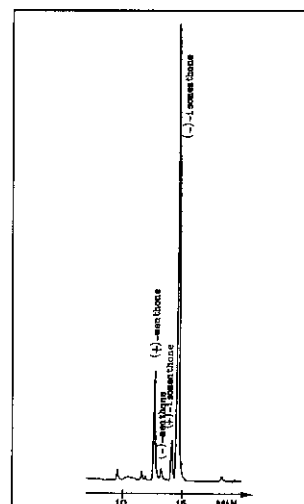


Figure 2. Enantiomer separation of menthone and isomenthone from commercial geranium Bourbon oil using a modified γ -cyclodextrin column (Lipodex E)⁴

(-)-menthone and (+)-isomenthone were detected in *Mentha* oils, high optical purities of (+)-menthone and (-)-isomenthone were detected in geranium oils. The difference between the enantiomeric distribution of menthone and isomenthone in mint and geranium oils is important for understanding the sensory qualities of the oils. Menthone, possessing an odor reminiscent of peppermint but harsher, and isomenthone, with its slightly musty odor, are significant marks in the quality control of mint and geranium oils.

Menthol: (+)-Neomenthol, (-)-menthol and (-)-menthyl acetate were enantiomerically pure or of very high enantiomeric purity in various peppermint and commint oils.

Carvone: (-)-Carvone has a warm-herbal-spicy-pungent odor with a slightly sweeter tone than (+)-carvone. It is the main component of spearmint oil. Very high enantiomeric purities of (-)-carvone were detected in six oils of spearmint from various sources.⁵ High enantiomeric purities of (-)-carvone were also detected in *M. longifolia* oils.

Adulteration of spearmint oils with synthetic (-)-carvone of high enantiomeric purity cannot be detected. The commercial enantiomer of (-)-carvone is synthesized from enantiomerically pure (+)-limonene, isolated from orange or other citrus peel oils, with retention of the integrity of the chiral center. Humans and mole rats⁶ can distinguish between supra-threshold concentration of the two enantiomers of carvone. Humans can detect (-)-carvone at lower concentrations than (+)-carvone.

α -Terpineol: (+)- α -Terpineol has a heavy floral, typically lilac, odor. The (-)-enantiomer has a coniferous, tarry odor character.

(+)- α -Terpineol is the predominant enantiomer in the four *M. citrata* oils (69-81%) (Table 3). In *M. aquatica*, (+)- α -terpineol was also the predominant (69%) enantiomer, while in Negev-type *M. longifolia*, the (-)-enantiomer was predominant (69%).⁷

In one case (the oil of *Micromeria fruticosa*), (+)- α -terpineol was detected as a pure enantiomer. To the best of my knowledge, enantiomerically pure α -terpineol has not been detected previously in essential oils or in other natural sources.

Other oxygenated monoterpenes: Enantiomerically pure (-)-linalyl acetate was detected in four oils of *M. citrata*.⁸ None of the analyzed commercial oils was adulterated with synthetic racemic linalyl acetate.

With the (+)-enantiomer at 53%, citronellol isolated from Israeli oil of fresh leaves of *M. citrata* was almost racemic.⁹

(-)-Borneol was the predominant enantiomer (94%) in *M. longifolia* oil, Negev type.¹⁰

Table 3. Enantiomeric composition (%) and relative quantity (%) of α -terpineol in *Mentha citrata* oils

Oil, source	Relative quantity	(4R)(+)- α -terpineol	(4S)(-)- α -terpineol
<i>Mentha citrata</i> Ehrh. oil, Portugal	2.7	72	28
<i>Mentha citrata</i> Ehrh. oil, England	3.9	69	31
<i>Mentha citrata</i> Ehrh. oil, Morocco	3.2	81	19
<i>Mentha citrata</i> Ehrh. oil, Israel	4.2	80	20

Conclusion

Capillary gas chromatography on optically active modified cyclodextrin phases is a highly sensitive method for enantiomeric analysis of chiral volatile

compounds in mint oils and in other essential oils.¹¹⁻¹³ The enantiomeric ratio of mint oils components is a reliable parameter to assess quality because it may be indicative of adulteration, contamination, aging, shelf life, technological process and the botanic source of the specific chiral compound.

The isolation of both chemically pure and optically pure compounds from oils of *Mentha* species may become a very interesting issue to flavor and fragrance companies because many regulatory authorities may recommend that these companies produce volatile chiral compounds in a single stereoisomer form or in specific enantiomeric ratios.

References

Address correspondence to Dr. Uzi Ravid, Agricultural Research Organization, Newe Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel.

1. MH Boelens, Sensory properties of optical isomers, *Perfum Flavor* **18**(6) 1-16 (1993)
2. U Ravid, E Putievsky and I Katzir, Enantiomeric distribution of piperitone in essential oils of some *Mentha* spp, *Calamintha incana* (Sm) Heldr and *Artemisia judaica* L, *Flav Fragr J* **9** 85-87 (1994)
3. U Ravid, E Putievsky and I Katzir, Chiral GC analysis of (1R)-(+)-pulegone with high enantiomeric purity in essential oils of some Lamiaceae aromatic plants, *Flav Fragr J* **9** 205-207 (1994)
4. U Ravid, E Putievsky and I Katzir, Chiral GC analysis of menthone and isomenthone with high enantiomeric purities in laboratory-made and commercial essential oils, *Flavour Fragr J* **9** 139-142 (1994)
5. U Ravid, E Putievsky, I Katzir, V Weinstein and R Ikan, Chiral GC analysis of (S)-(+)- and (R)-(-)-carvone with high enantiomeric purity in caraway, dill and spearmint oils, *Flav Fragr J* **7** 289-292 (1992)
6. G Heth, E Nevo, R Ikan, V Weinstein, U Ravid and H Duncan, Differential olfactory perception of enantiomeric compounds by blind subterranean mole rats (*Spalax ehrenbergi*), *Experientia* **48** 897-902 (1992)
7. U Ravid, E Putievsky and I Katzir, Determination of the enantiomeric composition of α -terpineol in essential oils, *Flav Fragr J* **10** 281-284 (1995)
8. U Ravid, E Putievsky and I Katzir, Chiral GC analysis of enantiomerically pure (R)-(-)-linalyl acetate in some Lamiaceae, myrtle and petitgrain essential oils, *Flav Fragr J* **9** 275-276 (1994)
9. U Ravid, E Putievsky, I Katzir, R Ikan and V Weinstein, Determination of the enantiomeric composition of citronellol in essential oils by chiral GC analysis on γ -cyclodextrin phase, *Flav Fragr J* **7** 235-238 (1992)
10. U Ravid, E Putievsky and I Katzir, Stereochemical analysis of borneol in essential oils using permethylated β -cyclodextrin as a chiral stationary phase, *Flav Fragr J* **11** 191-195 (1996)
11. A Mosandl, Capillary gas chromatography in quality assessment of flavours and fragrances, *J Chromatogr* **624** 267-292 (1992)
12. P Werkhoff, S Brennecke, W Bretschneider, M Güntert, R Hopp and H Surburg, Chiro-specific analysis in essential oil, fragrance and flavor research, *Z Lebensm Unters Forsch* **196** 307-328 (1993)
13. C Bicchi, V Manzin, A D'Amato and P Rubiolo, Cyclodextrin derivatives in GC separation of enantiomers of essential oil, aroma and flavour compounds, *Flav Fragr J* **10** 127-137 (1995)
14. R Emberger and R Hopp, in *Topics in Flavour Research*, RG Berger, S Nitz and P Schreier, eds, Marzling-Hangenhorn: H Eichhorn (1985) pp 201-218

