

# Enantiomeric Distribution of Five Monoterpenic Compounds in Nine Algerian *Eucalyptus* Essential Oils by Direct Multidimensional Gas Chromatography

Y. Foudil-Cherif\*

Université des Sciences et de la Technologie Houari Boumédiène, Institut de Chimie ; El-Alia BP 32, Bab-Ezzouar Alger, Algeria

B.Y. Meklati

C.R.A.P.C., BP 248 Alger RP 16004, Algeria

L. Mondello, G. Zappia and G. Dugo

Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, Italy

## Abstract

The enantiomeric distribution of sabinene,  $\beta$ -pinene, linalool, terpinen-4-ol, and  $\alpha$ -terpineol for nine *Eucalyptus* species has been determined by using a fully automated, multidimensional, double-oven (MDGC) system equipped with a SE-52 precolumn and a  $\beta$ -cyclodextrin main column. This system allows fractions to be multitransferred during the same GC analysis, and the use of the two GCs independently when the multitransfer option is rejected. The results obtained allowed a reliable differentiation between the nine eucalyptus oils.

## Key Word Index

*Eucalyptus punctata*, *Eucalyptus sideroxylon*, *Eucalyptus saligna*, *Eucalyptus cladocalix*, *Eucalyptus albens*, *Eucalyptus ovata*, *Eucalyptus leucoxylon*, *Eucalyptus blakelyi*, *Eucalyptus microcorys*, *Myrtaceae*, chiral analysis, enantiomeric distribution.

## Introduction

The specific character and the intensity of flavor and fragrance compounds is often related to their stereochemistry. In this way, the knowledge of the enantiomeric ratios of some constituents can be used as a potent genus chemotaxonomic marker (1-2). In addition, it could provide, for example, a better insight on the quality of the oil, on the isolation technique employed and on their geographic origin (3-14).

During the last decade, investigations dealing with the enantiomeric composition of the essential oils were carried out by gas chromatography (15). The availability and the increasing number of new chiral stationary phases (16-19) offer a means for direct, sensitive and accurate analysis of enantiomeric distribution of chiral components in complex mixtures.

More recently, the enantiomeric distribution of monoterpene biogenic volatile organic compounds in ambient air around Algerian *Eucalyptus globulus*, *Cedrus atlantica* and *Pinus halepensis* were evaluated by gas chromatography on a  $\beta$ -cyclodextrin chiral capillary column (20).

The direct enantiomeric separation of compounds using a simple gas chromatography technique with a single chiral

column is often difficult owing to the possible overlapping of the peaks. This difficulty can be avoided if a multidimensional gas chromatography (MDGC) is performed, since it permits the prefractionation of the sample, and the consequent chiral analysis of single components or at least to obtain simpler fractions than the whole sample, without problems of peak overlapping.

In this paper, in order to evaluate the stereoisomeric differentiation of nine Algerian *Eucalyptus* oils, the enantiomeric separation of  $\beta$ -pinene, sabinene, linalool,  $\alpha$ -terpineol, terpinen-4-ol was carried out by MDGC using a modified  $\beta$ -cyclodextrin stationary phase as main column. This system was previously used for the chiral separation of some components of citrus oils (22-26).

## Experimental

**Plant materials:** The adult leaves of the nine *Eucalyptus* species (*Eucalyptus punctata* DC, *E. sideroxylon* Cunn., *E. saligna* Smith, *E. cladocalix* F. Muell., *E. albens* Miq.ex Benth., *E. ovata* Labill., *E. leucoxylon* F. Muell., *E. Blakelyi* A. Cunn and *E. microcorys* F. Muell.) were collected from

\*Address for correspondence

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Table I. Relative amount of five monoterpenic compounds in nine Algerian *Eucalyptus* oils (21)

<i>Eucalyptus</i> Species	sabinene	$\beta$ -pinene	linalool	terpinen-4-ol	$\alpha$ -terpineol
<i>E.punctata</i>	< 0.1	4.1	0.1	0.7	1.6
<i>E.leucoxylo</i>	< 0.1	0.5	0.1	0.4	1.0
<i>E.cladocalyx</i>	0.6	0.2	0.5	1.5	0.6
<i>E.blakelyi</i>	0.2	0.1	0.1	1.2	0.3
<i>E.ovata</i>	< 0.1	0.4	2.2	0.5	10.3
<i>E.microcorys</i>	< 0.1	0.9	< 0.1	2.4	1.6
<i>E.saligna</i>	0.8	0.4	1.9	3.0	2.8
<i>E.sideroxylo</i>	< 0.1	0.2	0.1	0.5	1.3
<i>E.albens</i>	0.1	0.1	0.9	5.0	1.6

Table II. Enantiomeric ratios (%) of five monoterpenic compounds in the oils of nine Algerian *Eucalyptus* species

<i>Eucalyptus</i> Species	$\beta$ -pinene		sabinene		linalool		terpinen-4-ol		$\alpha$ -terpineol	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
<i>E.punctata</i>	3.8	96.2	39.6	60.4	88.2	11.8	36.5	63.5	49.4	50.6
<i>E.leucoxylo</i>	36.9	63.1	23.2	76.8	60.9	39.1	53.0	47.0	44.4	55.6
<i>E.cladocalyx</i>	63.2	36.8	45.4	54.6	92.9	7.1	39.4	60.6	52.1	47.9
<i>E.blakelyi</i>	24.1	75.9	52.1	47.9	46.7	53.3	39.0	61.0	<0.1	>99.9
<i>E.ovata</i>	35.3	64.7	32.6	67.4	8.0	92.0	40.4	59.6	62.6	37.4
<i>E.microcorys</i>	16.8	83.2	28.7	71.3	75.2	24.8	39.2	60.8	38.6	61.4
<i>E.saligna</i>	64.9	35.1	53.6	46.4	80.4	19.6	38.1	61.9	28.0	72.0
<i>E.sideroxylo</i>	18.5	81.5	52.1	47.9	76.9	23.1	46.0	54.0	58.8	41.2
<i>E.albens</i>	39.0	61.0	51.9	48.1	93.6	6.4	35.6	64.4	33.6	66.4

the trees located in Bainem forest (16 Km to the west of Algiers). Two or three trees of each species, 30-35 years old, were taken at random. Voucher specimens were cut off and housed in the arboretum of the Institut National de la Recherche Forestière (INRF). The oils were isolated from the fresh leaves by hydrodistillation. The qualitative and quantitative analyses of each oil were carried out using GC and GC/MS techniques (21).

### Instrumentation

The multidimensional system used consisted of two Shimadzu 17A gas chromatographs and a hot transfer line. The experimental conditions used were as follows.

**Gas chromatograph 1:** The chromatograph was equipped with two split/splitless injectors with two manual flow controllers. A fused silica SE - 52 capillary column (30 m x 0.32 mm, 0.40-0.45  $\mu$ m film thickness (MEGA, Legnano, Italy) was used as a pre-column. The oven temperature was programmed from 45°C isothermal for 6 min, then at 2°C/min to 220°C for 20 min, then at 10°C/min to 280°C for 5 min. The injector and detector (FID) temperature were 250°C. Helium was used as a carrier gas (90 kPa). An integrator Shimadzu C-R3A was connected to start and out signals on the gas chromatograph 1.

**Transfer line:** An aluminium thermoregulated block (200°C) equipped with a heater assay and a thermocouple assay was connected to the temperature auxiliary exit on GC-1.

**Gas chromatograph 2:** A Mega Dexdettbs  $\beta$  (Diethyltert-butylsilyl- $\beta$ -cyclodextrin) capillary column, 25 m x 0.25 mm,

0.25  $\mu$ m film thickness (MEGA, Legnano, Italy) was used as the main column. The oven temperature was programmed from 45°C isothermal for 6 min, then to 95°C at 2°C/min for 20 min, then at 2°C/min to 180°C for 15 min. The detector (FID) temperature was 280°C. Helium was used as a carrier gas (110 kPa). An integrator Shimadzu C-R3A was connected to start and out signals on gas chromatograph 2.

The nine oils and the reference compounds were analyzed by injecting 1  $\mu$ L of a pentane solution (1:100, V/V).

**Reference compounds:** The elution order of the enantiomers was assigned using enantiomerically pure reference compounds of definite chirality. The enantiomers of  $\beta$ -pinene, sabinene, linalool,  $\alpha$ -terpineol and terpinen-4-ol were purchased from Fluka (Italy).

### Results and Discussion

For each oil, the relative amount of the five monoterpenic compounds as reported in a previous paper (21) are shown in Table I. Table II reports the enantiomeric distribution of the analyzed components in the nine *Eucalyptus* oils.

As can be seen from data reported in Tables I and II, the quantitative amount and also the enantiomeric distribution of the five components analyzed in the nine oils is very different.

Under the chromatographic conditions applied for the simultaneous stereodifferentiation of genuine chiral compounds of *Eucalyptus* oils,  $\beta$ -pinene and sabinene are co-eluted in the nonchiral pre-column [Figure 1(a)]. These compounds are well separated and resolved into their enantiomers on the chiral main column [Figure 1 (b)] used in this investigation.

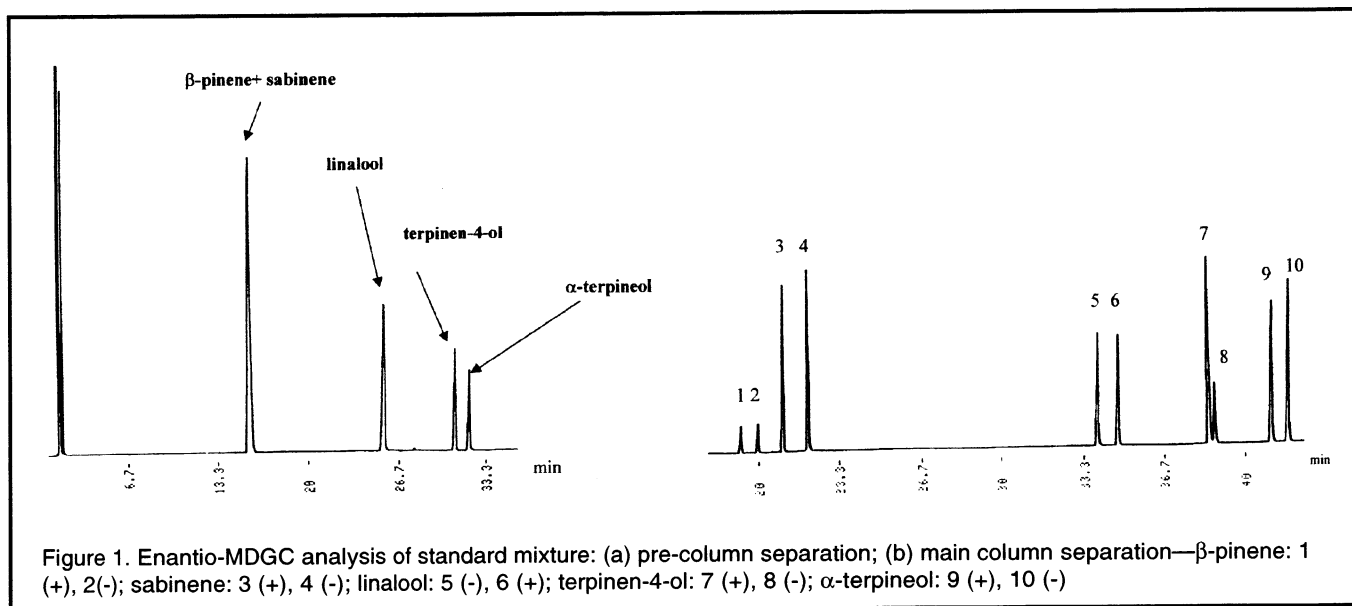


Figure 1. Enantio-MDGC analysis of standard mixture: (a) pre-column separation; (b) main column separation— $\beta$ -pinene: 1 (+), 2(-); sabinene: 3 (+), 4 (-); linalool: 5 (-), 6 (+); terpinen-4-ol: 7 (+), 8 (-);  $\alpha$ -terpineol: 9 (+), 10 (-)

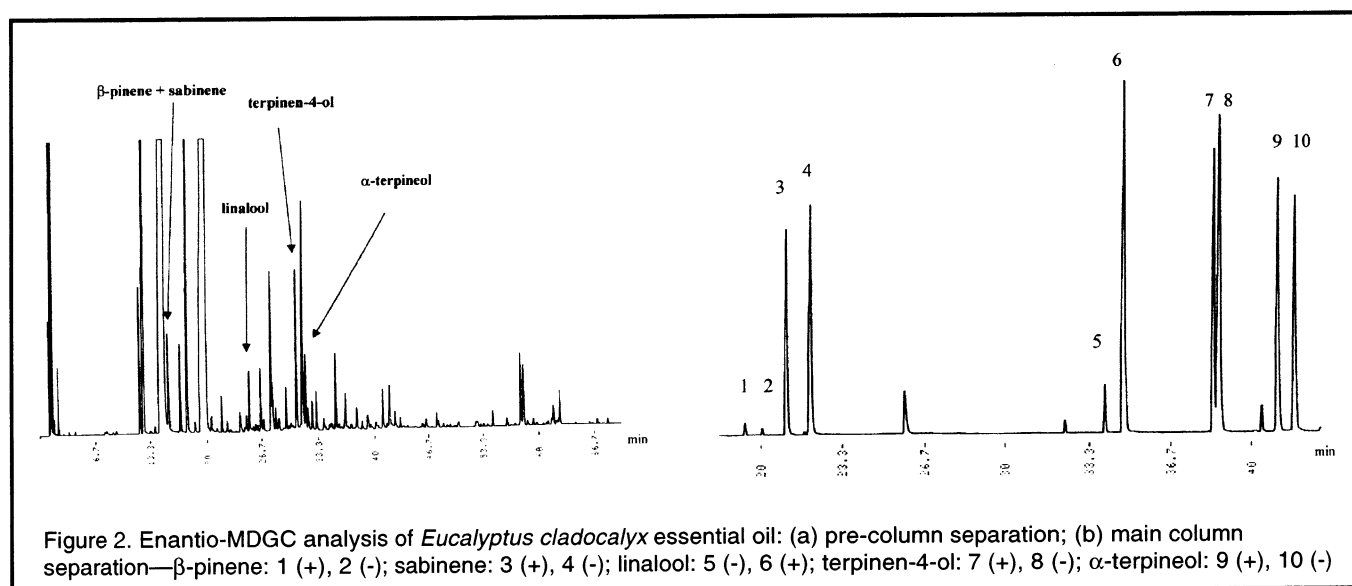


Figure 2. Enantio-MDGC analysis of *Eucalyptus cladocalyx* essential oil: (a) pre-column separation; (b) main column separation— $\beta$ -pinene: 1 (+), 2 (-); sabinene: 3 (+), 4 (-); linalool: 5 (-), 6 (+); terpinen-4-ol: 7 (+), 8 (-);  $\alpha$ -terpineol: 9 (+), 10 (-)

As an example, Figures 2(a) and 3(a) report the chromatograms of *E. cladocalyx* and *E. microcorys* oils obtained with the pre-column (SE-52). The chromatograms obtained with the chiral column for the fractions transferred from the SE-52 pre-column are shown in Figures 2(b) and 3(b).

As can be seen from Table II, (-)- $\beta$ -pinene is the most abundant isomer in many of the analyzed oils: *E. punctata*, *E. leucoxydon*, *E. blakelyi*, *E. ovata*, *E. microcorys*, *E. sideroxydon* and *E. albens*. The highest percentage was found in *E. punctata* (96.2%).

Regarding the enantiomeric distribution of sabinene, the levorotatory form was detected as major enantiomer in *E. punctata*, *E. leucoxydon*, *E. ovata* and *E. microcorys*. In all the other oils, a distribution very similar to a racemic mixture was found.

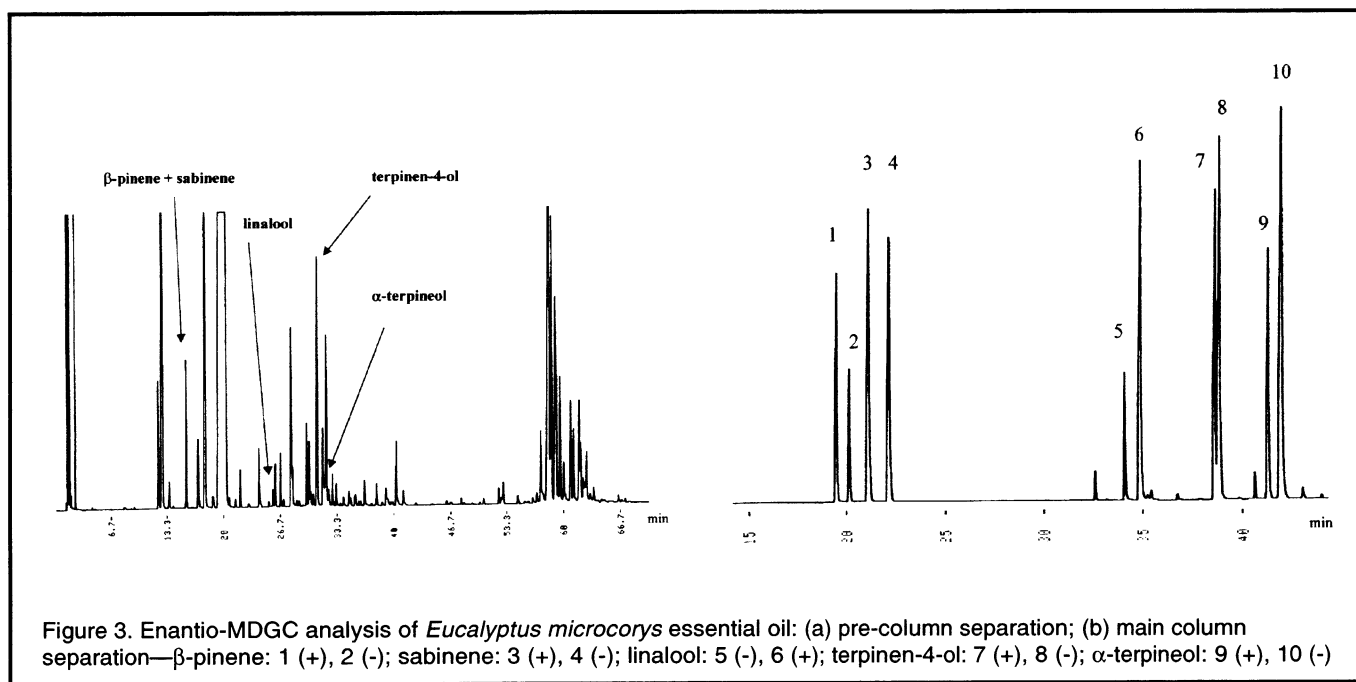
It is well known from the literature (8) that the linalool enantiomers differ considerably in odor. The (-)-linalool

has a typical and powerful petitgrain oil fragrance, whereas the (+) form possesses a woody fragrance and typical lavender note.

Table II shows that the (+)-linalool prevails in seven of the nine oils investigated; the higher amount was detected in *E. albens* (93.6%) and *E. cladocalyx* (92.9%). For *E. ovata* the (-)-linalool was predominant with 92.0%, and in *E. blakelyi* the racemic mixture was found.

The enantiomeric separation and enantiomeric distribution of terpinen-4-ol in *E. globulus* was reported in the literature (8). It was found that this oil contains an excess of (+)-terpinen-4-ol. In this study, the (-)-terpinen-4-ol prevailed in all the oils, except for *E. leucoxydon* and *E. sideroxydon* ones where the two enantiomeric forms had nearly the same ratio, very near to the racemate.

For  $\alpha$ -terpineol, the (-) form prevailed in *E. blakelyi*, *E. microcorys*, *E. saligna*, and *E. albens*. In contrast the (+) form



was predominant in *E. ovata* and *E. sideroxydon*. It is noteworthy that the (-)- $\alpha$ -terpineol was found with an excess of more than 99% in *E. blakelyi*. Approximately the racemic mixture of  $\alpha$ -terpineol was found in *E. punctata*, *E. leucoxydon* and *E. cladocalyx*.

It can be concluded that the simultaneous stereodifferentiation of the five *Eucalyptus* oil constituents was achieved by using a MDGC system. The characteristic difference in the nine *Eucalyptus* oils can be deduced from this enantio-MDGC analysis. The enantiomeric variation established allows a reliable differentiation of the nine *Eucalyptus* species with regard to the enantiomeric distribution of  $\beta$ -pinene, linalool and terpinen-4-ol. In addition, the enantiomeric ratio of (-)- $\alpha$ -terpineol in *E. blakelyi* [(+)/(–)- $\alpha$ -terpineol <0.1/>99.9%] and (-)-linalool in *E. ovata* [(+)/(–)-linalool 8/92] can be used as a chemotaxonomic marker for these species.

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