



Update on enantiomeric composition of (1R)-(+)- and (1S)-(-)-camphor in essential oils by enantioselective gas chromatography

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The enantiomeric ratios of camphor have been determined in authentic essential oils, using heptakis(6-*O*-*t*-butylsilyl-2,3-di-*O*-ethyl)- β -cyclodextrin as the chiral stationary phase. An enantiomeric excess of (1S)-(-) within 72–75% is characteristic of coriander oil (*Coriandrum sativum* L.). Contrary, an enantiomeric excess of (1R)-(+) characterizes the essential oils of sage (>90% for *Salvia sclarea* L. and 50–70% for *Salvia officinalis* L.) and of basil (>94% for *Ocimum basilicum* L.).

Introduction

Studies by various authors have concentrated on evaluation of the enantiomeric distribution of chiral compounds in various essential oils^{1–22} and chirality evaluation of some essential oil compounds has been adopted for quality assurance as an indicator of genuineness.

The enantiomeric forms (1R)-(+) and (1S)-(-) of camphor present no significant differences from the point of view of sensorial analysis: both enantiomers present the same profile²³ characterized by a pungent camphorous smell with a hint of mintiness and a fresh, bitter taste.^{24,25}

Bicchi *et al.*,²⁶ in particular, have published a review of the chirality subject, relating to the period 1989–1994, but the quotations concerning camphor in the bibliography are very limited. Kreis and Mosandl,¹¹ using enantioselective multi-dimensional gas chromatography, reported the stereodifferentiation of camphor in essential oils of *Lavandula* species.

Experimental papers published to date on the enantiomeric ratio of camphor concern mainly the essential oils of *Cinnamomum camphora* (L.) by J. Presl, *Ocimum canum* by Sims, *Sassafras albidum* (Nutt.) by Nees, in which the enantiomer (1R)-(+)^{24,25,27} is prevalent, and of *Salvia grandiflora*, *Salvia triloba* L., *Artemisia herba alba* by Asso, and *Artemisia ferganensis*, *Artemisia austrachanica*, *Lippia adoensis*, *Blumea balsamifera*, which reveal an enantiomeric excess of (1S)-(-).^{27,28}

According to Ravid *et al.*,¹⁰ only (1S)-(-)-camphor is present in *Chrysanthemum parthenium* L., while the species *Salvia tomentosa* Mill. contains (\pm)-camphor, and Furia and Bellanca²⁴ have also encountered camphor with a racemic distribution in *Chrysanthemum japonicum sinense*.

In three of the six samples of *Salvia officinalis* L. analyzed, Ravid *et al.*¹⁰ encountered an enantiomeric excess of (1S)-(-)-camphor, and in the other three an excess of the form (1R)-(+). We have carried out further research in this area. Evaluations have therefore been made of camphor in absolutely reliable essential oils relating to the species *Salvia officinalis* L. At the same time, evaluations have been made of the enantiomeric distributions of camphor in three other types of essential oil not referred to, so far, in the bibliography.

Identification of the two enantiomeric forms has been made by various authors with different GC systems and columns (β or

γ -cyclodextrin). With the heptakis(6-*O*-*t*-butyldimethylsilyl-2,3-di-*O*-acetyl)- β -cyclodextrin¹¹ column, the (1S)-(-) form is eluted first, followed by (1R)-(+), while for the octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)- γ -cyclodextrin (Lipodex E)^{10,29,30} column, the opposite is true. The experiments referred to here were carried out with heptakis(6-*O*-*t*-butylsilyl-2,3-di-*O*-ethyl)- β -cyclodextrin as the chiral stationary phase.

Experimental

The results obtained refer to pure essential oils of *Salvia officinalis* L. and *Salvia sclarea* L., *Ocimum basilicum* L., and *Coriandrum sativum* L., obtained from factory-distilled plants, accurately identified by botanists.

The essential oils were diluted in ethyl alcohol (10% v/v essential oil–ethyl alcohol), and were subjected to GC chiral analysis. GC-MS analysis confirmed the peak identity.

GC chiral analysis conditions

Enantiomeric ratios of camphor were obtained using a HRGC 5160 Mega Series (Carlo Erba Strumentazione, Rodano, Milan, Italy), equipped with a fused-silica capillary column 25 m \times 0.25 mm id, coated with heptakis(6-*O*-*t*-butylsilyl-2,3-di-*O*-ethyl)- β -cyclodextrin, 0.25 μ m film thickness (Mega@, Legnano, Milan, Italy); column temperature, 60 °C (10 min) to 120 °C at 3 °C min⁻¹, 120 to 230 °C at 4 °C min⁻¹; injector temperature, 200 °C; detector (FID) temperature, 220 °C; injection mode, split; split ratio, 1/20; injection volume, 0.2 μ l; carrier gas, H₂ 0.4 kg cm⁻².

GC MS analysis conditions

Analyses were performed on a Hewlett-Packard 5971A mass selective detector (MSD), equipped with an HP 5890 Series II gas chromatograph; GC conditions were: an SPBTM-5 fused-silica capillary column, 30 m \times 0.2 mm id, 0.2 μ m film thickness (SUPELCO, Milan, Italy); column temperature, 80 °C (10 min) to 120 °C at 1 °C min⁻¹, 120 °C (10 min) to 230 °C at 3 °C min⁻¹, 230 °C (10 min); injector temperature, 220 °C; injection mode, split; split ratio, 1/20; injection volume, 0.2 μ l; carrier gas, He at a constant pressure of 100 \times 10³ Pa.

MS conditions were: GC-MS interface temperature, 280 °C; acquisition parameters, full scan (40–300 u), ionization energy, 70 eV.

Results and discussion

The results obtained make it possible to acquire the sum data that had hitherto not been available for basil oil, coriander oil,

and clary sage oil. Only some of the data obtained to date are confirmed and concern *Salvia officinalis* L.

Table 1 gives the values of enantiomeric distribution of camphor in the essential oil samples analyzed, and Fig. 1 shows the enantiomeric distribution of camphor in the coriander oil samples [sample 1, (a)] and basil oil [sample 4, (b)].

The essential oils of *Salvia sclarea* L. present an enantiomeric excess of (1*R*)-(+), exceeding 90%; the oils of *Salvia officinalis* L. also present an enantiomeric excess of (1*R*)-(+), that is less marked but nevertheless exceeds 50%.

Only (1*R*)-(+)-camphor was found in three of the five samples of essential oils of basil analyzed (samples 1, 2 and 5), while in the other two, the presence, albeit very limited, of the enantiomer (1*S*)-(–) was found.

Table 1 Enantiomeric composition, enantiomeric excess and relative quantity of camphor in authentic essential oils.

| Source | Relative quantity (%) | Enantiomeric composition (%) | | Enantiomeric excess (%) |
|---|-----------------------|------------------------------|-------------------|-------------------------|
| | | (1 <i>S</i>)-(–) | (1 <i>R</i>)-(+) | |
| <i>Salvia sclarea</i> L. | | | | |
| Sample 1 (Italy) | 0.1 | 3.8 | 96.2 | 92.4 (1 <i>R</i>) |
| Sample 2 (Italy) | 0.1 | 4.9 | 95.1 | 90.2 (1 <i>R</i>) |
| <i>Salvia officinalis</i> L. | | | | |
| Sample 1 (France) | 11.8 | 23.4 | 76.6 | 53.2 (1 <i>R</i>) |
| Sample 2 (France) | 24.9 | 15.1 | 84.9 | 69.8 (1 <i>R</i>) |
| <i>Coriandrum sativum</i> L. | | | | |
| Sample 1 (Italy) | 5.4 | 86.3 | 13.7 | 72.6 (1 <i>S</i>) |
| Sample 2 (Italy) | 5.1 | 87.6 | 12.4 | 75.2 (1 <i>S</i>) |
| Sample 3 (Italy) | 5.2 | 87.0 | 13.0 | 74.0 (1 <i>S</i>) |
| <i>Ocimum basilicum</i> L. ^a | | | | |
| Sample 1 (Italy) | 0.9 | 0 | 100 | 100 (1 <i>R</i>) |
| Sample 2 (Italy) | 0.7 | 0 | 100 | 100 (1 <i>R</i>) |
| Sample 3 (Italy) | 0.4 | 2.5 | 97.5 | 95.0 (1 <i>R</i>) |
| Sample 4 (Italy) | 0.5 | 2.0 | 98.0 | 96.0 (1 <i>R</i>) |
| Sample 5 (Italy) | 0.5 | 0 | 100 | 100 (1 <i>R</i>) |

^a Of the basil oil samples analyzed, those of the so-called 'Genuese type' (samples 1 and 2) show mainly linalool (47–52%) and traces of estragol, while the so-called 'Neapolitan type' (samples 3, 4 and 5) show mainly linalool (47–63%) and between 9 and 12% estragol.

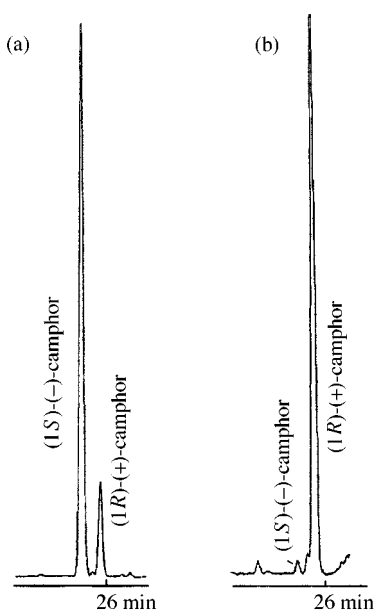


Fig. 1 Enantiomer GC separation of camphor isolated from (a) *Coriandrum sativum* L. and (b) *Ocimum basilicum* L., using heptakis(6-*O*-*t*-butylsilyl)-2,3-di-*O*-ethyl- β -cyclodextrin as the chiral stationary phase; for further conditions, see Experimental section.

In coriander oil, an enantiomeric excess of the form (1*S*)-(–) was found to be a characteristic.

Conclusions

Results concerning enantiomeric excess values obtained by GC chiral analysis of genuine *Salvia sclarea* L. and *Salvia officinalis* L. essential oils with a camphor content extending over a fairly wide range (0.1–25%), show an enantiomeric excess of the form (1*R*)-(+)-camphor. The enantiomeric excess values, however, seem to be characteristic of the species and, contrary to the results of other authors, have been found to conform to very narrow variability limits.

This conclusion is compatible with the biosynthetic pathway from geranyl pyrophosphate (GPP), the last step of which is the transformation of (1*S*)-(–)-borneol to (1*R*)-(+)-camphor by a dehydrogenase NAD-dependent.³¹

As regards *Ocimum basilicum* L. essential oils, with a camphor content below 1%, chiral analysis shows, even in this case, an enantiomeric excess of (1*R*)-(+)-camphor higher than 94%.

In contrast, results concerning *Coriandrum sativum* L. essential oils, show an enantiomeric excess of (1*S*)-(–)-camphor with very narrow variability limits.

It would seem possible to conclude that even the enantiomeric composition of camphor is a useful factor in assessing the genuineness, and is a characterizing element, of various botanical genuses.

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