Journal of Chromatography, 349 (1985) 3–8
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 695

RESOLUTION OF *cis*- AND *trans*-DIMETHYLCYCLOHEXANES BY PARTITION GAS CHROMATOGRAPHY THROUGH CYCLODEXTRIN COMPLEXES

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SUMMARY

α- and β-Cyclodextrins, dissolved in formamide or ethylene glycol, were applied as stationary phases in gas chromatography for the resolution of isomeric *cis*- and *trans*-1,2-, 1,3-, and 1,4-dimethylcyclohexanes. It was found that stereoselectivity arising from inclusion of dimethylcyclohexanes in cyclodextrin cavities is more distinct for β-cyclodextrin. Of the two matrix solvents, which influence not only the capacity factors but also the separation factors, formamide is the more efficient medium for stereoselective cyclodextrin inclusion processes. An evaluation of stability constants of (β-cyclodextrin · dimethylcyclohexane) complexes was attempted. Almost complete separation of a mixture containing the six isomers investigated was achieved by using concentrated β-cyclodextrin (1.48 mol. %) in formamide solution.

INTRODUCTION

The selective properties of cyclodextrins (CD), based on their ability to form inclusion compounds with various molecules and ions[1], have been widely used in many separation techniques, including liquid chromatography (LC)[2,3].

Our recent studies have shown that α- or β-CD can be successfully applied for analytical purposes, not only in LC but also in gas chromatography (GC), for imparting to a liquid stationary phase the stereoselectivity needed for the efficient separation of *o*-, *m*- and *p*-xylenes[4] and diethylbenzenes[5], as well as for the resolution of α- and β-pinenes into enantiomers[6].

These results encouraged us to undertake further systematic studies on the application of the same procedure for the separations of other kinds of isomer. Our aim is to establish some general principles that relate the shape and size of molecules to their chromatographic behaviour in GC systems containing α- or β-CD in the stationary phase solution.

In selecting compounds for our studies we have also taken into account the actual analytical needs and difficulties in their separation. This paper reports the results of the resolution of six isomeric *cis*- and *trans*-1,2-, 1,3-, and 1,4-dimethylcyclohexanes (DMCH), abbreviated respectively as: c-1,2; t-1,2; c-1,3; t-1,3; c-1,4; t-1,4.

0021-9673/85/$03.30

Analysis of DMCH mixtures is of crucial value in monitoring the course of xylene hydrogenation. The partial separation of DMCH by GC methods has already been achieved with a molecular sieve 13X porous-layer open-tubular (PLOT) column[7] or columns filled with graphitized thermal carbon black[8]. Numerous retention data relative to DMCH behaviour on various stationary phases have also been reported[9–13]. All these investigations concluded that the complete separation of DMCH mixtures poses difficulties.

EXPERIMENTAL

Reagents

α- and β-CD were supplied by Chinoin (Budapest, Hungary). Celite, 80–100 mesh, for GC was from BDH (Poole, U.K.). DMCH puriss. samples were obtained from Fluka (Buchs, Switzerland). All other materials were of analytical or reagent grade and were used without further purification.

Apparatus and procedures

Chromatographic studies were performed using a Hewlett-Packard 7620 A gas chromatograph, equipped with dual flame ionization detectors. Glass columns (2 m × 4 mm I.D. and 5 m × 4 mm I.D.) were used. The compounds were injected separately (0.2 μl) or as mixtures, with Hamilton microsyringes.

The stationary phases were prepared as follows. Celite, 80–100 mesh, was coated with solutions of α- or β-CD in formamide or ethylene glycol, using 4.54 g of the solvent and 20.0 g of Celite. The amounts of α- or β-CD varied from 0.0 to 1.5 g in different coated supports. The detailed preparation procedure was described earlier[4]. The mass of the coated support in the column was determined by weighing the columns before and after packing: the mean value for all the columns of 2-m length was 10.2 ± 0.5 g, and for the 5-m columns it was 24.5 g.

In all experiments two columns were used: the first with formamide or ethylene glycol solutions of α- or β-CD and the second (reference), containing only formamide or ethylene glycol, respectively. This two-column system enabled us to perform comparative measurements and excluded many sources of error.

The studies were carried out in the temperature range 45–70°C. Stability constants of the β-CD complexes were evaluated on the assumption that only complexes of 1:1 stoichiometry are formed, using the following equation

$$t'_{\beta\text{-CD}} = t'_0 (1 + K[\beta\text{-CD}]) \quad (1)$$

where $t'_{\beta\text{-CD}}$ and t'_0 are, respectively, the adjusted retention times of a solute on the column containing β-CD in a given solvent and on the reference column containing pure matrix solvent; K is the stability constant of a 1:1 β-CD complex with DMCH.

Special attention was paid to maintaining constant values of the inlet pressure (2.75 ± 0.05 atm) and the helium flow-rate (50 ± 0.5 ml/min). Under these conditions it was possible to evaluate and compare stability constants of CD complexes, although there was no other means of determining their exact values[14,15].

RESULTS AND DISCUSSION

Fig. 1 shows four chromatograms of a mixture of *cis*- and *trans*-1,2-, 1,3-, and 1,4-DMCH, which demonstrate how their separation is influenced by β-CD and α-CD in formamide. The corresponding chromatograms obtained from the column containining β- and α-CD in ethylene glycol are presented in Fig. 2. At 50°C, for each DMCH isomer, t'_{CD} is greater than t'_0. This means, according to eqn. 1, that under these experimental conditions both α- and β-CD form inclusion complexes.

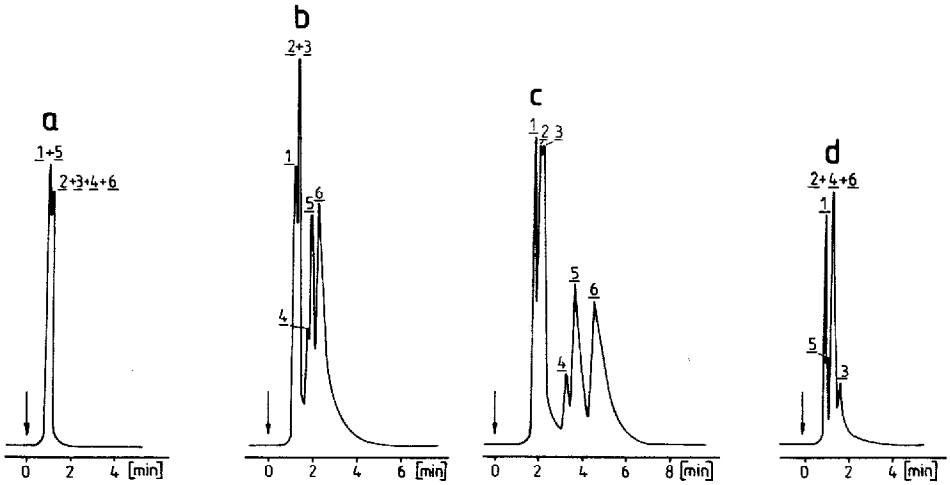


Fig. 1. Chromatograms of a mixture of DMCH: c-1,2 (6), t-1,2 (3), c-1,3 (1), t-1,3 (4), c-1,4 (2), t-1,4 (5), performed at 50°C on a column (2 m × 4 mm I.D.) packed with: (a) 0.0, (b) 0.10 and (c) 0.30 mol. % of β-CD and (d) 0.31 mol. % of α-CD in formamide, coated on Celite (4.54 g of formamide per 20 g Celite).

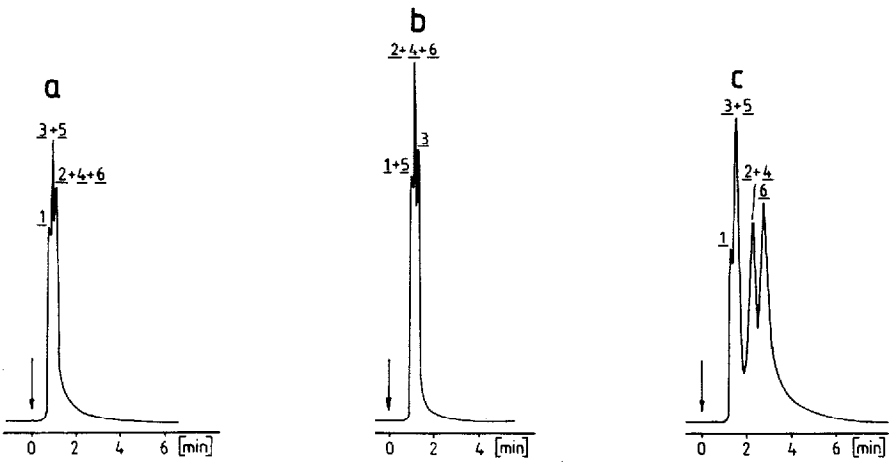


Fig. 2. Chromatograms of a mixture of DMCH: c-1,2 (6), t-1,2 (3), c-1,3 (1), t-1,3 (4), c-1,4 (2), t-1,4 (5), performed at 50°C on a column (2 m × 4 mm I.D.) packed with: (a) 0.0 and (b) 0.42 mol. % of α-CD and (c) 0.36 mol. % of β-CD in ethylene glycol solution, coated on Celite (4.54 g of ethylene glycol per 20 g Celite).

However, the selectivities that they impart to the liquid stationary phase towards DMCH are different. Thus, different elution orders are observed for α- and β-CD stationary phase solutions (*cf.* Figs. 1 and 2).

Separation factors (α) for neighbouring peaks, which illustrate the influence of β-CD complexation, are collected in Table I. For almost inseparable pair of isomers, *i.e.* t-1,2 and c-1,4 in the chromatograms shown in Figs. 1 and 2, α attains a value suitable for analysis (1.11) only in a solution of β-CD at very high concentration (1.48 mol. %).

TABLE I

SEPARATION FACTORS FOR NEIGHBOURING PEAKS OF DMCH ON COLUMNS WITH DIFFERENT CONCENTRATIONS OF β-CD IN FORMAMIDE

β-CD concentration in formamide solution (mol. %)	*Temp. (°C)*	$\alpha_{c\text{-}1,4/c\text{-}1,3}$	$\alpha_{t\text{-}1,2/c\text{-}1,4}$	$\alpha_{t\text{-}1,3/t\text{-}1,2}$	$\alpha_{t\text{-}1,4/t\text{-}1,3}$	$\alpha_{c\text{-}1,2/t\text{-}1,4}$
0.0	50	$\alpha_{c\text{-}1,2;t\text{-}1,2;t\text{-}1,3;c\text{-}1,4/c\text{-}1,3;t\text{-}1,4} = 1.24$				
0.0	60	$\alpha_{c\text{-}1,2;t\text{-}1,2;t\text{-}1,3;c\text{-}1,4/c\text{-}1,3;t\text{-}1,4} = 1.24$				
0.10	50	1.16	1.00	1.74	1.11	1.25
0.10	60	1.17	1.00	1.94	1.00	1.28
0.30	50	1.21	1.03	1.92	1.13	1.30
0.30	60	1.27	1.00	1.83	1.13	1.29
1.48	45	1.21	1.11	1.78	1.19	1.31

The selectivities arising from α-CD complexation are smaller, *e.g.* α values for neighbouring peaks, determined with a column containing 0.31 mol. % α-CD in formamide, are as follows: $\alpha_{t\text{-}1,4/c\text{-}1,3} = 1.3$; $\alpha_{t\text{-}1,3;c\text{-}1,4;c\text{-}1,2/t\text{-}1,4} = 1.4$; $\alpha_{t\text{-}1,3/t\text{-}1,4} \approx \alpha_{c\text{-}1,4/t\text{-}1,4} \approx \alpha_{c\text{-}1,2/t\text{-}1,4} \approx 1.0$; $\alpha_{t\text{-}1,2/t\text{-}1,3;c\text{-}1,4;c\text{-}1,2} = 1.6$.

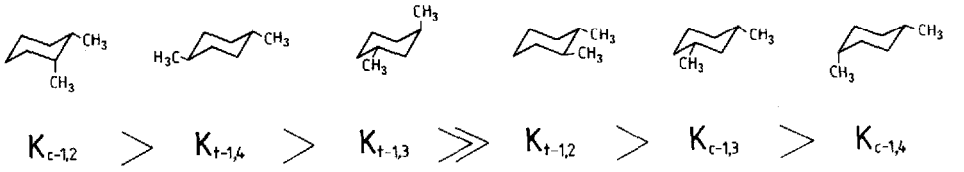
In general, β-CD complexes of DMCH are more stable than the corresponding complexes of α-CD. It should also be pointed out that β-CD inclusion processes are more stereoselective towards DMCH, *i.e.* more sensitive to changes in their structure. On account of this, the initial two peaks, corresponding to the DMCH resolution on a pure formamide-containing column (Fig. 1a) became five peaks (and a less marked sixth) on 0.30 mol. % of β-CD in formamide (Fig. 1c) and only three (and a less marked fourth) on 0.31 mol. % of α-CD (Fig. 1d).

Comparison of Figs. 1 and 2 leads to the conclusion that the matrix solvent influences not only capacity factors but also separation factors. The more polar solvent, formamide, seems to make a more efficient medium for CD inclusion processes than ethylene glycol; in formamide the effects of inclusion are more noticeable.

CD complexation equilibria are strongly affected by changes of temperature, as exemplified in Fig. 3 by the resolution of a DMCH mixture at 50, 60 and 70°C; at the highest temperature the inclusion effects are almost indistinguishable (Fig. 3c).

We have attempted to predict the stability constants of (β-CD · DMCH) complexes from the data obtained for more dilute solutions (up to 0.30 mol. %) of β-CD in formamide, where a 1:1 stoichiometry of complexes seems to be valid.

The predicted sequence is presented in Scheme 1.



Scheme 1.

The predicted stability constants are divided into two groups of distinctly different (about four times) values. Within each group the stability constants are similar. The division does *not* correspond to the *cis/trans* isomerization of DMCH molecules.

The most difficult resolution was between c-1,4 and t-1,2. On the columns with

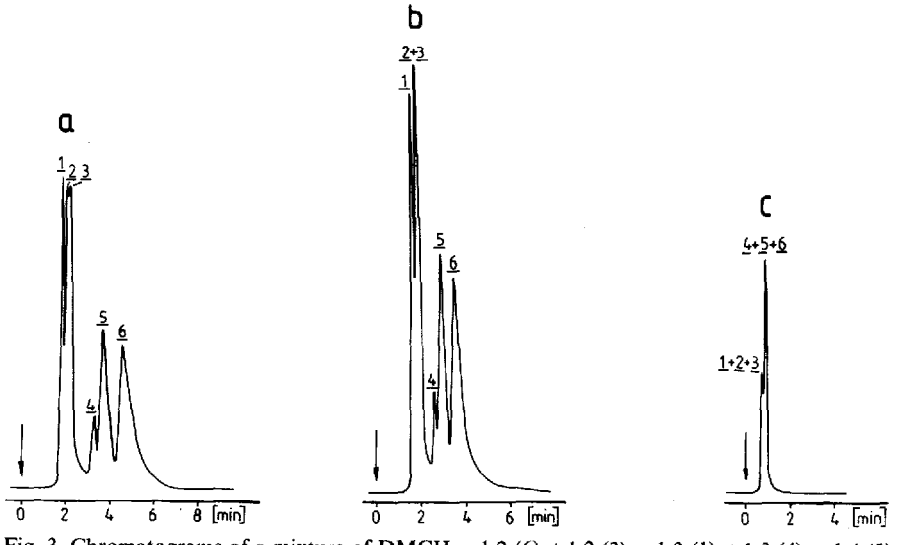


Fig. 3. Chromatograms of a mixture of DMCH: c-1,2 (6), t-1,2 (3), c-1,3 (1), t-1,3 (4), c-1,4 (2), t-1,4 (5) on a column (2 m × 4 mm I.D.) packed with 0.30 mol. % of β-CD in formamide, coated on Celite, at the following temperatures: (a) 50°C; (b) 60°C; (c) 70°C.

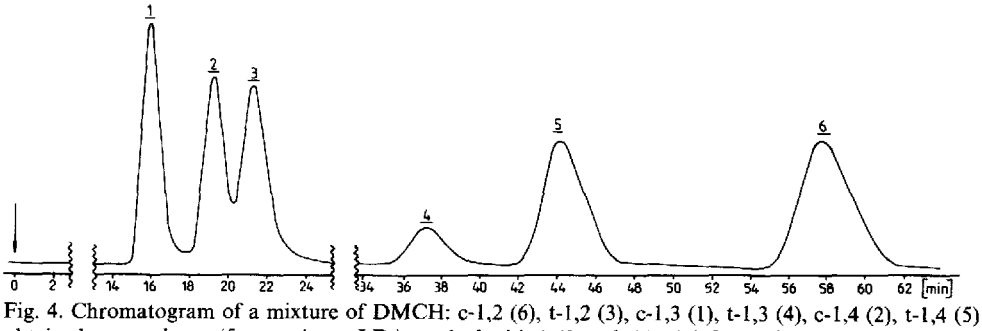


Fig. 4. Chromatogram of a mixture of DMCH: c-1,2 (6), t-1,2 (3), c-1,3 (1), t-1,3 (4), c-1,4 (2), t-1,4 (5) obtained on a column (5 m × 4 mm I.D.), packed with 1.48 mol. % of β-CD in formamide, coated on Celite. Temperature, 45°C.

low percentages of β-CD in formamide their peaks almost completely overlapped. Only on the columns with high percentages of β-CD (1.48 mol. %) was an adequate selectivity factor attained and complete separation achieved (Fig. 4).

The separation of c-1,4, c-1,2 and t-1,3 mixtures, which was earlier found to be inadequate[8], is easily accomplished on columns containing β-CD in formamide.

ACKNOWLEDGEMENTS

We are indebted to Professor J. Szejtli (Chinoin, Budapest, Hungary) for kindly providing α- and β-cyclodextrins.

This study was supported within Polish Academy of Sciences project 03.10.

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