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Note

Gas chromatographic separation of monoterpene hydrocarbon enantiomers on α-cyclodextrin[a,b]

MIKAEL LINDSTRÖM and TORBJÖRN NORIN
Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm (Sweden)
and
JOHAN ROERAADE*
Department of Analytical Chemistry, Royal Institute of Technology, S-100 44 Stockholm (Sweden)
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Monoterpene hydrocarbons are common natural products, and are the major constituents in turpentine and other essential oils[1]. Several monoterpenes are important mediators in the chemical communication between organisms and are essential in insect–plant interactions[2]. Most of these monoterpenes are chiral with the (+)- and (−)enantiomers often having different biological properties. The individual enantiomers of a compound such as α-pinene can be transformed to chiral auxiliaries, which are used in organic synthesis, *e.g.*, hydroboration reactions[3]. Hence there is a need for rapid and accurate methods for the determination of the enantiomeric composition of monoterpene hydrocarbons. The classical polarimetric method and NMR techniques require large amounts of sample and are not suitable for complex mixtures or impure compounds. Chromatographic techniques are therefore more attractive [4–6]. A perpentyl-β-cyclodextrin phase has been reported[5] for gas chromatographic (GC) separations of some chiral olefins, including α-pinene and limonene. However, the enantioselectivity of such columns is limited, requiring high plate numbers, which lead to long analysis times.

We have reported that the enantiomeric purity of monoterpene hydrocarbons can be determined by GC via transformation to carbamates that can be separated on a Chirasil-Val column[7]. Chiral olefins have also been converted to the corresponding diastereomeric ketal of (2*R*,3*R*)-2,3-butanediol that can be separated on standard GC columns[8]. However, these procedures are experimentally laborious and are therefore not suitable for routine analysis.

In 1983, Koscielski *et al.*[9] described a promising method for the enantiomeric separation of both α- and β-pinenes using α-cyclodextrin in formamide as the stationary phase. Later, the same workers separated the enantiomers of pinanes and

[a] Dedicated to Günther Ohloff on the Occasion of his 65th Birthday.
[b] Presented in part at the *1st International Symposium on the Separation of Chiral Molecules, Paris, 1988.*

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2-carene[10] using the same procedure. The aim of the present investigation was to study and develop further this method in order to obtain a reliable technique for the determination of the enantiomeric composition of monoterpene hydrocarbons in biological samples, such as spruce phloem.

EXPERIMENTAL

Apparatus

A Pye Unicam 304 gas chromatograph, equipped with a split/splitless injector and a flame ionization detector, was used with helium as the carrier gas. A Pye GCV chromatograph equipped with an effluent splitter (1:100) was used for preparative work and fraction collection. Merck–Hitachi D-2000 integrators were used. The different fractions from the biological samples were collected in gas-tight syringes (SGE, 100 μl) and cooled by wrapping in aluminium foil together with dry-ice. Before the analysis, the syringes were heated in an oven at 100°C.

Chemicals

α-Cyclodextrin was purchased from Aldrich as the hydrate. The individual enantiomers, (+)- and (−)-α-pinene, (−)-β-pinene, (+)- and (−)-limonene and (+)-3-carene, were commercial samples. (+)-2-Carene and racemic 2-carene were obtained by isomerization from (+)-3-carene and racemic 3-carene, respectively. Racemic 3-carene and (+)-β-pinene were gifts from A. M. Mosieenkow (Selinskii Institute, Moscow, U.S.S.R.) and P. Baeckström (Royal Institute of Technology, Stockholm, Sweden), respectively. The (+)- and (−)-enatiomers of camphene were obtained by preparative GC on an α-cyclodextrin column (2.1 m × 4 mm I.D.) at 45°C of a commercial racemate, and the identification of the enantiomers was carried out by NMR spectroscopy and polarimetry.

Procedure

To prepare the packing material for the analytical column, 200 mg of α-cyclodextrin hydrate were dissolved in 1 g of formamide, which was diluted with water (10 ml). This solution was poured into a round-bottomed flask containing 5 g of Chromosorb W AW (45–60 mesh). The slurry was dried under reduced pressure (15–20 mmHg) using a rotary evaporator at 50°C for 30 min, then packed into a glass capillary (1.8 m × 0.8 mm I.D.) using an ultrasonic bath. Fused-silica capillaries (20 cm × 0.53 mm I.D.) were epoxy-glued to the ends of the filled column before it was mounted in the chromatograph. The carrier gas was saturated with water vapour by passing it through a stainless-steel cylinder packed with water-impregnated glass-wool, and then through a second stainless-steel cylinder loosely packed with glass-wool to remove water aerosol.

Phloem samples (1 cm^2), taken 1.5 m above the ground, were collected from a Norway spruce [*Picea abies* (L.) Karst], then chopped with a knife and extracted with 1.5 ml of methanol in an ultrasonic bath for 15 min. Saturated aqueous sodium chloride (1.5 ml) and pentane (1 ml) were added to the methanol extracts. The mixture was shaken and the layers separated. The pentane phase was passed through a short silica column to remove oxygenated compounds in order to shorten the GC separation time. A 5-μl volume of the mixture was fractionated on a preparative column

(Carbowax 20M, 10% on Chromosorb W, 80–120 mesh) (2.1 m × 3 mm I.D.), and the individual monoterpenes were subsequently analysed on an α-cyclodextrin column.

RESULTS AND DISCUSSION

During initial attempts to separate the two enantiomeric pairs of α- and β-pinene at 35°C with dry helium, according to the method of Koscielski *et al.*[9], we were not able to obtain stable conditions. The retention times fluctuated, the peak shape deteriorated with time and the enantioselectivity was rapidly reduced. This behaviour was attributed to dehydration of the stationary phase matrix by the dry carrier gas. Earlier, Andera and Smolkova-Keulemansova[11] pointed out the beneficial effect of water in the carrier gas on the peak shape with cyclodextrin-based columns. Support for our assumption was obtained by measurement of the weight loss of the column. The column weight decreased from 11.38 to 11.24 g in 19 h, after which it remained almost constant. The selectivity factor, α, correspondingly fell from over 2.2 to less than 1.5 for α-pinene enantiomers. To improve this situation, the experiments were repeated with the carrier gas saturated with water vapour. Stable enantioselectivity and resolution were obtained with only a slight weight loss of the column. The increased stability of the "wetted" column allowed us to separate (+)- and (−)-limonene. For this separation, the humidification of the carrier gas is of critical importance, as can be seen in Fig. 1.

Initially, separations were carried out at 35°C, but better results can often be obtained at lower temperatures, as in Fig. 2, where the chromatograms of the enantiomeric pairs of 3-carene, camphene and limonene are shown. The elution order was determined by injecting the individual reference enantiomers. The retention times still fluctuated over a long period and also a slight deterioration of the peak shape after several days of use was noticed. This can be attributed to the pressure drop over the column, eventually resulting in an uneven water distribution along the column. It was

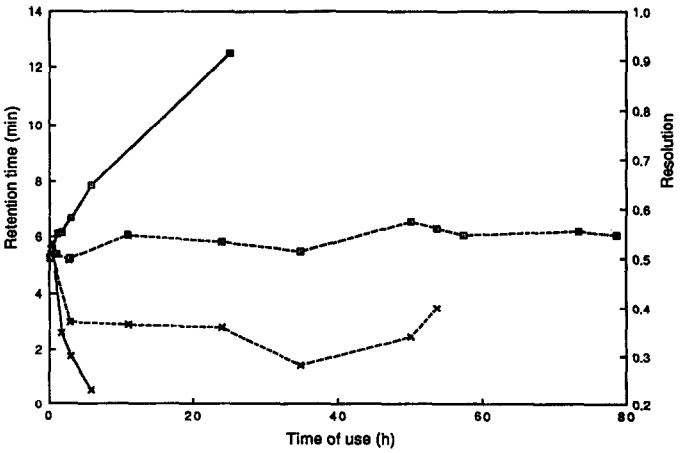

Fig. 1. (□) Change in retention time for (−)-limonene on α-cyclodextrin columns and (×) change in resolution of the limonene enantiomers with time in "unwetted" (solid line) and "wetted" (dashed line) systems.

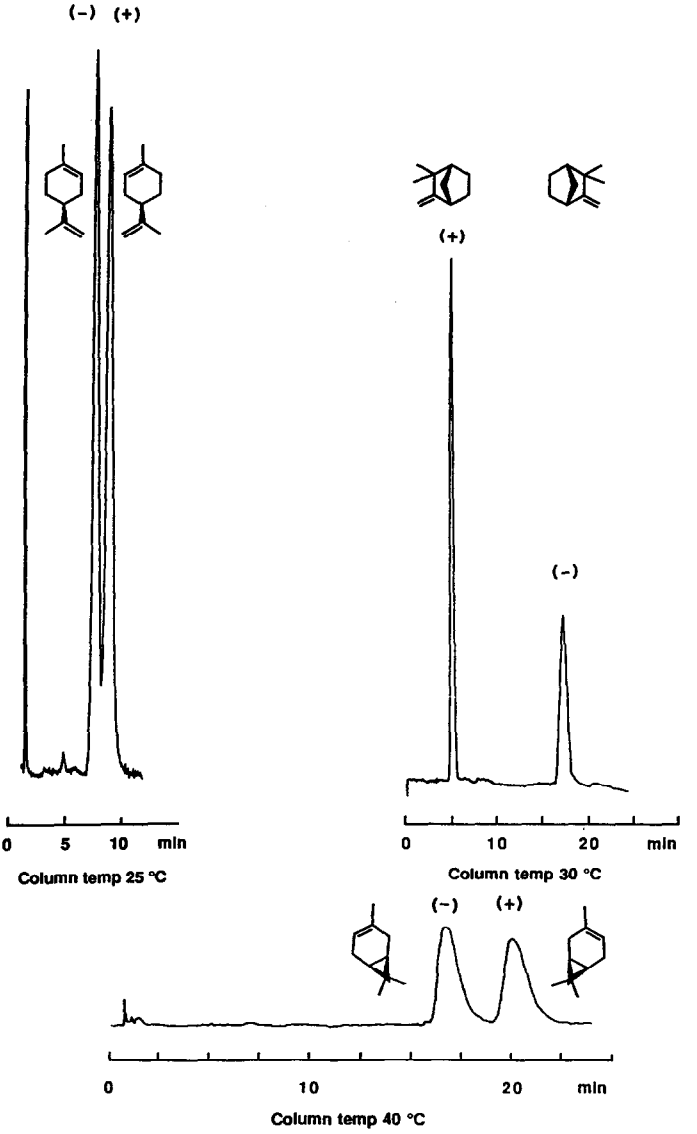

Fig. 2. Chromatograms of the enantiomeric pairs of some monoterpene hydrocarbons.

found that the peak shape could be restored by periodically reversing the direction of flow in the column.

The "wetted" α-cyclodextrin columns have the disadvantage of a limited temperature range and a low efficiency; however, this is outweighed by their high enantioselectivity. Thus, it is possible to obtain short analysis times making these columns suitable for preparative fractionation. Our pure (+)-and (−)-camphene reference material was obtained by such a preparative GC method from a commercial racemate of camphene.

TABLE I

THE ENANTIOMERIC COMPOSITION OF SOME CHARACTERISTIC MONOTERPENE CONSTITUENTS IN A PHLOEM EXTRACT OF *PICEA ABIES* (L.) KARST

Compounds are listed in elution order from the Carbowax column. Elution temperature on the cyclodextrin column was 30°C, except for 3-carene (40°C) and limonene (25°C).

Compound	*P. abies extract composition (%)*	*Enantiomeric composition (%) [(+):(−)]*	*Retention time α-CD column (min) [(+):(−)]*
α-Pinene	34	42:58	6.1:11.7
Camphene	9	8:92	5.4:17.6
β-Pinene	16	3:97	8.0:10.4
Myrcene	12	Not chiral	–
3-Carene	6	>99.5:<.5	19.8:16.5
Limonene	8	10:90	8.2:7.3
α-Phellandrene	13	[a]	–
Unidentified constituents	2	–	–

[a] The α-phellandrene enantiomers could not be determined owing to lack of reference material.

The improved "wetted" system was used to determine the enantiomeric composition of the major monoterpene hydrocarbons in a phloem extract of Norway spruce. A direct separation of the enantiomers was not possible owing to the complex nature of the extract. Therefore, the extract was first fractionated on the preparative Carbowax column and the individual monoterpene hydrocarbons were collected by a simple off-line procedure. The results from the final enantiomeric determinations on the α-cyclodextrin column are presented in Table I. In a previous paper[7] we have shown that there exists a large variation in the enantiomeric composition of α-pinene in Swedish spruce trees. A systematic investigation concerning the genetic dependence on the enantiomeric composition of monoterpene hydrocarbons in Swedish conifers is in progress.

To optimize the performance of α-cyclodextrin columns further, one can change the organic liquid in the stationary phase. Some promising results were obtained with triethanolamine instead of formamide as the stationary phase matrix component. This should be further investigated.

ACKNOWLEDGEMENTS

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