Journal of Chromatography, 441 (1988) 155–163
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1278

SEPARATION OF ENANTIOMERS ON DILUTED PERMETHYLATED β-CYCLODEXTRIN BY HIGH-RESOLUTION GAS CHROMATOGRAPHY

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SUMMARY

A number of volatile racemic solutes belonging to different classes of compounds have been resolved on permethylated β-cyclodextrin in OV-1701 on high-resolution glass open-tubular columns. The scope of the method is outlined.

INTRODUCTION

The gas chromatographic enantiomer separation of volatile chiral compounds[1–4] represents a versatile tool for modern enantiomer analysis[5,6] and the study of "chiral recognition phenomena" in contemporary stereochemistry[7]. Two complementary methods, *i.e.*, enantiomer separation on hydrogen-bonding stationary phases, *e.g.*, Chirasil-Val[8] or XE-60-L-Valine-*S*-α-phenylethylamide[9], and via coordination ("complexation gas chromatography"[10]), have been developed into routine procedures by employing high-resolution open-tubular columns in conjunction with sophisticated instrumentation and ancillary techniques such as gas chromatography–mass spectrometry with selected ion monitoring (GC–MS-SIM)[5]. Yet, a third approach to gas chromatographic enantiomer separation, based on chiral recognition via inclusion, is expected to gain increasing momentum in enantiomer analysis and in mechanistic studies concerned with enantioselectivity.

The separation of enantiomers on stationary phases containing cyclodextrins (CDs) by liquid and thin-layer chromatography is now well established[11–13]. The application of acylated CDs in gas chromatography for the separation of fatty esters was reported as early as 1961[14], while permethylated CDs were employed as column packings for the selective separation of hydrocarbons by inclusion[15]. Following the latter report we were interested in utilizing the inherent chirality of CDs for the gas chromatographic enantiomer separation of simple racemic hydrocarbons devoid of the potential functionalities necessary for conventional enantiomer separation by gas–liquid chromatography (GLC). However, an attempt to resolve racemic *n*-butyl-*tert.*-butylmethylmethane on permethylated β-cyclodextrin (20% in OV-101 and coated onto a 26 m × 0.25 mm I.D. stainless-steel open-tubular column, 60°C) via enantioselective inclusion failed, although the occurrence of chemical selectivity was evident from relative retention data[16].

Encouraged by recent results on the direct enantiomer separation of racemic

0021-9673/88/$03.50

saturated and unsaturated hydrocarbons, *i.e.*, *cis*- and *trans*-pinane, α- and β-pinene, respectively, on CD with packed columns[17,18] and of an oxygen-containing racemic solute on molten, permethylated β-cyclodextrin with an open-tubular column[19], we reinvestigated the use of permethylated β-cyclodextrins in polysiloxane solution for the separation of enantiomers by high-resolution GC.

EXPERIMENTAL

Instrumentation

A Carlo-Erba gas chromatograph, Fractovap 2350, equipped with flame ionization detection (FID) and suitable for operation with glass open-tubular columns, was used. The carrier gas was high-purity nitrogen, which was used without further purification, and the splitting ratio was set to 1:100. In order to avoid overloading, the instrument was set at its highest sensitivity at a tolerable signal-to-noise ratio.

Open-tubular columns[20]

Duran glass tubings (Schott Ruhrglas, Mainz, F.R.G.) were drawn to capillaries of 0.25 mm I.D., using a Hupe & Busch glass-drawing machine.

Acid leaching and rinsing. The capillary column was filled to 90% of its capacity with aqueous 6 *M* hydrochloric acid, sealed under vacuum and heated to 150°C for 24 h. The ends were opened and the capillary column was rinsed with *ca.* 3 volumes of 0.01 *M* hydrochloric acid and 1 ml of methanol. Finally, the column was dried for 2 h at 250°C under a stream of dry nitrogen.

Deactivation with DPTMDS (diphenyltetramethyldisilazane). The column was dynamically coated with a solution of DPTMDS in *n*-pentane (1:1, v/v), then sealed under vacuum and heated to 200°C. The temperature was increased to 390°C at a rate of 3°C/min and held for 12 h. The column ends were opened, and the capillary column was rinsed with 1 ml of *n*-pentane, 3 ml of methanol and 2 ml of diethyl ether[21].

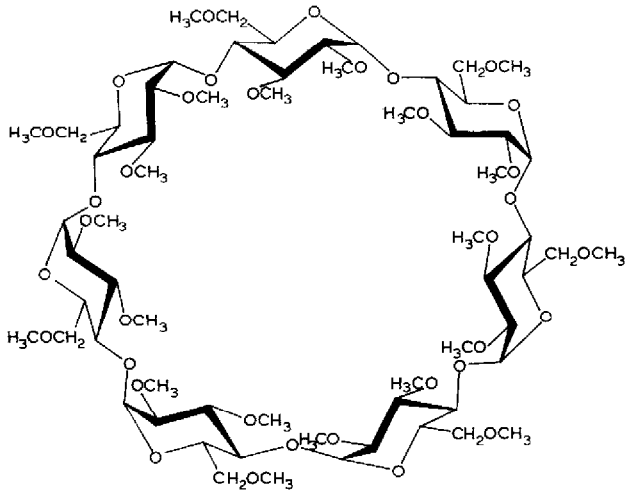
Coating. The column was coated with a 0.05–0.06% solution of the permethylated β-cyclodextrin and the required amount of OV-1701 (0.5–0.6%) in *n*-pentane–dichloromethane (10:1, v/v) by the static method.

Solutes

Unless otherwise stated the solutes used were commercial samples. Pinenes were obtained from the Dragoco Co. (Holzminden, F.R.G.).

RESULTS AND DISCUSSION

The use of permethylated β-cyclodextrin as a selective stationary phase in GC may have some advantages compared to underivatized CD or to peracylated CD: (i) low polarity and solubility in moderately polar polysiloxanes, (ii) low melting point, and (iii) improved thermal and chemical stability. These desirable properties are complemented by the invariably high tendency of permethylated β-cyclodextrins to form molecular inclusion compounds. The increase in hydrophobicity at the entrance of the chiral cavity upon permethylation may be regarded as an additional advantage in the use of permethylated CD.



Heptakis(2,3,6-tri-O-methyl)β-cyclodextrin (permethylated β-cyclodextrin) was prepared and characterized according to the literature[22–25]. It is soluble in methylphenylpolysiloxanes.. We discern the following advantages of using solutions of permethylated β-cyclodextrin in moderately polar polysiloxanes as stationary phases as compared to molten CD[19]: (i) the retention increase due to inclusion is less pronounced and the analysis time is therefore reduced, (ii) the temperature range is extended below the melting point of permethylated β-cyclodextrin, and (iii) reliable thermodynamic data on enantioselectivity are readily accessible from relative retention data when diluted solutions of the chiral selector are employed, as shown in complexation GC[4].

As our previous results with stainless-steel open-tubular columns proved rather unsuccessful[16], we decided to resort to surface-treated glass capillary columns, which were obtained in essentially the same way as in complexation GC[20], (*cf.*, Experimental). Solutions of permethylated β-cyclodextrin in the following polysiloxanes have been investigated: OV-101 (methylpolysiloxane); DC-550 (25% phenylmethylpolysiloxane); OV-17 (50% phenylmethylpolysiloxane) and OV-1701 (5% cyanopropyl/7% phenylmethylpolysiloxane). Stationary phases containing permethylated β-cyclodextrin in OV-1701 gave the best results with regard to peak shape and long-term stability.

In Figs. 1–10 some representative gas chromatograms featuring enantiomer separation of different classes of chiral compounds on permethylated β-cyclodextrin in OV-1701 are shown, *cf.*, also Table I. The number of effective plates, N, has been determined for the first eluted peak of α-pinene, *cf.*, Fig. 1

$$N = 5.545\left(\frac{t'}{w_h}\right)^2 = 26\,650 \qquad (1)$$

corresponding to a height equivalent to an effective plate $h = 0.94$ mm for the 25-m column employed [w_h = peak width at half-height, t' = adjusted retention time (for dead volume)].

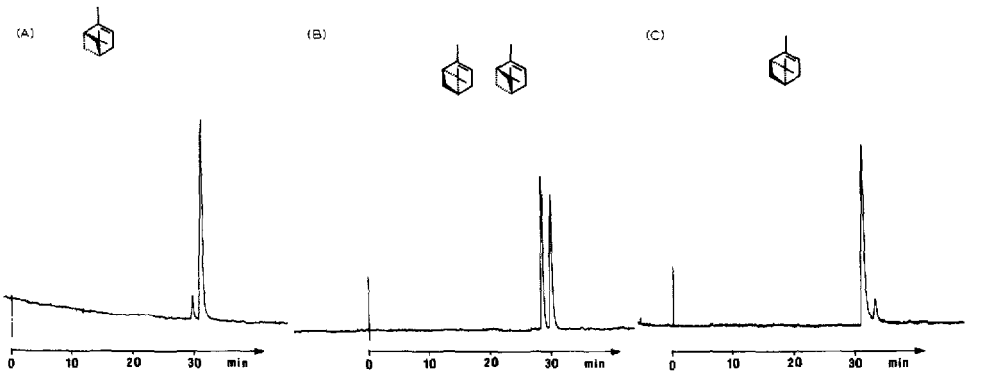


Fig. 1. Enantiomer separation of racemic α-pinene on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 50°C; inlet pressure, 0.5 bar nitrogen. (A) (−)-α-pinene (peak integration, *ee* = 79.8%); (B) racemic α-pinene (peak integration, *ee* = 0.00%); (C) (+)-α-pinene (peak integration, *ee* = 86.2%).

Noteworthy is the nearly quantitative enantiomer separation of 4-isopropenyl-1-methylcyclohexene ("dipentene"), which occurs in its optically active forms as limonene in biological matrices. To our knowledge, this diolefin has not previously been separated by complementary methods. Comparison of the enantiomer separation of *trans*-2,5-dimethyltetrahydrofuran and *trans*-2,5-dimethoxytetrahydrofuran

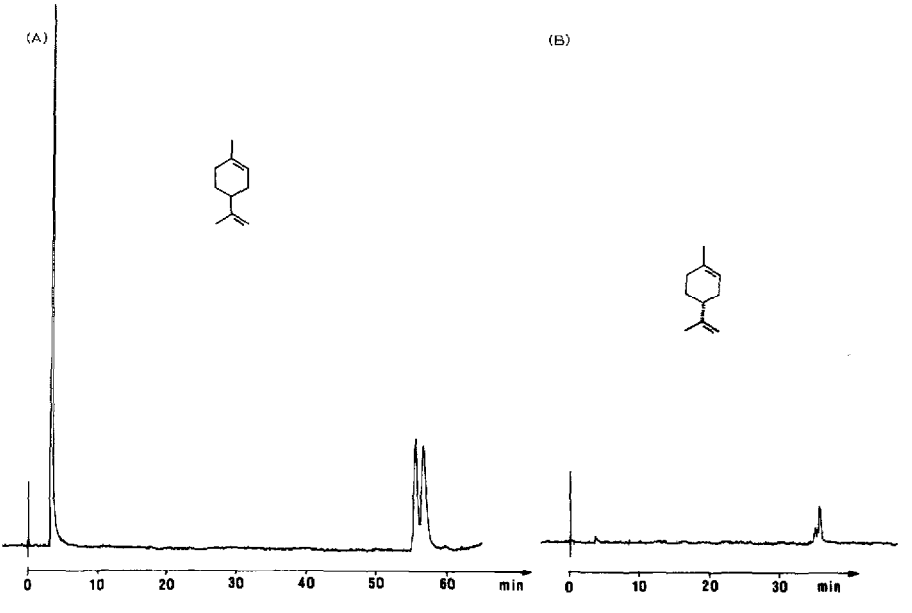


Fig. 2. Enantiomer separation of racemic dipentene on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 60°C; inlet pressure, 0.5 bar nitrogen. (A) racemic sample (50°C); (B) enantiomerically impure (+)-limonene.

(Figs. 3 and 4) implies that the enantiomer selectivity imparted by permethylated β-cyclodextrin is sensitive to the shape of the molecules rather than to the presence of certain chemical functionalities. It should be noted that the order of elution of the diastereomers is inverted. Thus, the achiral *cis* isomer, which is eluted first in the case of 2,5-dimethyltetrahydrofuran, is eluted last in the case of 2,5-dimethoxy- and 2,5-diethoxytetrahydrofuran.

While racemic β-pinene is not resolved, the racemic α-isomer can be quantitatively resolved on permethylated β-cyclodextrin, although the separation coefficient, α, is much smaller than that reported for underivatized CD[18]. However, the high peak resolution of the enantiomers on permethylated β-cyclodextrin with glass open-tubular columns, *cf.*, Fig. 1, offsets the low separation coefficient. The commercial samples investigated, *i.e.*, (+)- and (−)-α-pinene, have been found to be

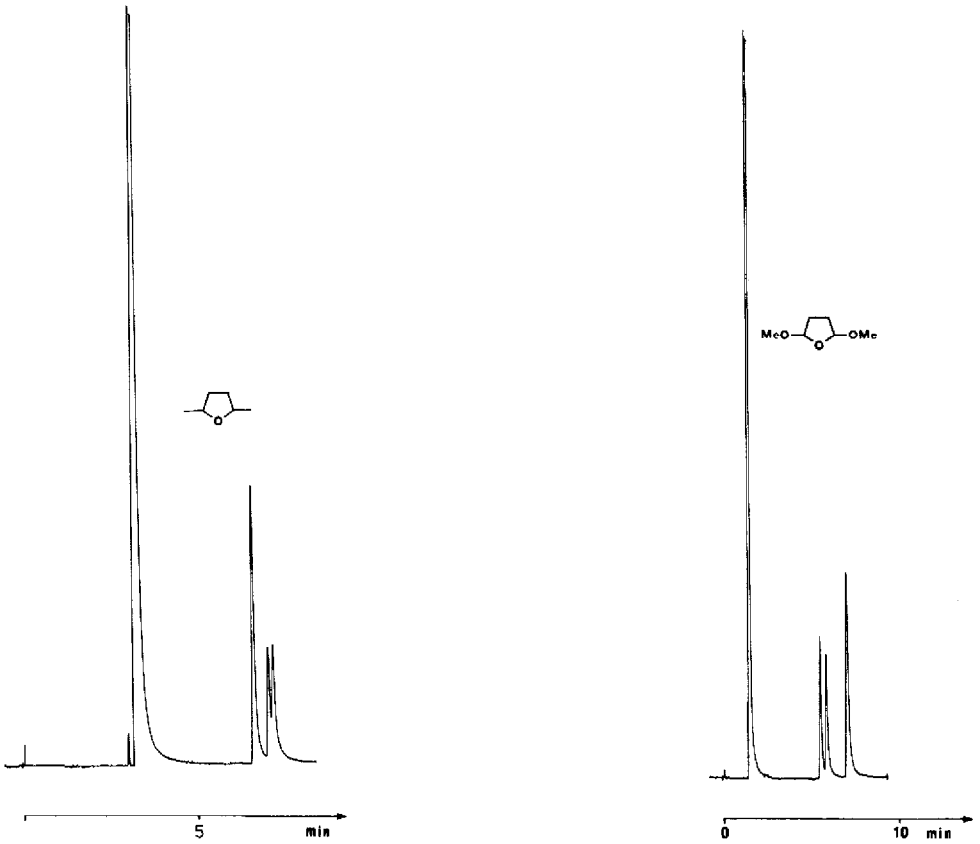


Fig. 3. Diastereomer and enantiomer separation of 2,5-dimethyltetrahydrofuran on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin) in OV-1701 (0.08 m). Oven temperature, 60°C; inlet pressure, 0.5 bar nitrogen.

Fig. 4. Diastereomer and enantiomer separation of 2,5-dimethoxytetrahydrofuran on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 70°C; inlet pressure, 1.0 bar nitrogen. Me = methyl.

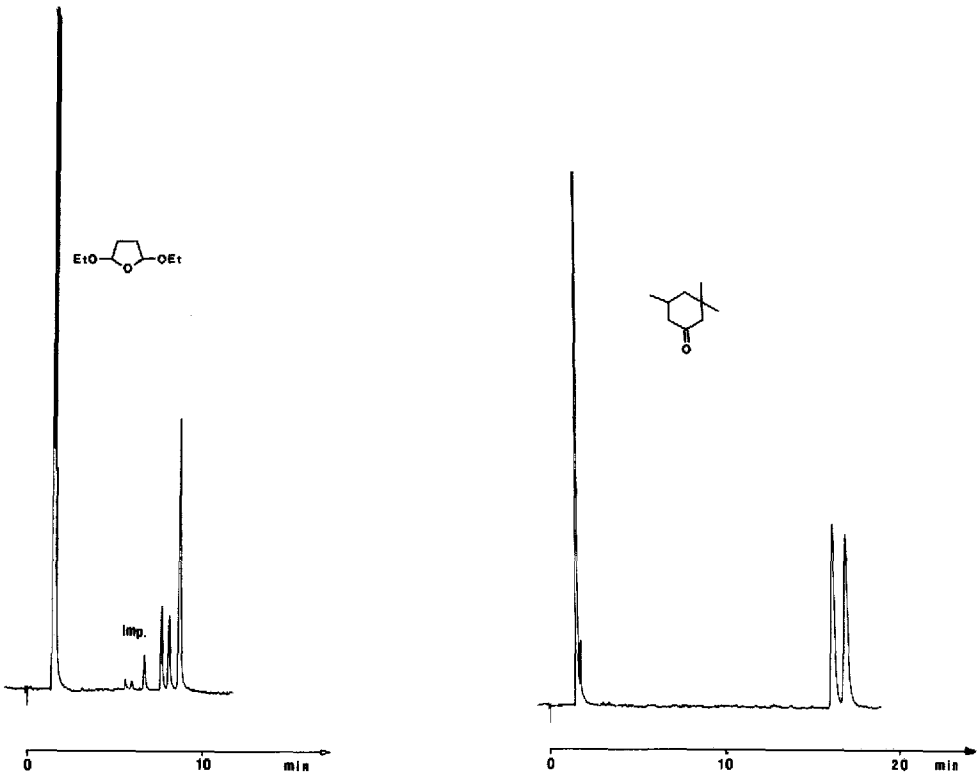


Fig. 5. Diastereomer and enantiomer separation of 2,5-diethoxytetrahydrofuran on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 80°C; inlet pressure, 1.0 bar nitrogen. Et = ethyl.

Fig. 6. Enantiomer separation of racemic 3,3,5-trimethylcyclohexanone on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 80°C; inlet pressure, 1.0 bar nitrogen.

considerably contaminated with the respective antipodes. The enantiomeric compositions, *ee*, were determined by digitalized integration to be 79.8% for (−)-α-pinene and 86.2% for (+)-α-pinene. The *ee* values are lower than those reported previously for commercial samples, based on chiroptical evidence[26]. It should be noted that the screening of *ee* by GC[6] is much more straightforward than that by polarimetry[27]. For future developments of gas chromatographic enantiomer separation on CD it may be desirable to combine the high separation coefficients found with underivatized α-CD and the high peak resolution experienced with diluted permethylated β-cyclodextrin, coated on high-resolution open-tubular columns. The screening of solutions of permethylated α-cyclodextrin as well as permethylated γ-cyclodextrin in polysiloxanes for solutes with different molecular sizes is also warranted. The present investigations have shown that the size of the cavity in permethylated β-cyclodextrin leads to the highest chiral recognition for the solutes listed in Table I.

Hitherto, cyclodextrins have only been accessible via the enzymatic degrada-

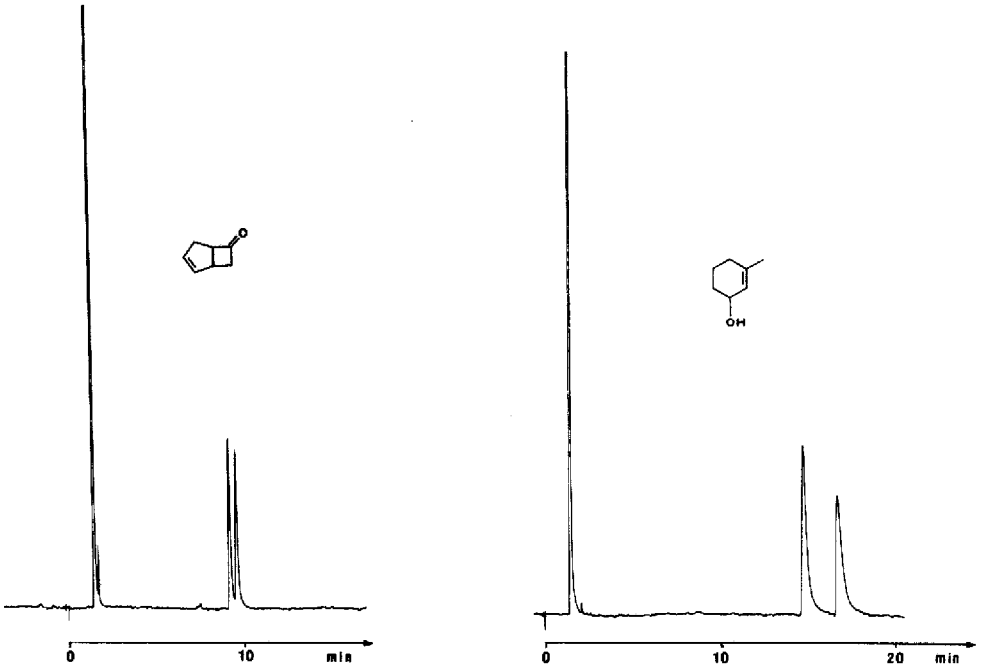


Fig. 7. Enantiomer separation of racemic bicyclo[3.2.0]hept-2-en-6-one on a 25 m × 0.25 mm I.D. Glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 mm). Oven temperature, 80°C; inlet pressure, 1.0 bar nitrogen.

Fig. 8. Enantiomer separation of racemic 3-methyl-2-cyclohexen-1-ol ("seudenol") on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 80°C; inlet pressure, 1.0 bar nitrogen.

tion of starch. The inaccessibility of the unnatural chiral form restricts the use of CD in enantiomer analysis, because it is sometimes useful to reverse the order of elution of enantiomers by inverting the chirality of the stationary phase[6].

An important aspect of enantiomer separation by CD is the possibility to mimic enantioselectivities found in nature in a chromatographic flow system. Systematic studies on the influence of the size of the CD cavity and the chemical structure of the racemates on enantioselectivity in combination with correlation of the order of elution and absolute configuration, may allow insights into pertinent mechanisms of chiral recognition. The anchoring of CD to the polysiloxane backbone represents another challenge for the future.

CONCLUSION

It has been shown that a solution of permethylated β-cyclodextrin in OV-1701 represents a versatile stationary phase for the enantiomer separation of different classes of compounds by high-resolution glass capillary gas chromatography. Although only a selected array of racemic compounds is presently amenable to enantiomer separation by this approach, the method will nevertheless complement the use

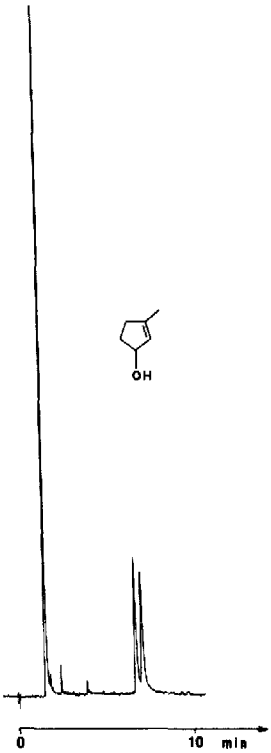


Fig. 9. Enantiomer separation of racemic 3-methyl-2-cyclopenten-1-ol on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 80°C; inlet pressure, 1.0 bar nitrogen.

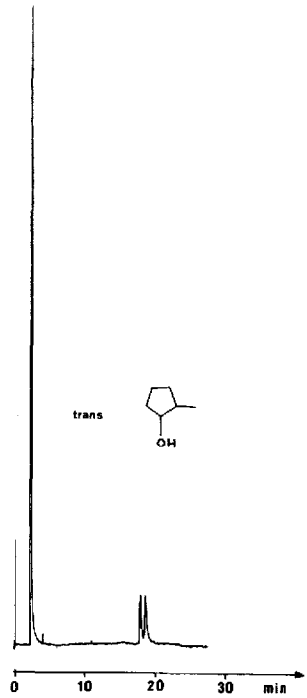


Fig. 10. Enantiomer separation of racemic *trans*-2-methylcyclopentanol on a 25 m × 0.25 mm I.D. glass open-tubular column, coated with 10% heptakis(2,3,6-tri-O-methyl)β-cyclodextrin in OV-1701 (0.08 m). Oven temperature, 60°C; inlet pressure, 0.7 bar nitrogen.

TABLE I

GAS CHROMATOGRAPHIC DATA ON THE SEPARATION OF ENANTIOMERS ON PERMETHYLATED β-CYCLODEXTRIN IN OV-1701

Solute	*Temperature (°C)*	*Inlet pressure (bar)*	*Separation coefficient* α	*Peak resolution* R_s
α-Pinene	50	0.5	1.06	1.15
Dipentene	50	0.5	1.03	0.35
2,5-Dimethyltetrahydrofuran	60	0.5	1.04	—*
2,5-Dimethoxytetrahydrofuran	70	1.0	1.09	0.68
2,5-Diethoxytetrahydrofuran	80	1.0	1.08	1.12
3,3,5-Trimethylcyclohexanone	80	1.0	1.06	1.28
Bicyclo[3.2.0]-2-hepten-6-one	80	1.0	1.06	0.72
3-Methyl-2-cyclohexen-1-ol	80	1.0	1.15	1.66
3-Methyl-2-cyclopenten-1-ol	80	1.0	1.09	0.65
trans-2-Methylcyclopentanol	60	0.7	1.06	0.73
exo-Norborneol	80	1.0	1.05	0.91
iso-Borneol	80	1.0	1.04	1.04

* Low peak resolution.

of hydrogen-bonding stationary phases (Chirasil-Val[8] or XE-60-L-Valine-*S*-α-phenylethylamide[9]) and of coordinating stationary phases ("complexation gas chromatography"[10]) in certain instances.

ACKNOWLEDGEMENTS

Generous support of this work by Deutsche Forschungsgemeinschaft and Fonds der chemischen Industrie is acknowledged. We thank the Dragoco, Co., Holzminden, F.R.G. for specimens of pinenes.

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