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Short Communication

Chiral compounds of essential oils

XV. Stereodifferentiation of characteristic compounds of *Mentha* species by multi-dimensional gas chromatography

Birgit Faber, Armin Dietrich and Armin Mosandl*

Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität Frankfurt, Marie-Curie-Strasse 9, D-60439 Frankfurt (Germany)

ABSTRACT

The direct enantioselective analysis of menthone, isomenthone, menthol, neomenthol, neoisomenthol and menthylacetate in *Mentha* oils is achieved, using multi-dimensional gas chromatography with heptakis (2,3-di-O-methyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin in PS 268 as the chiral stationary phase.

INTRODUCTION

The chiral monoterpenoids menthone (**1**,**2**), neomenthol (**3**,**4**), isomenthone (**5**,**6**), menthol (**7**,**8**), neoisomenthol (**9**,**10**) and menthylacetate (**11**,**12**) are characteristic compounds of the essential oil of peppermint (*Mentha x piperita* L.) (see Scheme 1).

The enantiomeric ratio of these compounds has proved to be an indicator for the genuineness of mint and peppermint oil. As shown in previous investigations, mint oil of natural origin contains enantiomerically pure (−)-menthone (**1**), (+)-isomenthone (**5**), (−)-menthol (**8**) and (−)-menthylacetate (**11**) [1,2].

This article describes the stereodifferentiation of these monoterpenoids and of neomenthol and neoisomenthol directly from the essential oil, using enantioselective multi-dimensional gas chromatography (MDGC), as a well-established technique for the analysis of different kind of chiral volatile compounds [3–6].

EXPERIMENTAL

Materials

Self-prepared peppermint oils of different species of *Mentha piperita*, kindly provided by the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising, Germany, are used as authentic samples. The essential oils are ob-

* Corresponding author.

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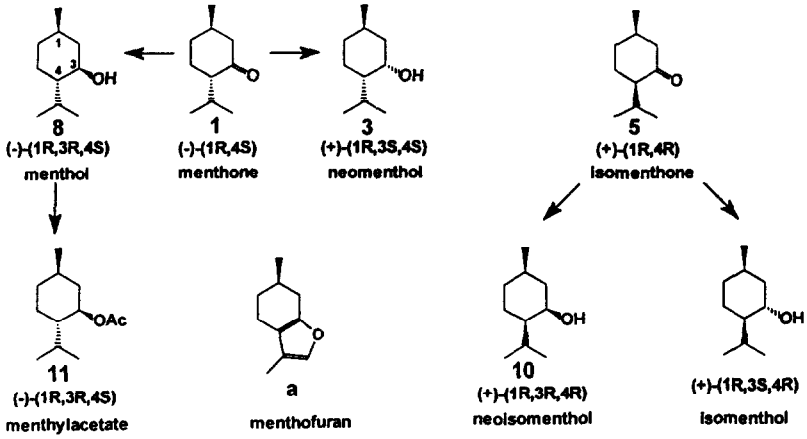

Scheme 1.

tained by steam distillation of dried peppermint leaves.

Column preparation

Column preparation follows according to refs. 7 and 8.

Instrumentation

The following instrumentation was used: Siemens SiChromat 2-8 double-oven system with two independent temperature controls, two flame ionization detectors and a live switching coupling piece (live-T-piece). Injector: split, 260°C; detectors: 300°C each.

Precolumn

A 30 m × 0.23 mm I.D. Duranglass capillary, coated with a 0.65-μm film of SE-52 (Riedel-de Haen, Seelze-Hannover, Germany) was used as precolumn; carrier gas: hydrogen 1.8 bar; temperature programme: 120°C isothermal for 20 min, then at 4°C/min to 230°C.

Main column

A 30 m × 0.23 mm I.D. Duranglass capillary, coated with a 0.23-μm film of heptakis (2,3-di-O-methyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin (15%) in PS 268 (85%) (a diphenylmethylsilicone; ABCR, Karlsruhe, Germany) was used as the main column, synthesized according to ref. 9; carrier gas: hydrogen 1.2 bar; temperature programme: 47°C isothermal for 19 min, then at 20°C/min to 70°C, isothermal for 15 min, then at 1.5°C/min to 210°C.

Heart cutting

Heart cutting times are given in Table I.

Elution order (main column)

The elution order is as follows: menthone **1**, (−)-(1*R*,4*S*); menthone **2**, (+)-(1*S*,4*R*); neomenthol **3**, (+)-(1*R*,3*S*,4*S*); neomenthol **4**, (−)-(1*S*,3*R*,4*R*); isomenthone **5**, (+)-(1*R*,4*R*); isomenthone **6**, (−)-(1*S*,4*S*); menthol **7**, (+)-(1*S*,3*S*,4*R*); menthol **8**, (−)-(1*R*,3*R*,4*S*); neoisomenthol **9**, (−)-(1*S*,3*S*,4*S*); neoisomenthol **10**, (+)-(1*R*,3*R*,4*R*); menthylacetate **11**, (−)-(1*R*,3*R*,4*S*); menthylacetate **12**, (+)-(1*S*,3*S*,4*R*).

TABLE I

HEART CUTTING TIMES

Compound	Cut times (min)	
	Standard mixture	Essential oils
Menthone (**1**,**2**)	17.00–17.10	17.03–17.09
Neomenthol (**3**,**4**)	17.68–17.80	17.66–17.81
Isomenthone (**5**,**6**) and menthol (**7**,**8**)	18.00–18.40	18.00–18.36
Neoisomenthol (**9**,**10**)	19.50–19.72	19.50–19.78
Menthylacetate (**11**,**12**)	27.73–27.90	27.74–27.95

Reference compounds

The order of elution was assigned, using racemates, enantiomerically pure or enriched references of definite chirality.

Racemates: menthone/isomenthone (Roth, Karlsruhe, Germany; 4226); neoisomenthol synthesized according to ref. 10.

Optically active references: (−)-menthone (Aldrich, Steinheim, Germany; 21,823-5); isomenthone: $[\alpha]_{20}^{D} = +94.3°$ ($c = 2.52$; $CHCl_3$), (Roth, 6458); (−)-neomenthol (Fluka, Neu Ulm, Germany; 72139); (+)-neomenthol (Fluka, 72134); (−)-menthol (Fluka, 63660); (+)-menthol (Fluka, 63658); (−)-menthylacetate (Fluka, 45985); (+)-menthylacetate (Fluka, 45987); (+)-neoisomenthol synthesized according to ref. 10; menthofuran (Aldrich, Milwaukee, WI, USA; W32350-0).

RESULTS AND DISCUSSION

Authenticity control of flavour and fragrances is of considerable interest, because naturalness of food and beverages is highly demanded by the customer. Therefore, legal regulations of the European Community require differentiation between natural and nature-identical flavouring substances. While natural compounds are of natural origin, the nature identical flavourings are chemically identical with their natural models, but obtained by chemical synthesis or isolated by chemical processes. In this paper the simultaneous enantioselective analysis of the typical chiral mint oil constituents is used for the differentiation of natural and nature identical origin [11,12].

We describe the determination of menthone (**1,2**), neomenthol (**3,4**), isomenthone (**5,6**), menthol (**7,8**), neoisomenthol (**9,10**) and menthylacetate (**11,12**) using MDGC with the column combination of a non-chiral precolumn (SE-52) and a chiral main column (heptakis (2,3-di-O-methyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin). This technique allows the chirospecific analysis, directly from the essential oil, without any other purification or isolation procedure. In this way the separation of all six chiral monoterpenoids into their enantiomeric pairs is achieved with just one chromatographic run (Fig. 1).

Sixteen samples of self-prepared peppermint oils of different origin and kind were analyzed.

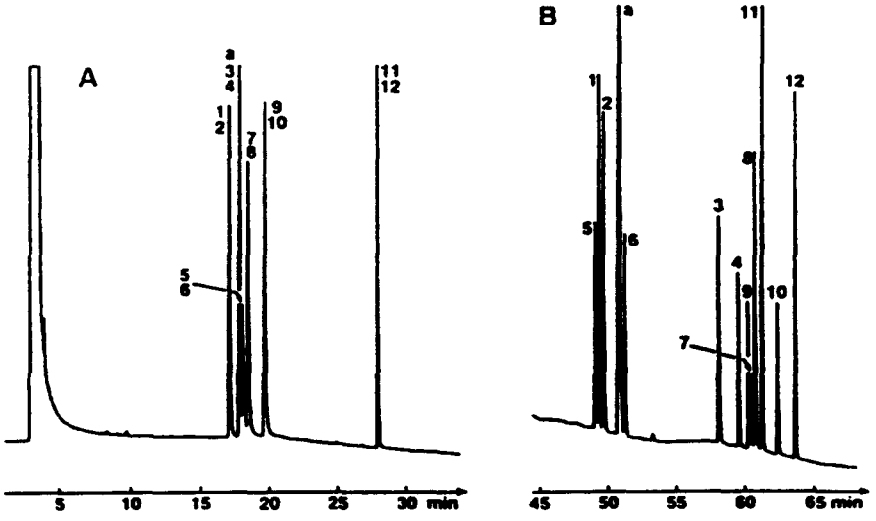

Fig. 1. Simultaneous stereoanalysis of mint oil constituents, using enantioselective MDGC (standard mixture); (−)-menthone (**1**); (+)-menthone (**2**); menthofuran (**a**); (+)-neomenthol (**3**); (−)-neomenthol (**4**); (+)-isomenthone (**5**); (−)-isomenthone (**6**); (+)-menthol (**7**); (−)-menthol (**8**); (−)-neoisomenthol (**9**); (+)-neoisomenthol (**10**); (−)-menthylacetate (**11**); (+)-menthylacetate (**12**). (A) Precolumn: preseparation of the racemic compounds; (B) main column: separation of the racemic compounds into their enantiomeric pairs. For conditions see Experimental section.

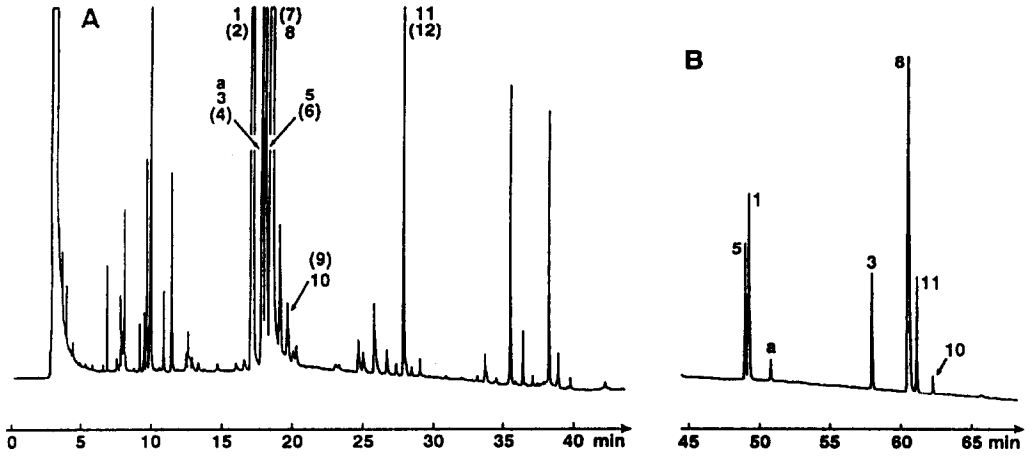

Fig. 2. Enantioselective MDGC analysis of a self-prepared oil of *Mentha piperita*. (A) Precolumn; (B) main column separation: detection of (−)-menthone (**1**); (+)-neomenthol (**3**); (+)-isomenthone (**5**); (−)-menthol (**8**); (+)-neoisomenthol (**10**); (−)-menthylacetate (**11**) and menthofuran (**a**) as genuine peppermint oil constituents. For conditions see Experimental section.

All of them yielded the same result. In accordance with previous investigations [1], enantiomerically pure (−)-menthone (**1**), (+)-isomenthone (**5**), (−)-menthol (**8**) and (−)-menthylacetate (**11**) are detected (Fig. 2). Fig. 2 shows that neomenthol as well as neoisomenthol occur as the pure (+)-enantiomers (**3**,**10**). This result comes up to our expectations, because (−)-menthone (**1**) is the precursor of (+)-neomenthol (**3**) and (+)-isomenthone (**5**) is the precursor of (+)-neoisomenthol (**10**) (Scheme 1). Peppermint oil of natural origin contains up to 0.9% neoisomenthol. Furthermore it contains also isomenthol with just an amount up to 0.4% [13]. Because of this small amount the stereodifferentiation of isomenthol is still under investigation. Its biosynthesis should result from the reduction of (1*R*)-configurated (+)-isomenthone (**5**). Thus, the (1*R*)-configurated isomenthol is expected too.

Menthofuran (**a**), a further peppermint oil constituent, coelutes with neomenthol (**3**,**4**) at the precolumn. Therefore it is transferred together with neomenthol (**3**,**4**) to the main column (Figs. 1–3).

The enantiomeric distribution of some commercially available mint oils is strange to our results. Fig. 3 shows such an oil, which contains (+)-menthylacetate (**12***). In Fig. 4 the essential oil contains (+)-menthylacetate (**12***), as well as (+)-menthol (**7***). This clearly indicates an adulteration with racemic compounds. The absence of menthofuran (**a**) at the latter oil is typical for mint oils, produced by *Mentha arvensis* [14,15]. According to the quality demands of refs. 16 and 17, mint and peppermint oils have to contain a minimum amount of alcohols (calculated as menthol) and a minimum amount of esters (calculated as menthylacetate). As natural

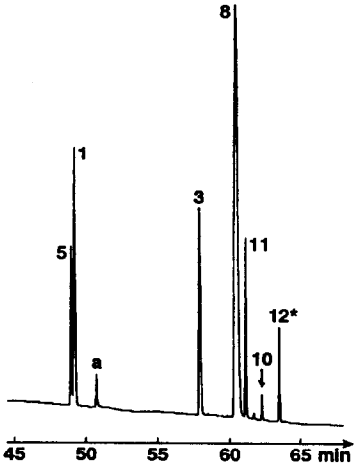

Fig. 3. Enantioselective MDGC analysis of a commercially available peppermint oil, containing the strange enantiomer (+)-menthylacetate (**12***). For conditions see Experimental section.

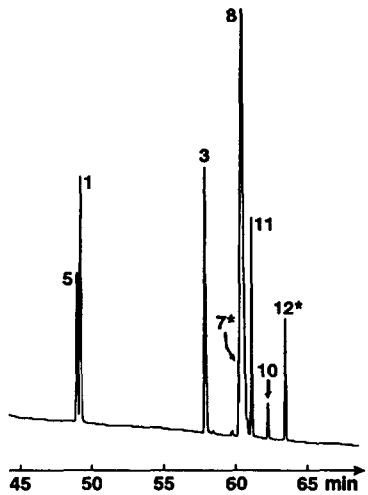

Fig. 4. Enantioselective MDGC analysis of a commercially available mint oil, proving the adulteration with the strange enantiomers (+)-menthol (**7***) and (+)-menthylacetate (**12***). For conditions see Experimental section.

mint and peppermint oils do not achieve these quality levels in any case, the fraudulent addition of racemic menthol (**7,8**) as well as menthylacetate (**11,12**) conclusively explains the detection of the strange enantiomers **7*** and **12*** (Figs. 3 and 4).

CONCLUSIONS

The simultaneous stereodifferentiation of six characteristic mint oil constituents is achieved, using MDGC with the column combination SE-52/heptakis (2,3-di-O-methyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin. All six chiral monoterpenoids occur as pure (1*R*)-configurated enantiomers in genuine mint oils. Thus, the occurrence of the strange (1*S*)-enantiomers of (+)-menthylacetate and (+)-menthol in commercially available mint oils has to be regarded as an adulteration with the corresponding racemates.

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