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Chromatographic analysis of some commercial samples of camphene via cyclodextrin inclusion processes

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ABSTRACT

A gas-liquid chromatographic system containing a-cyclodextrin in formamide medium (coated on Celite) was found to be useful for the separation of camphene enantiomers, with a separation factor a $[t'_R(-)/t'_R(+)]$ equal to 3.6 (at 30°C). The packing was applied in the analytical mode for a study of the composition of five commercial camphenes, and in the micropreparative mode to establish the enantiomeric composition of separated camphenes.

INTRODUCTION

Terpenes are found in large amounts in most plants, and are of special interest because of their industrial uses and on account of their chemical properties and biological activity. The very large number of possible stereoisomers within each group of terpenes makes it very difficult to obtain pure reference compounds, especially enantiomers. In fact, most commercially available terpenes derived from natural sources are of variable composition and variable enantiomeric purity. Hence methods for their exact analysis and the determination of their enantiomeric purity are of crucial importance.

Nevertheless, the chromatographic separation of enantiomeric hydrocarbons presents a number of difficulties, because they are very resistant to diastereoisomer formation. The use of optically active organic stationary phases cannot be applied on the basis of the 'three-point attachment' concept of Dalgliesh [1] because hydrocarbon molecules do not contain any functional groups able to form the necessary hydrogen bonds for specific attachment of enantiomers.

Recently, chiral metal complexes were applied in high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC) for the resolution of some olefins into enantiomers [2–7]. However, in this instance n-donor-acceptor interactions, being possible only for unsaturated hydrocarbons, take place. Chiral saturated hydrocarbons cannot be recognized in this way.

Subsequently it was discovered that the stereoselective fitting of α- and β-pi-

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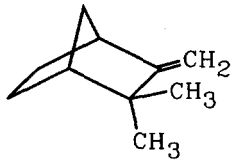
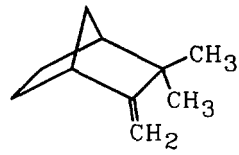
nenes and *cis*- and *trans*-pinanes to the a-cyclodextrin (a-CD) cavity permits the very efficient resolution of their enantiomers under GLC conditions [8–11]. Another approach was initiated recently by König and co-workers [12,13] and subsequently by Armstrong and co-workers [14,15] involving the use of some molten CD derivatives of relatively low fusibility in capillary GC. Although numerous efficient separations of various chiral compounds have been reported [12–15], only one example concerns terpenoic hydrocarbons, viz., limonene.

This paper reports some further systematic studies on the application of CD solutions to enantioselective separations of various hydrocarbons and it focuses attention on the enantiomeric camphenes.



(+)-camphene



(-)-camphene

EXPERIMENTAL

Reagents

CDs were supplied by Chinoin (Budapest, Hungary), Celite for GLC by BDH (Poole, U.K.) and samples of camphenes by Fluka (Buchs, Switzerland), Aldrich (Milwaukee, WI, U.S.A.), S.C.M. Glidco (Jacksonville, FL, U.S.A.) and Chemipan (Warsaw, Poland). All materials were used as received.

Apparatus and procedures

Analytical chromatographic studies were performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with duel flame ionization detectors. The peak areas and retention times were measured by means of a Hewlett-Packard Model 3390A integrator. Glass columns (2 m × 4 mm I.D.) were used. The compounds were injected with Hamilton microsyringes (0.02 μl). A constant inlet pressure (2.75 ± 0.05 atm) and helium flow-rate (40 ± 0.5 ml/min) were maintained.

Micropreparative separations were performed on a Hewlett-Packard Model 7620A gas chromatograph, adapted for this purpose, equipped with a thermal conductivity detector and a glass column (8 m × 7 mm I.D.) [16].

The amount of coated support in each analytical column was *ca.* 11 g, and the micropreparative column was filled with *ca.* 100 g of packing.

The column packings, *i.e.* Celite (30-80 mesh) coated with a formamide solution of *a*-, *β*- or y-CD, were prepared as described previously [8]. The amounts of formamide (4.54 g) and Celite (20 g) were constant. The amounts of CDs used were as follows: (I) none (reference); (II) a-CD, 0.60 g (0.79 mol%); (III) *β*-CD, 0.23 g (0.22 mol%); and (IV) y-CD, 0.90 g (0.79 mol%).

As indicated previously [17], an a-CD then acts as an efficient separating agent under GLC conditions when its formamide solution contains 3–4% of water. Hence

the packing for column II contained cu. 4% of water and lithium nitrate (0.45 g) was added as a stabilizing agent. The contents of formamide and water in the final column packings were determined by thermogravimetric analysis with a DuPont Type 1090B apparatus.

Optical rotation was measured using a JASCO DIP 360 polarimeter.

^{1}H and ^{13}C NMR spectra were recorded with a Bruker AM-500 spectrometer at 500.14 and 125.76 MHz, respectively.

RESULTS AND DISCUSSION

Analytical approach

Fig. la shows a chromatogram obtained on the chiral column II containing α-CD solution (0.79 mol%) in formamide medium.

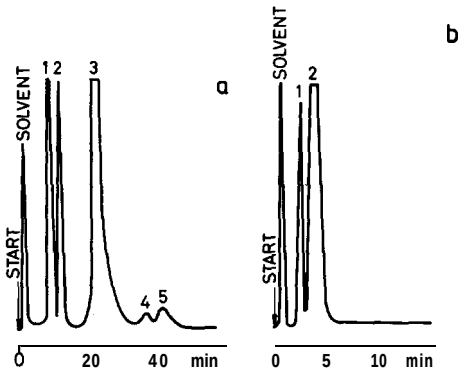


Fig. 1. Chromatograms of a heptane solution of (+)-camphene from Fluka obtained at 40°C on (a) chiral column II (2 m × 4 mm I.D.) loaded with Celite (30–80 mesh) covered with a solution of a-CD (0.79 mol%) in formamide and (b) a reference achiral column with Celite (30-80 mesh) covered with formamide only. Injected samples, 0.02 μl; helium flow-rate, 40 ml/min.

The sample was (+)-camphene from Fluka, dissolved in heptane. It is seen that in addition to the peak of the solvent, several peaks were unregistered. At first, it was very difficult to identify the maxima of elution of camphene enantiomers from chiral column II or even to answer the question of whether resolution of the enantiomers had been achieved or not. In order to elucidate this matter, a second reference achiral column was used, working in parallel, as shown in Fig. 1 b. This achiral reference column, containing pure matrix medium, *i.e.*, formamide, should permit the elution of the sum of (+)- and (−)-camphene. In this simplified consideration, impurities by other hydrocarbons that may be eluted together with camphene were not taken into account. Thus, on the basis of the results shown in Fig. la and b, it was possible to suggest tentatively that maybe peaks 1 and 3 in Fig. la and 2 in Fig. 1 b are the suspected ones, but it was very uncertain. For this reason we undertook further studies on other camphene samples, derived from different sources. Their chromatograms, obtained on chiral column II (0.79 mol% α-CD), are presented in Fig. 2.

The adjusted retention times (t'_R) of the components of the investigated samples

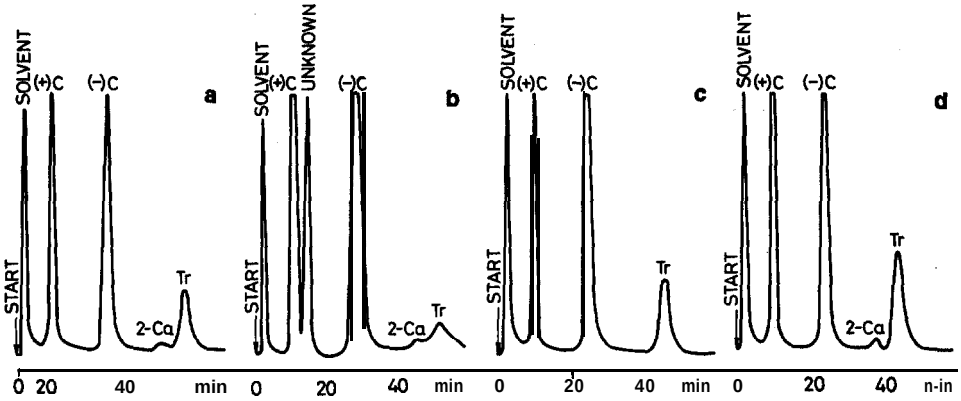


Fig. 2. Chromatograms of heptane solutions of camphenes obtained under the same conditions as in Fig. la. (+)C, (+)-camphene; (−)C, (-)-camphene; 2-Ca, 2-carene; Tr, tricyclene. (a) (+)-Camphene from Aldrich; (b)(−)-camphene from Aldrich; (c) camphene from S.C.M. Glidco; (d) camphene from Chemipan.

TABLE I

ADJUSTED RETENTION TIMES (t'_R) OF THE MAIN COMPONENTS OF THE COMMERCIAL CAMPHENES DETERMINED AT 40°C ON CHIRAL COLUMN II (0.79 mol% κ-CD) AND THEIR CONTENTS EVALUATED FROM THE PEAK AREAS

Flow-rate, 40 ml/min

Source	t'_R (min)	Content (%)
Aldrich (+)	5	60
	7	
	14	28
	23	1
	26	11
Aldrich (−)	5	40
	7	13
	14	43
	23	1
	26	6
Chemipan	5	56
	7	–
	14	31
	23	1
	26	12
Fluka	5	42
	7	11
	14	44
	23	1
	26	3
S.C.M. Glidco	5	41
	7	2
	14	43
	23	–
	26	13

as eluted from the a-CD column, and their percentage contents calculated from peak areas, are given in Table I. In the calculations the share of the solvent was subtracted and it was not taken into account.

Total declared and found contents of (+)- and (-)-camphene enantiomers are given in Table II. The found values were evaluated at 60°C using the y-CD (0.79 mol%) column IV, which separates camphene from impurities of other terpenoic hydrocarbons especially well.

TABLE II

TOTAL CONTENTS OF (+)- AND (-)-CAMPHENE DETERMINED AT 60°C ON COLUMN IV WITH y-CD (0.79 mol%)

source	Total content of camphene (%)	
	Declared	Determined
Aldrich (+)-	80	79
Aldrich (−)-	95	80
Chemipan	Not given	75
Fluka	90-95	83
S.C.M. Glidco	Not given	76

The chromatograms in Figs. 1 and 2 and the data in Tables I and II lead to the conclusion that in fact peaks with adjusted retention times of 5 and 14 min (peaks 1 and 3, respectively, in Fig. la) correspond to enantiomers of camphene. Nevertheless, the absolute configuration of the enantiomeric camphenes, *i.e.a,* the sequence of their elution, remained unsolved. Our earlier experiments on *a-* and *β*-pinenes and *cis-* and *trans-*pinanes indicated that the compounds of *R* configuration form more stable complexes with a-CD than those of S configuration. In order to make this aspect clear, we have indicated the enantiomers of camphene in Fig. 2 according to this supposition, although this statement precedes true verification, which will be discussed late.

Enantiomeric compositions found using column II with 0.79 mol% a-CD are presented in Table III.

In fact the enrichment of the samples denoted (+) or (-) is poor, and they seemed to be closer to a racemic mixture. From a comparison of the data in Tables II and III, it is seen that the total contents of camphene determined on the y-CD column IV are always smaller than those found as the sum of (+) and (−)-camphenes using the a-CD column II. This may suggest that some small impurities are eluted from column II together with enantiomers. In some instances rationalization of the measured optical rotation with the true enantiomeric composition suggests that there must be another component involved, imposing its optical activity. This may be exemplified by camphene from Fluka or (−)-camphene from Aldrich.

In additional chromatographic runs we have identified peaks with retention times (Figs. la and 2a-d) of 23 and 26 min as 2-carene (2-Ca) and tricyclene (Tr), respectively, while that with a retention time of 7 min remained unidentified.

The values of the separation factor $a\,[t'_R(-)/t'_R(+)]$ determined on column II

TABLE III

ENANTIOMERIC COMPOSITION OF (+)- AND (-)-CAMPHENE IN THE SAMPLES INVESTIGATED

Source	Optical rotation (°)		Enantiomeric composition from chromatograms evaluated on a-CD column II	
	Declared	Measured directly	(+)-	(−)-
Aldrich (+)-	+ 12.6	+ 13.7	60	28
Aldrich (−)-	- 6.6	− 20.7	40	43
Chemipan	Not given	+ 10.7	56	31
Fluka	+17	− 19.5	42	44
S.C.M. Glidco	Not given	-21.5	41	43

(a-CD) at different temperatures are given in Table IV. Two aspects are notable. The first is the remarkable enantioselectivity of the α-CD inclusion process towards camphene, e.g., $\alpha = 3.6$ at 30°C. Such values are very rarely achieved for enantiomers, where separation factors of the order of 1. l-l .2 are considered satisfactory. Second, the considerable changes in the values of the selectivity factor with temperature should be mentioned. This phenomenon may suggest that the enantioselective differentiation processes of camphenes via a-CD complexation are mainly enthalpic.

Micropreparative approach

Micropreparative attempts were made to confirm the absolute configuration of enantiomeric camphenes, ***i.e.,*** to verify the sequence of their elution from the α-CD column assumed above and to identify the compound denoted (Figs. 1 and 2) as unknown on the chromatograms.

Fig. 3 shows an example of the separation of 10 μl of *ca.* 60% camphene in heptane solution, performed on a micropreparative scale. The inset shows a chromatogram of a heptane solution of a collected fraction of (-)-camphene.

Starting from the Fluka camphene, using the micropreparative approach, we obtained (+)-camphene of 82% optical purity and of 90% chemical purity (both

TABLE IV

VALUES OF ENANTIOSELECTIVITY FACTOR a AT DIFFERENT TEMPERATURES

Temperature (°C)	a
30	3.6
35	3.1
40	2.9
45	2.3

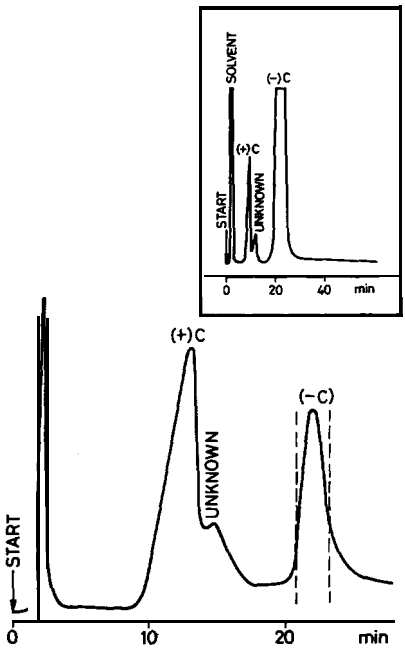


Fig. 3. Micropreparative separation of 10 μl of camphene from Fluka at 40°C on a column (8 m × 1 cm I.D.) filled with Celite (30–80 mesh) covered with a-CD solution (0.59 mol%) in formamide. Helium flow-rate, 40 ml/min. Inset: chromatogram of indicated collected fractions of (−)-camphene.

determined chromatographically) and (−)-camphene of 94% optical purity and 98% chemical purity (both determined chromatographically). The optical rotations of (+)- and (-)-camphene were $[\alpha]_D^{20}$ = + 7.8" (c = 1, CCl_4) and $[\alpha]_D^{20}$ = − 8.5" ($c = 1$, CCl_4), respectively. Supplementary ^1H and ^{13}C NMR spectra confirmed the above-mentioned purities.

Hence the earlier assumed absolute configuration of camphenes appeared unequivocally to be correct. Unfortunately, our attempts to identify the 'unknown' compound have so far failed. Nevertheless, both the contents of camphenes in the collected fractions and their optical purity seem to be worthy of mention, especially with regard to our analytical data for commercial samples.

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REFERENCES

1 C. E. Dalgliesh, *J. Chem. Soc.*, (1952) 3940.
2 V. Schurig, *Angew. Chem., Int. Ed. Engl., 16 (1977)* 110.
3 V. Schurig, *Chromatographia, 13 (1980) 263.*

4 J. Köhler and G. Schomburg, *Chromatographia*, 14 (1981) 559.
5 J. Köhler and G. Schomburg, *J.* Chromutogr., 255 (1983) 331.
6 J. Kiihler, A. Deege, G. Schomburg, *Chromutogruphiu, 18 (1984)* 119.
7 V. Schurig and R. Weber, *J. Chromutogr., 289 (1984) 321.*
8 T. Kościelski, D. Sybilska and J. Jurczak, *J. Chromutogr., 280 (1983) 131.*
9 T. Kościelski, D. Sybilska, S. Belniak and J. Jurczak, *Chromutogruphiu, 19 (1984) 292.*
10 T. Kościelski, D. Sybilska, S. Belniak and J. Jurczak, *Chromutogruphiu, 21 (1986) 413.*
11 T. Kościelski, D. Sybilska and J. Jurczak, *J. Chromutogr., 364 (1986) 299.*
12 W. A. König, S. Lutz and G. Wenz, *Angew. Chem., Int. Ed. Engl., 27 (1988) 979.*
13 W. A. König, S. Lutz, C. Colberg, N. Schmidt, G. Wenz, E. van der Bey, A. Mosandl, G. Günther and A. Kustermann, *J. High Resolut. Chromatogr. Chromutogr. Commun.*, 11 (1988) 621.
14 D. A. Armstrong, W. Li and J. Pitha, *Anal. Chem.*, 62 (1990) 217.
15 D. W. Armstrong, W. Li, C.-D. Chang and J. Pitha, *Anal. Chem., 62* (1990) 914.
16 D. Sybilska, J. Goronowicz and M. Cieślak, *Pol. Put. Appl., P-277627, 1989.*
17 D. Sybilska and J. Jurczak, *Carbohydr. Res., 192 (1989) 243.*