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Enantioselective gas chromatographic analysis of monoterpenes in essential oils of the family *Myrtaceae*

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ABSTRACT: The elution profiles of several essential oils of the *Myrtaceae* family were acquired using a single enantioselective stationary phase column. These chromatograms were compared with those obtained by using a two-column coupled system, which comprised a low-polarity stationary phase column connected to the terminal end of the enantioselective column. The enantiomeric distributions of β-pinene, sabinene, α-phellandrene, limonene, *trans*- and *cis*-sabinene hydrate, linalool, terpinen-4-ol and α-terpineol were determined. The use of a two-column coupled system is demonstrated to be useful for improving the resolution of target (chiral) compounds from otherwise interfering matrix components. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: enantioselective gas chromatography; cyclodextrin; essential oil, tea tree; *Melaleuca*; *Leptospermum*; *Agonis*

Introduction

In the present investigation, the enantiomeric distributions of the monoterpenes and monoterpene alcohols in commercial-type tea tree essential oils, and other essential oils from the family *Myrtaceae* were determined using enantioselective gas chromatography. *Melaleuca alternifolia* and *M. linarifolia* are widely recognized because they are a source of commercial tea tree essential oil. Here the lesser known essential oils of *M. decussata*, *M. uncinata*, *M. armillaris*, *M. quinquinervia* were also analysed, as well as five *Agonis* essential oils, and two essential oils from the genus *Leptospermum*. *Agonis* is a small genus of 11 species, all of which occur naturally only in southwestern Australia. *Leptospermum* is a genus of about 86 species, distributed throughout Australia and extending to Malaysia and New Zealand.

Enantioselective GC analysis of terpenoid compounds is commonly performed using capillary columns coated with cyclodextrin derivatives (CDD) diluted in a suitable polymeric stationary phase.[1] Whilst the use of CDD coated columns often provides adequate resolution of the individual enantiomers of chiral compounds easily, the analysis of complex matrices such as essential oils can be problematic. There is an increased demand on the available resolving power of the column to ensure that the target (chiral) compounds are well resolved from matrix interferences. To circumvent this problem many analysts have adopted multi-dimensional enantioselective GC (*enantio*-MDGC) approaches for the analysis of essential oils.

The *enantio*-MDGC approach, which was first described by Schomburg and co-workers[2] requires unresolved target components to be heart-cut from the primary (achiral) column and delivered to the (chiral) analytical column, which provides resolution and quantitative measurement of the individual enantiomers. There have been numerous applications of *enantio*-MDGC for food, flavour and fragrance analysis,[3] including its recent use for the analysis of *M. alternifolia*.[4] A variant of the multidimensional GC technique is comprehensive gas chromatography (GC × GC). Enantioselective essential oils analysis has been reported using comprehensive *enantio*-GC × GC[5] (where the first column contained the enantioselective stationary phase), and comprehensive GC × *enantio*-GC[6] (where the enantioselective stationary phase column was applied in the more conventional second dimension position). The former of these two examples was applied to the analysis of *M. alternifolia* essential oils.

In the present study a less technically demanding approach to achieving adequate resolution of the target (chiral) compounds in these essential oils was investigated. Dugo and co-workers[7,8] previously showed that a two-column combination (which essentially provides a capillary column of mixed stationary phase) was useful in enhancing the resolution of target compounds from matrix interferences. A two-column approach, similar to the work of Dugo *et al*. but improved with the use of a

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chiral-non chiral stationary phases combination, will be taken here.

Experimental

Materials

Samples were provided by Australian Botanical Products (Hallam, Australia). Prior to injection all samples were diluted 1:100 (v/v) in *n*-hexane.

Instrumental

All analyses were performed on a Shimadzu GC-17A gas chromatograph (Shimadzu Italia, Milan, Italy) equipped with FID detector operated at 280 °C.

Method 1 used a diethyl-*tert*-butylsilyl-β-cyclodextrin enantioselective stationary phase column (Mega, Milan, Italy). This column is prepared in a base polymer of PS086. The column dimensions were 25 m × 0.25 mm (0.25 μm film thickness). The oven was temperature programmed from an initial oven temperature of 45 °C (constant for 6 min) to 200 °C at 2 °C/min. The carrier gas was hydrogen, which was supplied at a head pressure of 43 kPa. An injection volume of 0.1 μl was used for all analyses and the split ratio was 100:1.

Method 2 used two capillary columns which were serially coupled using a zero dead-volume glass press-fit capillary column joiner. The first column was a diethyl-*tert*-butylsilyl-β-cyclodextrin enantioselective stationary phase column. The column dimensions were 25 m × 0.25 mm (0.25 μm film thickness). Column 2 was an Rtx5-MS low-polarity stationary phase column. The column dimensions were 30 m × 0.25 mm (0.25 μm film thickness). The oven was temperature programmed from an initial oven temperature of 80 °C (constant for 6 min) to 200 °C at 1.2 °C/min. The carrier gas was hydrogen, which was supplied at a head pressure of 104 kPa. An injection volume of 0.1 μl was used for all analyses and the split ratio was 100:1.

Results and Discussion

The two-column approach produced more satisfactory results than analysis using only the enantioselective stationary phase column. Typical results from the two approaches are contrasted below.

Method 1

The diethyl-*tert*-butylsilyl-β-cyclodextrin enantioselective stationary phase was able to separate the individual pairs of enantiomers of numerous components of tea tree essential oil. (±)-Sabinene, (±)-β-pinene, (±)-α-phellandrene, (±)-limonene, (±)-*trans*- and (±)-*cis*-sabinene hydrate, (±)-linalool, (±)-terpinen-4-ol and (±)-α-terpineol were all adequately sepatated (baseline-resolution-or-better). However the determination of the enantiomeric distribution of each of these compounds (using Method 1) was problematic because there were several interfering matrix components. A typical chromatogram of *Melaleuca alternifolia* essential oil using the diethyl-*tert*-butylsilyl-β-cyclodextrin stationary phase column is shown in Fig. 1. (+)-Sabinene co-eluted with 1,8-cineole, and (−)-sabinene was poorly resolved from myrcene. Likewise, (+)-limonene was poorly resolved from *para*-cymene, and (+)-*trans*-sabinene hydrate could not be resolved from terpinolene. In each case the possibility of correctly determining the enantiomeric distribution of the target compound was hindered.

Method 2

The chromatogram of the essential oil shown in Fig. 1 is presented in Fig. 2 using the two-column combination. Using the new experimental setup, (−)-*trans*-sabinene hydrate co-eluted with γ-terpinene. Although *trans*-sabinene hydrate is abundant in the flush growth sample, there appears to be only small amounts present in the other oils analysed,[9] and hence this component was considered to be less important for the characterization of the mature-type essential oils. Whilst problematic in the former experiment, both isomers of sabinene eluted without matrix interference from the two-column combination. In the high terpinen-4-ol/low 1,8-cineole type oils, (+)-limonene was not quantifiable, because it could not be resolved from *para*-cymene. Both isomers of limonene could be satisfactorily quantified in the high 1,8-cineole/low terpinen-4-ol oils, which typically contained lower amounts of *para*-cymene. For example, *L. petersonii*, which is commonly called the lemon-scented tea-tree, produces an essential oil with a high limonene content. Thus the *para*-cymene interference was small with respect to the (+)-limonene content. It may be possible to have resolution of both (+)- and (−)-limonene from *para*-cymene by reversing the order of the capillary columns, as this was achieved previously using a similar set-up.[7,8] However, for the present study the enantioselective stationary phase column was purposely installed at the injector side of the two-column combination. This ensured that components had a lower effective elution temperature from the column, and hence better resolution of enantiomers was expected.[10] Also in this manner, the second column should not affect the enantiomeric resolution achieved (provided that the same temperature program is used), but the second column will affect the resolution of enantiomers from the matrix. Having

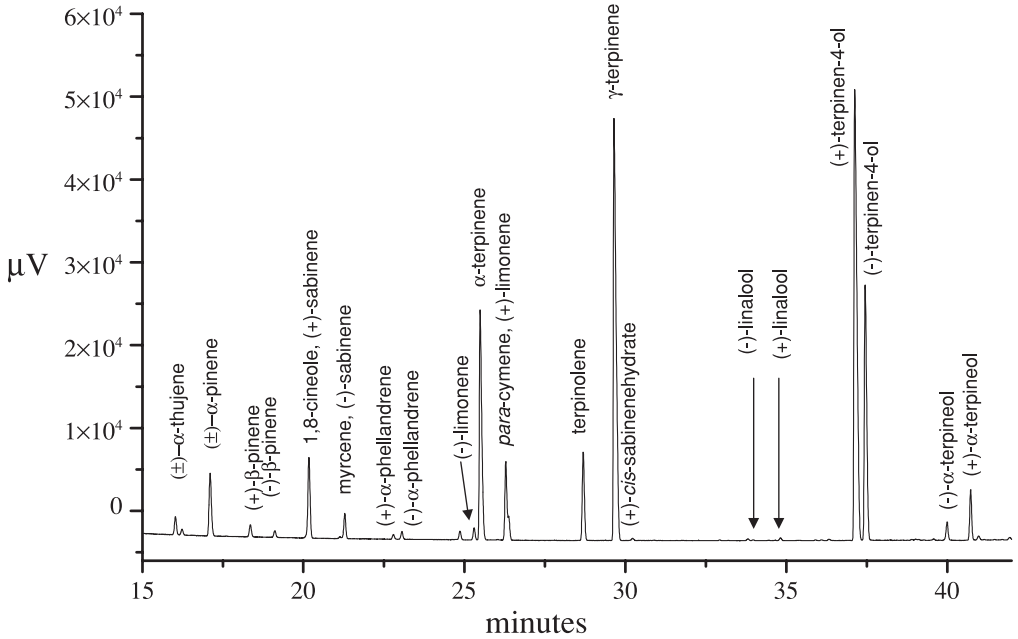

Figure 1. Typical chromatogram of a *M. Alternifolia* essential oil using method 1

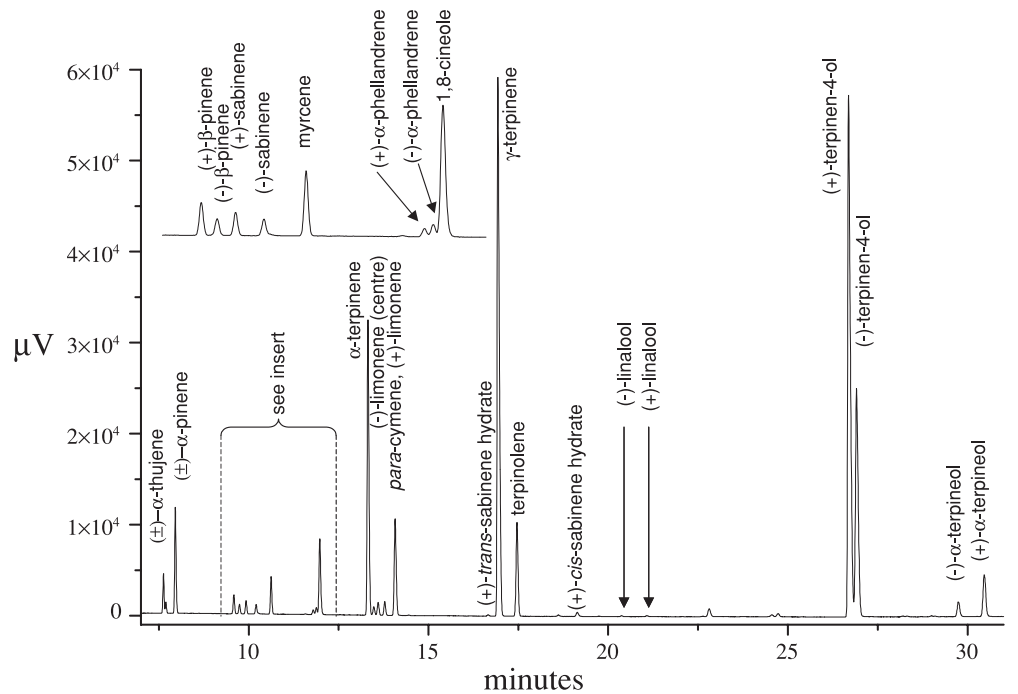

Figure 2. Typical chromatogram of a *M. Alternifolia* essential oil using method 2. The expanded insert shows the separation of (±)-sabinene, (±)-β-pinene and (±)-α-phellandrene

the achiral column second also allows for very easy changing of the second column if further separation optimization is required, without affecting the primary column result.

Table 1 lists the enantiomeric distribution of the monoterpenes and monoterpene alcohols in a range of *Melaleuca* essential oils, and the enantiomeric distribution of the monoterpenes and monoterpene alcohols in a range of *Leptospermum* and *Agonis* essential oils are reported in Table 2.

Table 1. Enantiomeric distribution [data presented as percentage (+)-isomer] of monoterpenes and monoterpene alcohols in a range of *Melaleuca* essential oils

	M. alternifolia[a]	M. alternifolia[b]	M. linarifolia	M. decussata	M. uncinata	M. armillaris	M. quinquenervia
Sabinene	42–62	56	82	29	44	22	30
β-Pinene	64–67	55	46	15	30	10	11
α-Phellandrene	39–41	40	42	59	—[e]	—[e]	—[e]
Limonene	—[c]	—[c]	—[c]	73	78	89	58
trans-Sabinene Hydrate	—[d]	—[d]	—[d]	—[e]	—[e]	—[e]	—[e]
cis-Sabinene Hydrate	Predominantly (+)	95	59	—[e]	—[e]	—[e]	—[e]
Linalool	52–68	47	36	85	74	62	99
Terpinen-4-ol	68–69	72	69	53	67	68	67
α-Terpineol	74–75	77	60	59	60	58	47

[a] Range obtained from the analysis of six *Melaleuca alternifolia* essential oils. [b] High pH distillation of flush growth (new leaves). [c] (+)-Limonene co-eluted with *para*-cymene. [d] (−)-*trans*-Sabinene hydrate co-eluted with γ-Terpinene. (+)-*cis*-Sabinene hydrate was major isomer. [e] Not detected or present as trace only. Assignment of absolute configuration based on injection of authentic reference standards.

Table 2. Enantiomeric distribution [data presented as percentage (+)-isomer] of monoterpenes and monoterpene alcohols in a range of *Leptospermum* and *Agonis* essential oils

	L. petersonii	L. polygalifolium	A. pariceps	A. juniperina	A. flexuosa	A. species swamp	A. species rose
Sabinene	33	93	44	48	67	Predominantly (−)	22
β-Pinene	9	81	6	40	26	23	30
Limonene	93	—[a]	95	88	81	77	60
Linalool	69	52	82	90	94	82	31
Terpinen-4-ol	20	73	48	51	54	57	51
α-Terpineol	43	61	68	55	62	78	70

[a] (+)-Limonene co-eluted with *para*-cymene.

Conclusions

The enantiomeric distributions of several monoterpenes and monoterpene alcohols present in 13 different essential oils from the family *Myrtaceae* were determined using a two-column configuration. The two-column experiment required only a minimum amount of method optimization, and was shown to improve the resolution of key target components from the matrix. The monoterpenes and monoterpene alcohols were eluted within ca. 30 min.

Alternative stationary phase column combinations, for example CDD/WAX, were not used here, nor were reversed-column-order experiments, but it would be useful to investigate such options to further enhance the separation of target compounds from matrix interferences. The present experimental configuration cannot match the superior resolution capabilities of multi-dimensional GC, which permits separation of target components and matrix interferences; however where there is a requirement to improve the resolution of a small number of target analytes from the matrix, a two-column one-dimensional analysis such as that described here is useful. The experiment described here is inexpensive because it requires only the existing instrumentation, and is less technically demanding than the more sophisticated multidimensional approaches. It is relatively simple to check a range of second column types if an analysis requires further separation optimization.

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