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# Stereoselectivity of α-, β- and γ-cyclodextrin complexation relative to *cis–trans* acyclic alkenes and cyclooctenes under conditions of gas–liquid chromatography

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## ABSTRACT

Solutions of α-, β- and γ-cyclodextrins in formamide were used as stationary phases in gas–liquid chromatographic systems to observe the effects of complexation on the retentions of acyclic ($C_6$–$C_9$) alkenes and cyclooctenes which differ in structure and geometry. On adding β-cyclodextrin to a liquid stationary phase, substantial stereoselectivity with respect to *cis–trans* geometry of acyclic alkenes was found, whereas double bond positions were less recognizable. Almost all the investigated isomers of acyclic alkenes were separated using β-cyclodextrin under appropriate conditions. Small, but distinguishable, effects of γ-cyclodextrin complexation, improving the resolution of *cis–trans* isomers, were observed for 2-octenes and 2-onenes. α-Cyclodextrin influences the resolution of cyclooctenes, enhancing the separation factors for *cis–trans* geometrical isomers and showing substantial enantioselectivity towards optical isomers of *trans*-cyclooctene. Baseline resolutions were achieved for mixtures of *cis*-cyclooctene and two enantiomeric *trans*-cyclooctenes.

## INTRODUCTION

The behaviour of alkenes on conventional gas chromatographic stationary phases has been the subject of many studies which have provided some qualitative information [1–3]. It has been established that the retention of alkenes depends on the length of the alkene chain, the double bond position, the *cis*–trans geometry and the stationary phase polarity. Recently, attemps have been made to find some quantitative descriptions, *i.e.*, to evaluate relationships between retention indices of alkenes (determined on different commonly used stationary phases) and the parameters describing their structure [4].

Nevertheless, resolutions of isomeric alkenes on known conventional phases are very poor and thus the separations are not easy to perform. More promising selectivities have been observed recently for alkenes by applying some special gas

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chromatographic systems, *e.g.*, using liquid crystal glass capillaries [5–11] and copper complexes bonded to silica [12–14].

On the other hand, cyclodextrin (CD) complexation [15,16] may be considered as a possible choice for the separation of various isomers [17–20], which can distinguish their shape more than their polarity under gas–liquid chromatographic conditions [21–23]. Therefore, it is of interest to investigate how such steroselective CD inclusion will recognize isomers of alkenes.

In this work we studied agents that improve the differentiation of isomeric alkenes and the relationship between the structure of a guest molecule (double bond position, *cis*–trans geometry, cyclization and chirality) and its ability to form inclusions in α-, β- and γ-CD cavities.

## EXPERIMENTAL

### *Reagents*

α-, β- and γ-CD were supplied by Chinoin (Budapest, Hungary). Celite was obtained from BDH (Poole, UK), Chromosorb P AW from Chrompak (Netherlands) and pure formamide from Merck (Darmstadt, Germany).

All the investigated compounds, except *trans*-cyclooctene, were commercial products from different sources and were used without further purification.

Racemic *trans*-cyclooctene was generated in methanolic solution by singlet photosensitized isomerization of *cis*-cyclooctene, according to the procedure described in detail by Inoue *et al.* [24]; methyl benzoate was used as an active sensitizer.

### *Apparatus and procedures*

Chromatographic studies were performed using Hewlett-Packard Model 5890 and Carlo Erba Model 4200 gas chromatographs, both equipped with flame ionization detectors. Glass columns (2 m × 4 mm I.D. and 1 m × 3.2 mm I.D.) were used. The peaks areas and the retention times were measured with a Hewlett-Packard Model 3390 integrator. The compounds were injected separately (0.02 μl) or as mixtures with Hamilton microsyringes.

The stationary phases were prepared as follows. Celite (30–80 mesh) and Chromosorb P AW (80–100 mesh) were coated with α-, β-, γ-CD dissolved in formamide. In order to obtain a good separation, the α-CD column contained 4% of water together with lithium nitrate (0.45 g) used as a stabilizing agent.

The amounts of CDs in stationary phases containing 4.5 g of formamide and 20 g of Celite were as follows: column I, none; column II, 0.6 g of α-CD; column III, 1.2 g of β-CD and column IV, 1.2 g of γ-CD. The contents of formamide and water in each stationary phase were determined by thermogravimetric analyses using a DuPont Thermal Analysis System 1090. The amount of coated support contained in the 2-m columns was 11.5 ± 0.5 g.

The column used for special separations of hexenes and heptenes is described in the caption of Figure 1.

In all experiments special care was devoted to maintaining a constant helium inlet pressure (2.75 ± 0.05 atm) and flow-rate (50 ± 0.5 ml/min) in order to make possible a comparison between the stability constants of CD complexes if the isomeric alkenes, although their exact values could not be determined [25,26].

## RESULTS AND DISCUSSION

The relative stabilities of CD complexes can be compared on the assumption that only 1:1 stoichiometric complexes are formed using the following equation:

$$t'_R = t'_{Ro}(1 + k[CD])$$

were $t'_R$ and $t'_{Ro}$ are the adjusted retention times of the solute on the column containing CD in formamide and on the reference column containing pure formamide, respectively, $k$ is the stability constant of the solute–CD complex and [CD] is the CD concentration (molar fraction) in formamide solution.

The separation factor, $\alpha$, for two solutes, A and B, can be calculated by the ratio of their adjusted retention times when the same column and identical separation conditions are used:

$$\alpha = t'_{R(B)}/t'_{R(A)}$$

The adjusted retention times of *n*-alkenes, reported in Table I, show the influence of their structure on their ability to be complexed by α-, β- and γ-CD. The selectivities are increased when β-CD in formamide is used as a stationary phase. Chromatograms of the elutions of *cis* and *trans* isomers (Fig. 1) exemplify the behaviours of α-, β- and γ-CD with respect to *n*-alkenes.

It can be observed that α-CD influences very slightly the retentions of *n*-alkenes as an index of the low stability of the complex formed. Small but distinguishable effects of γ-CD activity, which improves the resolution of *cis–trans* isomers, can be

TABLE I

ADJUSTED RETENTION TIMES ($t'_R$) OF ACYCLIC ALKENES DETERMINED ON COLUMNS (2 m × 4 mm I.D.) FILLED WITH CELITE (30–80 mesh) COATED WITH FORMAMIDE (F, COLUMN I) AND FORMAMIDE SOLUTIONS MODIFIED BY α-CD (COLUMN II), β-CD (COLUMN III) AND γ-CD (COLUMN IV) AT 40°C AND A HELIUM FLOW-RATE OF 40 ml/min

| Compound | $t'_R$ | | | |
|---|---|---|---|---|
| | Column I, F | Column II, F, α-CD | Column III, F, β-CD | Column IV, F, γ-CD |
| *cis*-2-Hexene | 0.09 | 0.11 | 1.8 | 0.39 |
| *trans*-2-Hexene | 0.06 | 0.12 | 0.9 | 0.27 |
| *cis*-3-Hexene | 0.09 | 0.09 | 1.2 | 0.28 |
| *trans*-3-Hexene | 0.09 | 0.06 | 0.6 | 0.17 |
| *cis*-2-Heptene | 0.19 | 0.47 | 3.8 | 0.7 |
| *trans*-2-Heptene | 0.16 | 0.42 | 2.1 | 0.7 |
| *cis*-3-Heptene | 0.20 | 0.43 | 3.3 | 0.6 |
| *trans*-3-Heptene | 0.16 | 0.34 | 1.7 | 0.4 |
| *cis*-2-Octene | 0.42 | 0.58 | 7.9 | 1.3 |
| *trans*-2-Octene | 0.25 | 0.62 | 4.4 | 0.9 |
| *cis*-2-Nonene | 1.1 | 1.1 | 15.2 | 2.5 |
| *trans*-2-Nonene | 0.9 | 1.6 | 8.9 | 1.8 |

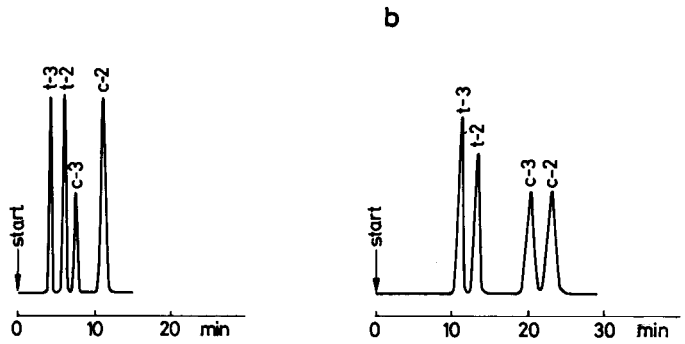


Fig. 1. Chromatograms of mixtures of (a) *cis*- and *trans*-2- and -3-hexenes and (b) *cis*- and *trans*-2- and -3-heptenes obtained under optimum conditions: β-CD column (2 m × 4 mm I.D.) containing 15.7 g of filling prepared using 20 g of Chromosorb P AW, 80–100 mesh, 1.5 g of β-CD and 4.5 g of formamide; temperature, 30°C; helium flow-rate, 40 ml/min; injected samples, 0.08 × 0.1 μl.

observed for 2-octenes and 2-nonenes. This behaviour may suggest that a major selectivity of γ-CD might appear towards larger molecules of *n*-alkenes (> $C_9$).

In contrast to α-CD and γ-CD, β-CD appears to be a true powerful selector for *n*-alkenes. The stereoselectivity imparted to a liquid stationary phase by β-CD complexation is considerable with respect to both *cis–trans* geometry and alternation of the double bond position. The data in Table I indicate that the *cis–trans* geometry is better recognized than double bond position by β-CD . Nevertheless the differentiation of all the investigated isomers was sufficient to perform the analyses, as is exemplified by the chromatograms of 2- and 3-hexenes and -heptenes.

The relatively indifferent behaviour of β-CD towards homologous compounds seems worth mentioning. The mean value of the separation coefficient for homologous series ($C_{n+1}/C_n$) on a β-CD estimated from the retention of $C_6$–$C_9$ alkenes of corresponding configuration is about 2 both for the compounds of *cis*-2 and for those of *trans*-2 configuration. Its comparison with the corresponding $\alpha_{(C_{n+1}-C_n)}$ values observed with pure matrix solvent (2–3) leads to the conclusion that the separation of homologous series achieved using the applied systems with β-CD is mainly due to the matrix solvent contribution, *i.e.*, formamide used in this study. This suggestion was confirmed in a separate set of experiments performed with $C_5$–$C_9$ 1-alkenes, where $\alpha_{(C_{n+1}-C_n)}$ was found to be *ca.* 2 for the columns containing either formamide or β-CD formamide solution.

*Resolution of cyclooctenes*

Cyclooctenes are interesting compounds because of the considerable difference (9.3 kcal/mol) in the strain energy between *trans* and *cis* isomers [27]. One of the most fascinating aspects of their structure is the inherent chirality of *trans*- or (*E*)-cyclooctene [28]. Therefore, it seemed interesting to establish how the cyclization and the difference in the structures of stereoisomers and enantiomers of cyclooctene will be reflected in their abilities to form CD inclusion complexes.

Fig. 2 shows chromatograms of *cis*-cyclooctene and of its mixtures with racemic *trans*-cyclooctene, generated in solution via a sensitized photoisomerization process [25]. Stereoselective inclusions in α-, β- and γ-CD cavities are confirmed.

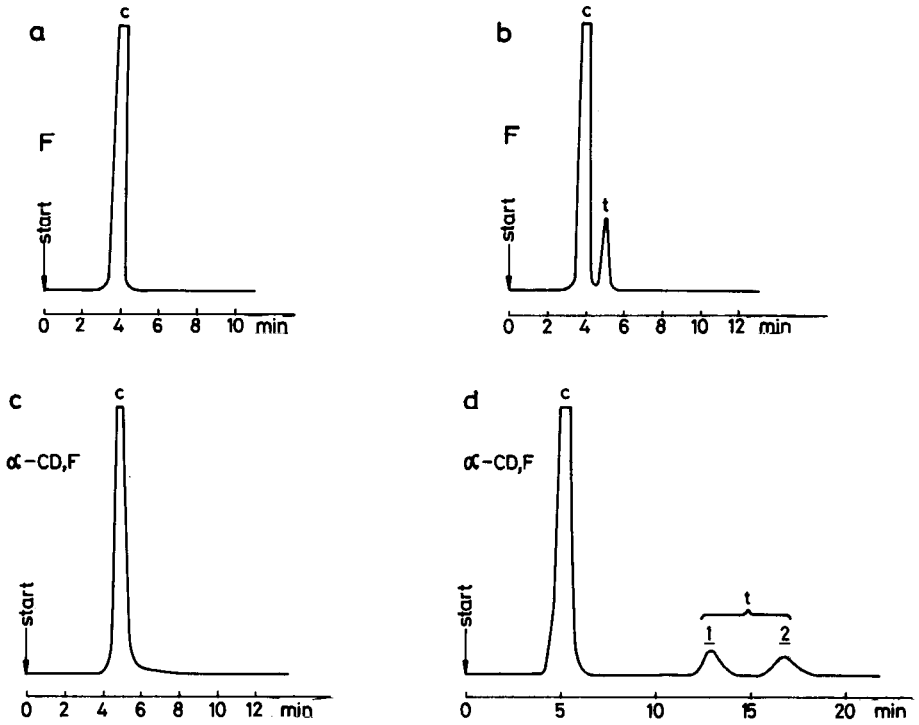


Fig. 2. Chromatograms of methanolic solution of *cis*-cyclooctene (a and c) unirradiated and (band d) afer *ca.* 16 h of irradiation performed at 40°C on (c and d) column II with α-CD in formamide and (a and b) on the reference column I with formamide. Helium flow-rate, 40 ml/min; injected samples, 0.1 μl. Peaks of methanol, which eluted much later than cyclooctene, are not shown.

It should be explained that for chromatographic studies of *trans*-cyclooctene behaviour we used the mixtures of *cis–trans* cyclooctenes obtained directly after the irradiation period (*ca.* 18 h). Under the conditions of the sensitized photoisomerization process applied in this work, the content of *trans* form in the stationary state was *ca.* 12% (*cis ca.* 88%).

These data indicate a high stereoselectivity of α-CD complexation with respect in the isomeric cyclooctenes, which leads to efficient baseline separations of (+)- and (−)-*trans*-cyclooctenes and their mixtures with *cis*- cyclooctene.

The kinetic data, reported in Table II show that the racemic *trans*-cyclooctene, determined on column I, is split into equal peaks on column II. This supports the hypothesis that the α-CD column really works for the separation of racemic *trans*-cyclooctene into enantiomers. To the best of our knowledge, this is the first example of the direct chromatographic resolution of *cis*-syclooctene and enantiomeric *trans*-cyclooctenes.

Further studies aimed at the determination of the absolute configuration of enantiomer 1 and 2 (indicated here according to the sequence of their elution) are in progress. Attempts have also been made to elaborate an assay method for monitoring chiral sensitized photoisomerization processes using optically active sensitizers.

TABLE II

CHANGES IN COMPOSITION OF *CIS*- AND *TRANS*-CYCLOOCTENE MIXTURE (CALCULATED FROM CHROMATOGRAPHIC DATA, IN %) DURING IRRADIATION PROCESSES

| Sample | Time of irradiation (h) | Composition determined at 40°C on the achiral column I covered with formamide | | Composition determined at 40°C on column II covered with α-CD solution in formamide | | |
|---|---|---|---|---|---|---|
| | | *cis*, $t'_R$ = 3.8 min | *trans*, $t'_R$ = 5.6 min | *cis*, $t'_R$ = 4.2 min | *trans* 1, $t'_R$ = 12.2 min | *trans* 2, $t'_R$ = 16.3 min |
| Initial solution[a] | 0 | 97.4 | – | 99.9 | – | – |
| Sample 1 | 6 | 93.1 | 4.4 | 94.8 | 2.4 | 2.3 |
| Sample 2 | 12 | 87.2 | 9.2 | 89.4 | 5.1 | 4.8 |
| Sample 3 | 18 | 83.9 | 12.8 | 86.7 | 6.7 | 6.6 |
| Sample 4 | 24 | 82.4 | 13.9 | 84.9 | 7.6 | 7.7 |
| Sample 5 | 32 | 82.0 | 13.4 | 86.8 | 6.6 | 6.5 |

[a] Initial solution containing 0.4 g of *cis*-cyclooctene dissolved in 5 ml of methanol and 50 mg of methyl benzoate used as a sensitizer. Irradiation process was performed following strictly ref. 24. Each value quoted is a mean value of three determinations. The peak of methanol, eluted at a retention time of 50 min from column I and 60 min from column II was not taken into account.

In contrast with the acyclic alkenes investigated, efficient separations of *cis*- and racemic *trans*-cyclooctene can be simply acieved on conventional liquid polar stationary phases, as has been reported elsewhere [29,30] and confirmed in this work using pure formamide liquid stationary phase ($\alpha_{trans-cis}$ = 1.46). This phenomenon is due to the substantial polarity of the *trans*-cyclooctene molecule. For this reason, the sequence of elution from the reference formamide column, first *cis* and then *trans*, is the opposite of that observed for acyclic *trans*- and *cis*-2-octenes.

Although γ-CD and espacially β-CD form much more stable complexes than α-CD with cyclooctenes, their discrimination of *cis–trans* geometry is very poor and there is no chiral recognition of *trans*-cyclooctene optical isomers. The very weak discrimination of *cis–trans* geometry arising from β-CD or γ-CD complexation is the opposite in direction to that imposed by formamide medium. As a consequence, the stereoselectivity towards *cis–trans* geometry exhibited by pure formamide ($\alpha_{trans-cis} \approx$ 1.46) can be annihilated by β- or γ-CD complexation, as has been found for columns III ($\alpha_{trans-cis} \approx 1$) and IV ($\alpha_{trans-cis} \approx 1$). On increasing the β-CD concentration a decrease in selectivity is observed, which leads to a change in $\alpha_{trans-cis}$ values from 1.46 to 1.0.

This behaviour confirms again the suggestion that there is no relationship between strength of α-, β- and γ-CD complexation of various organic compounds and their ability to differentiate selectively the isomers of these compounds.

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