

## **Approach to direct chiral recognition of some terpenic hydrocarbon constituents of essential oils by gas chromatography systems via $\alpha$ -cyclodextrin complexation**

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### ABSTRACT

The application of a very selective chiral stationary phase (Celite coated with  $\alpha$ -cyclodextrin in formamide solution) in gas chromatography is described. Using this phase it is possible to separate enantiomeric mixtures of  $\alpha$ -pinene,  $\beta$ -pinene, limonene and camphene. The separation of these compounds in essential oils is also demonstrated.

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### INTRODUCTION

Terpenes are an important group of compounds found in large amounts in plants. For the separation and analysis of their very complex mixtures derived from natural sources such as essential oils, gas chromatography (GC) is the method of choice, especially for compounds with boiling points ranging from 140 to 300°C. Nevertheless, owing to the large number of possible isomers within each group of terpenes and the lack of pure standard compounds, the problem of the analysis of essential oils is too difficult to solve by simple classical GC methods [1]. For this reason, improved GC techniques have been applied, *e.g.*, capillary GC, GC–mass spectrometry [2,3] and GC–Fourier transform infrared spectrometry [4–6].

However, many of the terpenes are chiral compounds and hence they can be present in natural mixtures in one or two enantiomeric forms and in various proportions. The chirality of the terpenes may have a strong influence on their various biological interactions because antipodes often differ in their biological activity. This phenomenon is understandable in view of the high stereospecificity required by a “lock-and-key” type of relationship between two partners, when one of them has a rigidly defined chirality. As a consequence, the important problem of chiral

discrimination leads to additional difficulties in the analysis of essential oils, especially when terpenic hydrocarbons are considered. To our knowledge, the chromatographic separation and direct chiral recognition of terpenic hydrocarbons in essential oils have not previously been attempted, mainly because of the lack of convenient enantioselective chromatographic stationary phases.

We found previously that using  $\alpha$ -cyclodextrin ( $\alpha$ -CD) under appropriate conditions of partition GC, very efficient separations of  $\alpha$ -pinene and  $\beta$ -pinene into enantiomers can be achieved [7]. Recently, efficient separations of enantiomers of  $\alpha$ -pinene and limonene achieved using permethylated  $\beta$ -cyclodextrin [8] or  $\alpha$ -cyclodextrin [9] and the separation of enantiomeric camphenes [10] have been reported. The applications of cyclodextrins and their derivatives for the separation of enantiomers under GC conditions have been thoroughly reviewed by Schurig and Nowotny [11]. This paper reports our attempts to apply this method using  $\alpha$ -CD for the chiral recognition of the above-mentioned enantiomeric hydrocarbons when they are present in complex mixtures such as essential oils.

Two essential oils from species of the conifer family Pinaceae were studied, silver fir needle oil obtained from *Abies alba* Mill. and pine needle oil obtained from *Pinus sylvestris* L. The chemical compositions of both oils have recently been studied by several workers [12–15], terpenic hydrocarbons, *i.e.*,  $\alpha$ -pinene,  $\beta$ -pinene, limonene and camphene, were found to be the main components.

## EXPERIMENTAL

### Reagents

$\alpha$ -,  $\beta$ - and  $\gamma$ -CD were supplied by Chinoin (Budapest, Hungary). Celite (30–80 mesh) for GC was from BDH (Poole, U.K.). Silver fir needle oil and pine needle oil obtained by steam distillation of needles of *Abies alba* Mill. and *Pinus sylvestris* L., respectively, were from Herbapol (Łódź, Poland). All other materials were of analytical-reagent grade and were used without further purification.

### Apparatus and procedure

Chromatographic studies were performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a dual flame ionization detector. The peak areas and retention times were measured by means of a Hewlett-Packard 3390A integrator. Glass columns (2 m  $\times$  4 mm I.D.) were used. The compounds (0.02–0.15  $\mu$ l) were injected with Hamilton microsyringes separately or as mixtures. A constant inlet pressure ( $2.75 \pm 0.05$  atm) and helium flow-rate ( $40 \pm 0.5$  ml/min) were maintained. The column packings, *i.e.*, Celite (30–80 mesh) coated with a formamide solution of  $\alpha$ -CD,  $\beta$ -CD and  $\gamma$ -CD, were prepared as described previously [16]. The amounts of formamide (4.54 g) and Celite (20 g) were constant. Stationary phases contained CDs as follows: I, none (control); II,  $\alpha$ -CD (0.79 mol%); III,  $\beta$ -CD (0.23 mol%); and IV,  $\gamma$ -CD (0.79 mol%). The amount of coated support in each analytical column was *ca.* 11 g.

In contrast to the behaviour of  $\beta$ -CD and  $\gamma$ -CD,  $\alpha$ -CD forms an efficient separating agent under GC conditions when its formamide solution contains 3–4% of water [16]. Consequently, the stationary phase for column II contained 4% of water and lithium nitrate (0.45 g) was added as the stabilizing agent. The contents of

formamide and water in the final packings were determined by thermogravimetric analysis with a DuPont Model 1090 thermal analysis system.

## RESULTS AND DISCUSSION

### Preliminary studies

In separate sets of experiments performed using a supplementary column with glycerine as stationary phase in the temperature range 70–80°C, it was found that the content of monoterpene hydrocarbon was 88% in pine needle oil and 82% in fir needle oil. These data correspond with the earlier results of thermogravimetric studies [17]. These hydrocarbon fractions were the main objectives of our investigations. The remaining, more polar, terpenic derivatives in the oils (12 and 18%, respectively) were strongly adsorbed on columns containing formamide medium (at 30°C) and were not eluted in the form of distinguishable peaks.

### Artificial mixtures

Fig. 1 shows the chromatogram of an artificial mixture of enantiomers of  $\alpha$ -pinene,  $\beta$ -pinene, camphene and limonene obtained on chiral discriminating column II. A chromatogram of the same mixture obtained on the reference achiral column with pure formamide (I, F) is presented in Fig. 2.

Mixture I studied was prepared from pure compounds or from compounds of known composition and previously determined optical purity and contained, respectively, (+)- and (–)- $\alpha$ -pinene (13% and 5%), (+)- and (–)- $\beta$ -pinene (6% and 22%), (+)- and (–)-limonene (23% and 19%) and (+)- and (–)-camphene (5% and 7%). The adjusted retention times of the compounds on columns with  $\alpha$ -CD,  $\beta$ -CD and  $\gamma$ -CD are given in Table I. The data indicate that only  $\alpha$ -CD recognizes distinctly enantiomers on the investigated monoterpene hydrocarbons. In contrast, no distinguishable enantioselectivity could be observed for  $\beta$ -CD and  $\gamma$ -CD, although their

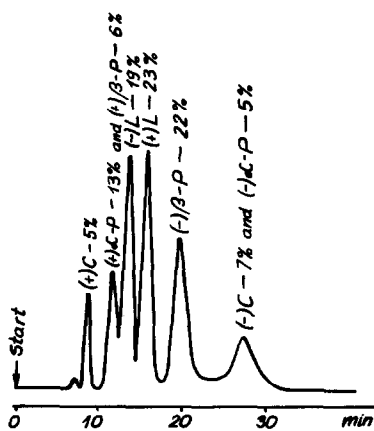


Fig. 1. Chromatogram of artificial mixture I containing (+)- and (–)- $\alpha$ -pinene ( $\alpha$ -P), (+)- and (–)- $\beta$ -pinene ( $\beta$ -P), (+)- and (–)-limonene (L) and (+)- and (–)-camphene (C) obtained at 30°C on column II. Injected sample, 0.12  $\mu$ l.

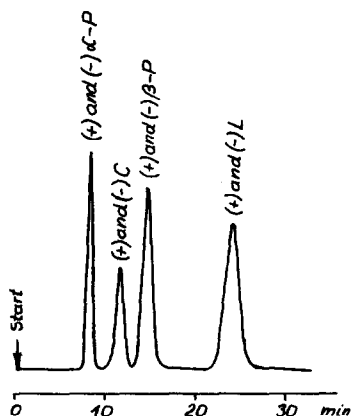


Fig. 2. Chromatogram of mixture I obtained at 30°C on reference column I. Injected sample, 0.04  $\mu$ l.

complexes with  $\alpha$ - and  $\beta$ -pinene, camphene and limonene are much more stable than the corresponding complexes with  $\alpha$ -CD.

For this reason, higher temperatures and lower  $\beta$ -CD concentration were applied to elute these compounds at reasonable retention times from  $\beta$ -CD (0.23 mol%) and  $\gamma$ -CD (0.79 mol%) columns, as can be seen in Table I. Overall these data seem to be satisfactory.

Under the experimental conditions very efficient discrimination of particular compounds can be achieved via  $\alpha$ -CD complexation at 30 and 40°C on column II, *e.g.*, at 30°C with a separation factor  $\alpha_{-/+} = 2.4$  for  $\alpha$ -pinene, 1.7 for  $\beta$ -pinene, 3.2 for camphene and  $\alpha_{+/-} = 1.2$  for limonene. Such high separation factors for enantiomers are very rarely encountered, especially when hydrocarbons are involved.

The chromatogram in Fig. 1 shows that the situation is less satisfactory for mixtures. In fact (+)-camphene, (+)-limonene, (-)-limonene and (-)- $\beta$ -pinene are

TABLE I

ADJUSTED<sup>a</sup> RETENTION TIMES ( $t'_R$ , min) OF ENANTIOMERS OF  $\alpha$ -PINENE,  $\beta$ -PINENE, LIMONENE AND CAMPHENE DETERMINED AT 30°C AND/OR 60°C COLUMNS I-IV

Compound	Column I		Column II, 30°C	Column III, 60°C	Column IV, 60°C
	30°C	60°C			
(+)- $\alpha$ -Pinene	8.0	1.9	11.4	10.0	13.0
(-)- $\alpha$ -Pinene	8.0	1.9	22.6	10.0	13.0
(+)-Camphene	11.4	2.4	8.5	20.7	35.2
(-)-Camphene	11.4	2.4	22.6	20.7	35.2
(+)- $\beta$ -Pinene	14.4	2.8	11.4	23.6	28.8
(-)- $\beta$ -Pinene	14.4	2.8	19.5	23.6	28.8
(+)-Limonene	23.9	3.9	15.5	17.9	9.3
(-)-Limonene	23.9	3.9	13.3	17.9	9.3

<sup>a</sup> Adjusted with the dead time of the column,  $t_0$  ( $t'_R = t' - t_0$  where  $t'$  is the observed retention time).

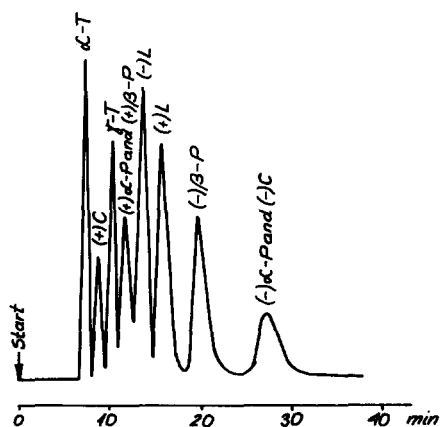


Fig. 3. Chromatogram of artificial mixture II, *i.e.*, mixture I supplemented with  $\alpha$ -terpinene ( $\alpha$ -T) and  $\gamma$ -terpinene ( $\gamma$ -T), obtained at 30°C on chiral column II with  $\alpha$ -CD. Injected sample, 0.12  $\mu$ l.

eluted as separate peaks and thus they can be directly identified and determined. (+)- $\alpha$ -Pinene give a combined peak with (+)- $\beta$ -pinene and (-)- $\alpha$ -pinene gives a combined peak with (-)-camphene; hence they can only be determined as a sum. In order to overcome this difficulty, a supplementary achiral column was used, working in parallel, which allowed the separation of  $\alpha$ -pinene,  $\beta$ -pinene and camphene. As indicated in Table I and from literature data, the problem of the separation of these three hydrocarbons is relatively simple and for this purpose reference column I (F) can be used. Further confirmations can be obtained by using column III (0.23 mol%  $\beta$ -CD) or IV (0.79 mol%  $\gamma$ -CD).

The results obtained on two columns ( $\alpha$ -CD and reference) may serve as a basis for further approximate evaluations of the relative amounts of (+)- $\alpha$ -pinene and (+)- $\beta$ -pinene in a mixture of unknown composition.

Preliminary studies of essential oils and literature data suggest that in addition to the above-mentioned compounds, other compounds may also be present, including achiral molecules. In order to investigate this problem, we studied mixture I supplemented with various terpenic hydrocarbons. This preliminary identification of unknown peaks is exemplified by Fig. 3.

#### Essential oils

Figs. 4 and 5 show chromatograms of pine needle oil and fir needle oil, respectively, obtained on column II (0.23 mol%  $\alpha$ -CD) under similar conditions to those applied for standard mixtures (Figs. 1 and 3). Table II gives the contents of the main components as identified by direct measurements on the  $\alpha$ -CD column and Table III those on the reference column. It is seen that the main components of fir needle oil (overall *ca.* 75.8%) are laevorotatory monoterpene hydrocarbons: (-)-limonene (27.7%), (-)- $\beta$ -pinene (14.4%), (-)- $\alpha$ -pinene and (-)-camphene (33.7%). In contrast, in the pine needle oil dextrarotatory hydrocarbons dominate (overall *ca.* 59.1%): (+)- $\alpha$ -pinene and (+)- $\beta$ -pinene (56.3%), (+)-camphene (0.9%) and (+)-limonene (1.9%).

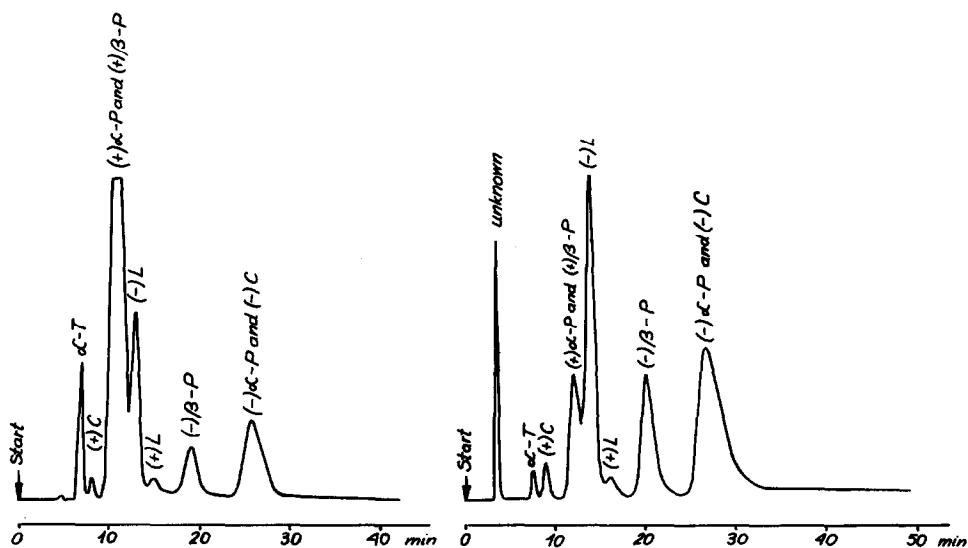


Fig. 4. Chromatogram of a 0.15- $\mu$ l sample of pine needle oil obtained at 30°C on chiral column II with  $\alpha$ -CD.

Fig. 5. Chromatogram of a 0.12- $\mu$ l sample of fir needle oil obtained at 30°C on chiral column II with  $\alpha$ -CD.

TABLE II

CONTENTS (%) OF THE MAIN COMPONENTS OF PINE NEEDLE OIL AND FIR NEEDLE OIL (IN %) AS DETERMINED ON CHIRAL COLUMN II WITH  $\alpha$ -CD

Conditions as in Table I.

Compound	Pine needle oil	Fir needle oil
$\alpha$ -Terpinene	5.3	1.3
(+)-Camphene	0.9	1.2
(+)- $\alpha$ -Pinene and (+)- $\beta$ -pinene	56.3	10.7
(-)-Limonene	12.4	27.7
(+)-Limonene	1.9	1.2
(-)- $\beta$ -Pinene	5.8	14.4
(-)- $\alpha$ -Pinene and (-)-camphene	13.7	33.7

TABLE III

CONTENTS (%) OF THE MAIN COMPONENTS OF PINE NEEDLE OIL AND FIR NEEDLE OIL AS DETERMINED ON REFERENCE COLUMN I

Conditions as in Table I.

Compound	Pine needle oil	Fir needle oil
$\alpha$ -Terpinene	4.5	1.5
(+)- $\alpha$ -Pinene and (-)- $\alpha$ -pinene	35.8	35.1
(+)- $\beta$ -Pinene and (-)- $\beta$ -pinene	38.3	14.8
(+)-Limonene and (-)-limonene	14.5	27.2
(+)-Camphene and (-)-camphene	0.9	1.2

The results presented above suggest that the difficult problem of the direct recognition of enantiomers of particular terpenic hydrocarbons present in essential oils could be resolved on the basis of  $\alpha$ -CD complexation. To achieve this goal very detailed optimization studies should be performed.

#### ACKNOWLEDGEMENT

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