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# Molecular Modeling as a Tool to Discriminate between Enantiomers Resolved by Derivatized Cyclodextrins in Gas Chromatography

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**Abstract.** The elution order of a series of enantiomers, determined by gas chromatography using permethylated and trifluoroacetylated $\beta$- and $\gamma$-cyclodextrins as stationary phases, was tentatively correlated with the lowest energies of the host–guest complex models resulting by including the enantiomers into the cyclodextrin cavity by means of the molecular mechanics method using standard software packages SYBYL and SPARTAN. The modeling data of cyclic isomers such as proline derivatives and $\gamma$-lactones correlate with the gas chromatographic data. Those of open isomers such as other aliphatic D,L-amino acids and alcohols, give contradictory results.

**Key words:** Modified cyclodextrins, enantioselective separations, chiral recognition, molecular modeling.

## 1. Introduction

The complexation properties and the chirality of $\alpha$-, $\beta$- and $\gamma$-cyclodextrins and of several alkylated derivatives have been applied both for chromatographic [1] and gas chromatographic separations of chiral isomers [2–5]. While the mechanisms of the chiral recognition have been investigated and understood for several amide ligands, both for models [6] and real systems [7,8], as being due to diastereospecific hydrogen bonding interactions, studies of the cyclodextrins consider two different chiral recognition mechanisms for gas chromatographic separations: the formation of an inclusion complex in the cavity and an external association [9]. Referring to the forces implied in complexation with cyclodextrins in an aprotic environment, short-range London forces are proposed as the main ones responsible for complexation, while smaller, longer-range electrostatic and hydrogen bonding contributions are considered for enantiodiscrimination [10]. In any case, neither of the theories

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furnish sufficient information to predict the potential resolution of a cyclodextrin and the elution order ($R > S$ or $S > R$) of analytes in chromatography.

This work has been planned to investigate whether the chiral analyte with the lower retention time in gas chromatography could be that which is less included in the cyctodextrin cavity, mainly because of its higher steric hindrance. In particular, attention was turned to cyclic isomers lacking —NH or —OH groups responsible for hydrogen bonding, such as proline derivatives and $\gamma$-lactones, which show high separation factors on $\beta$- or $\gamma$-cyclodextrin phases derivatized with methyl and trifluoroacetyl groups (Figure 1).

The inclusion level of the analytes was investigated by molecular modeling and was measured by comparing the energies of the host–guest complexes of the enantiomers introduced in the cavity [11,12]. Models of hosts were prepared starting from X-ray parameters of derivatized cyclodextrins drawn from the Cambridge Structural Data Base System [13]; twenty pairs of optical isomers were considered as guests.

## 2. Experimental

### 2.1. Synthesis of permethylated cyclodextrins

Cyclodextrin derivatives were synthesized by modification of methods previously proposed [14]. Briefly, permethylated $\beta$-cyclodextrin was synthesized by treating a dimethylsulphoxide (DMSO) solution (20 mL) of commercial $\beta$-cyclodextrin (1 mmol) with sodium hydride (20 mmol) and then with methyl iodide in excess (50 mmol), added slowly with stirring at room temperature. After 24 h the reaction mixture was diluted with 100 mL water and extracted twice with 30 mL of methylene chloride. The organic phase was washed with 30 mL of water and evaporated to dryness The residue was fractionated on silica gel and crystallized from a hexane/ethyl acetate mixture (1/1). Pure product (0.4 mmol) was recovered. Analytical data corresponded to those given in the literature [15]. Permethylated $\gamma$-cyclodextrin was synthesized similarly.

### 2.2. Synthesis of 2,6-di-*O*-methyl-3-*O*-trifluoroacetyl $\beta$- and $\gamma$-cyclodextrins

The synthesis was performed under the same conditions as for permethylated cyclodextrins, substituting sodium hydride reagent by sodium hydroxide (30 mmol per mmol of cyclodextrin). The organic phase obtained by extracting the reaction mixture with dichloromethane contained a mixture of permethylated and dimethylated cyclodextrins. Separation was done on silica gel (43–60 mm) by column chromatography (1.5 × 20 cm), eluting with a dichloromethane/dimethoxyethane mixture (1/1). Two main fractions were recovered; the first gave crystals of permethylated cyclodextrin, the last (200 mg), showing two methoxy groups per unit of glucose ($^1$H-NMR), was dissolved in pyridine (1 mL) and treated with triflu-

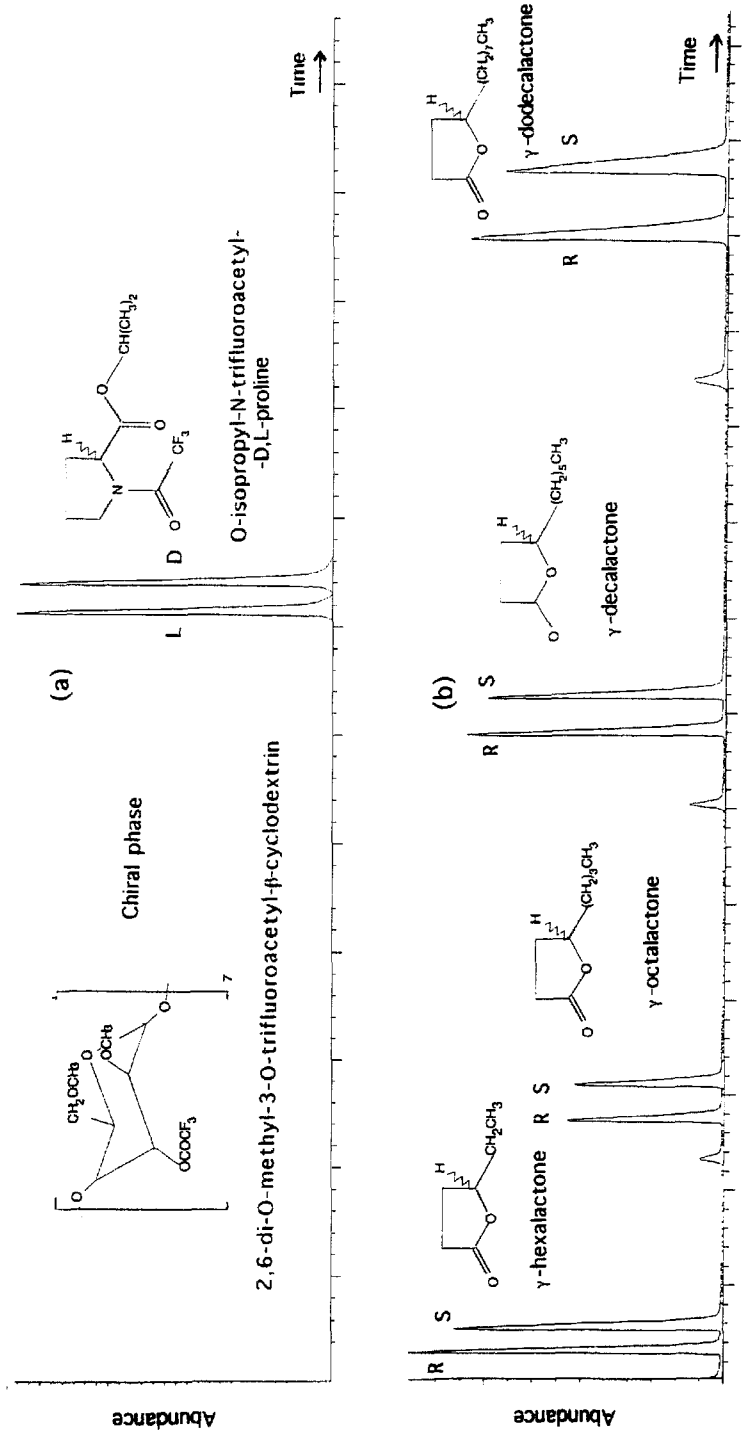


*Figure 1.* GC separation of *O*-isopropyl-*N*-trifluoroacetyl D,L-proline derivatives (a) and γ-lactones (b) on capillary columns with 2,6-dimethyl-3-trifluoroacetyl-β-cyclodextrin/Carbowax 20M (1/1).

oroacetic anhydride (0.5 mL) at 60 °C for 4 h. The reaction mixture was diluted with 10 mL of dichloromethane and mixed with 20 mL of 2N aqueous HCl. The organic phase was recovered with a separating funnel and washed twice with 10 mL of water, dried on $CaCl_2$, and concentrated. The clear, pale-yellow, viscous liquid recovered showed the stretching IR band of C=O at 1720 $cm^{-1}$ and appeared to contain mainly the 2,6-di-*O*-methyl-3-*O*-trifluoroacetyl cyclodextrin.

### 2.3. Gas chromatography

Fused silica columns (25 m long) of untreated capillary tubing from Supelco (Bellefonte, PA USA) were dynamically coated with a 8% dichloromethane solution of the derivatized cyclodextrin mixed with Carbowax 20M (1/1 wt.). One tenth of the column was filled with the solution which was slowly advanced (80 cm/min) by pushing with a segment of mercury (40 cm) and nitrogen. After elution, the column was kept under flowing nitrogen for 10 min at 60 °C, then was conditioned at 160 °C for 2 h. The Grob test was performed at 120 °C and efficiency was measured by testing the dodecane peak. All columns were filled with the same method and the efficiency was generally higher than 1000 plates per meter.

### 2.4. Molecular modeling

$\beta$- and $\gamma$-cyclodextrin structures were recovered from the Cambridge Structural Data Base [11]. The cyclodextrin skeleton was modified by introducing methyl and trifluoroacetyl groups, using the SPARTAN [16] package software (Figure 2).

Before simulation of interactions, guests and hosts were fully minimized using the Tripos force field method. Studies were done using different software packages: SYBYL [17] and SPARTAN. SYBYL runs on Silicon Graphics Indigo Extreme II work stations with 32 Mbytes RAM and 3D graphics board with Z-buffer; SPARTAN runs on IBM RISC 6000/250 machines with 32 Mbytes RAM and 3D 24 bit graphics card with Z-buffer. Docking experiments were done both with SYBYL and BIOSYM [18] packages. The force field used was the SPARTAN version of Tripos 5.2, SYBYL version 5.3(19), while CVFF was used in BIOSYM.

## 3. Results and Discussion

An initial conformational analysis of the guests was performed, to deduce the population of the conformers and to choose the most abundant for inclusion and docking. Conformers of proline were determined in detail both by SYBYL and SPARTAN using a systematic search on C—C bonds, and gave similar results (Table I).

The study has been limited to conformers arising from a systematic search rotation (360°/6) around the first C—C bond of the side chain (C—C=O for prolines and C—C—R for lactones) (Figure 1). The predominant conformer (>50%) for lactones and proline derivatives was used for inclusion experiments. Noncyclic

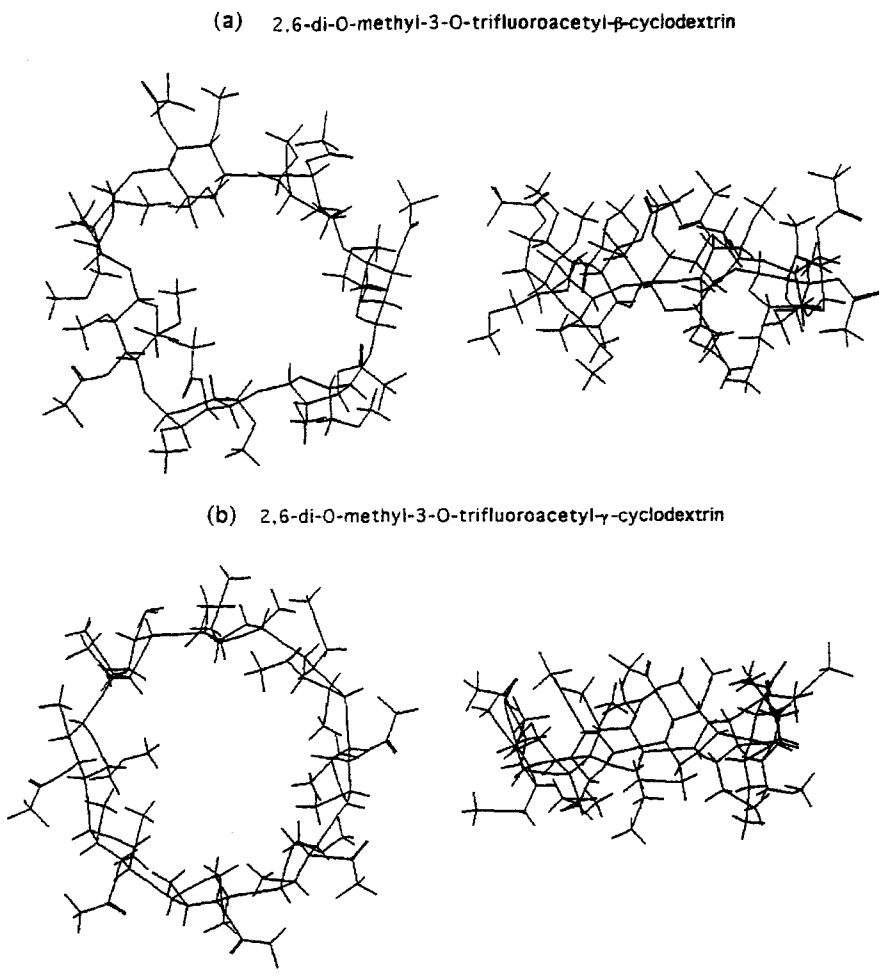


*Figure 2.* Stick models of 2,6-dimethyl-3-TFA-$\beta$-CD (a) and 2,6-dimethyl-3-TFA-$\gamma$-CD (b) minimized by SPARTAN package software.

compounds generally showed a larger variety of conformers, with lower energy differences. Compounds were introduced in the cyclodextrin cavity along a fixed axis, against the dipole moment orientation, according to the line described in the guideline shown in Scheme I.


Scheme I. Guideline followed to insert guest in the cyclodextrin cavity.

Table I. Population and energy of conformers of proline derivatives and lactones calculated by SYBYL.

| Compound | C—C bond rotation (360°/6) | Number of conformers | % Population and energy (kJ $mol^{-1}$) |
|---|---|---|---|
| L-Proline-*N*-TFA/*O*-*i*Pr | C—C=O | 2 | 86 (42.17), 14 (46.86) |
| | C—$CF_3$; C—C=O | 5 | 57, 19, 8, 8, 6 |
| | O—$CH(CH_3)_2$; C—$CF_3$; C—C=O | 15 | 19, 19, 19, 6, 6, 6, 2, 2, 2, 2, 2, 2, 2, 1, 1 |
| D-Proline-*N*-TFA/*O*-*i*Pr | C—C=O | 2 | 86 (42.17), 14 (46.86) |
| L-Proline-*N*-TFA/*O*-Me | C—C=O | 2 | 81 (41.34), 19 (41.34) |
| D-Proline-*N*-TFA/*O*-Me | C—C=O | 2 | 81 (41.34), 19 (41.34) |
| L-Proline-*N*-PFP/*O*-*i*Pr | C—C=O | 2 | 72 (41.46), 28 (43.85) |
| D-Proline-*N*-PFP/*O*-*i*Pr | C—C=O | 2 | 72 (41.46), 28 (43.85) |
| $\gamma$-hexalactone (*R*) | C—$CH_2CH_3$ | 2 | 53 (31.76), 47 (32.05) |
| $\gamma$-hexalactone (*S*) | C—$CH_2CH_3$ | 2 | 53 (31.63), 47 (31.92) |
| $\gamma$-octalactone (*R*) | C—$CH_2CH_2CH_2CH_3$ | 2 | 53 (33.15), 47 (33.43) |
| $\gamma$-octalactone (*S*) | C—$CH_2CH_2CH_2CH_3$ | 2 | 53 (33.01), 47 (33.35) |
| $\gamma$-decalactone (*R*) | C—$CH_2(CH_2)_4CH_3$ | 2 | 53 (34.73), 47 (34.98) |
| $\gamma$-decalactone (*S*) | C—$CH_2(CH_2)_4CH_3$ | 2 | 53 (34.60), 47 (34.85) |
| $\gamma$-dodecalactone (*R*) | C—$CH_2(CH_2)_6CH_3$ | 2 | 53 (36.32), 47 (36.53) |
| $\gamma$-dodecalactone (*S*) | C—$CH_2(CH_2)_6CH_3$ | 2 | 53 (36.15), 47 (36.44) |

The end point corresponds to the position with a minimum of energy of the system. It was checked easily and automatically, by fixing the guest in a previously determined centroid. The energies of the system were minimized with geometrical optimization. Host–guest interactions were considered as mainly being due to London forces. Several computational methods were tested. The manual positioning of the guest was repeated several times because the SPARTAN version did not allow automatic docking. In fact, not all the software packages provide a docking function; generally this function guides the operator by displaying the best direction to position the guest in the cavity, simultaneously showing the energy values. In other cases, the operator fixes several points in the cavity and moves the guest from point to point by computing the geometry optimization, the energy contributions and the other parameters needed. The list and the minimum energies of the host–guest complexes calculated by the SPARTAN software package are reported in Table II.

Separation factors $\alpha$ represent the ratios $t'_2/t'_1$ of the retention times of the pairs of enantiomers determined by gas chromatography, under isothermal conditions, on capillary columns dynamically coated with a 8% solution of the cyclodextrin derivatives mixed 1/1 with Carbowax 20M.

As far as proline derivatives and $\gamma$-lactones are concerned, the gas chromatographic elution order recorded on 2,6-dimethyl-3-TFA-$\beta$-CD correlated well with the energies of the host–guest systems: the enantiomer which deeply penetrates into the cavity and shows the lower complex energy is the one with greater retention in

Table II. Minimum energy of the host–guest complexes determined by SPARTAN, separation factors $\alpha$, and sign of the correlation with the GC elution order.

| Guest | | Host | Host–guest min. energy kJ mol$^{-1}$ | GC data $\alpha$ (temp. °C) | GC el. order | Correlation GC-data/ min. energy |
|---|---|---|---|---|---|---|
| **1.** Proline (*O*Me/TFA) | (D) | $\beta$-CD(3-TFA | 299.11 | 1.15 (120) | L < D | positive |
| | (L) | 2,6-dimethyl) | 329.15 | | | |
| **2.** Proline (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | 293.42 | 1.40 (120) | L < D | positive |
| | (L) | 2,6-dimethyl) | 294.80 | | | |
| **3.** Proline (*Oi*Pr/EFB) | (D) | $\beta$-CD(3-TFA | 288.44 | 1.20 (120) | L < D | positive |
| | (L) | 2,6-dimethyl) | 289.57 | | | |
| **4.** $\gamma$-hexalactone | (*R*) | $\beta$-CD(3-TFA | 305.72 | 1.12 (150) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 289.20 | | | |
| **5.** $\gamma$-octalactone | (*R*) | $\beta$-CD(3-TFA | 325.85 | 1.12 (150) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 294.51 | | | |
| **6.** $\gamma$-decalactone | (*R*) | $\beta$-CD(3-TFA | 322.92 | 1.06 (150) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 276.94 | | | |
| **7.** $\gamma$-dodecalactone | (*R*) | $\beta$-CD(3-TFA | 296.44 | 1.05 (150) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 273.63 | | | |
| **8.** Alanine (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | −47.78 | 1.03 (100) | D < L | negative |
| | (L) | 2,6-dimethyl) | 29.04 | | | |
| **9.** Valine (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | 301.08 | 1.06 (100) | D < L | positive |
| | (L) | 2,6-dimethyl) | 264.47 | | | |
| **10.** Norvaline (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | 298.03 | 1.04 (100) | D < L | positive |
| | (L) | 2,6-dimethyl) | 260.78 | | | |
| **11.** Leucine (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | 279.91 | 1.08 (100) | D < L | positive |
| | (L) | 2,6-dimethyl) | 199.24 | | | |
| **12.** Norleucine (*Oi*Pr/TFA) | (D) | $\beta$-CD(3-TFA | 296.22 | 1.06 (100) | D < L | negative |
| | (L) | 2,6-dimethyl) | 335.01 | | | |
| **13.** 2-Cl-propionicacid (*O*Me) | (*R*) | $\beta$-CD(3-TFA | 265.56 | 1.12 (80) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 261.67 | | | |
| **14.** 1-phenylethanol | (*R*) | $\beta$-CD(2,3,6- | −121.25 | 1.07 (110) | *R* < *S* | positive |
| | (*S*) | trimethyl) | −171.29 | | | |
| **15.** 2,4-dimethylphenyl | (*R*) | $\beta$-CD(2,3,6- | 652.79 | 1.09 (110) | *R* < *S* | positive |
| ethanol | (*S*) | trimethyl) | 774.92 | | | |
| **16.** 2-pentanol(TFA) | (*R*) | $\beta$-CD(2,3,6- | −41.46 | 1.05 (60) | *R* < *S* | positive |
| | (*S*) | trimethyl) | −52.38 | | | |
| **17.** Proline (*Oi*Pr/TFA) | (D) | $\gamma$-CD(3-TFA | −34.98 | 1.38 (120) | L < D | positive |
| | (L) | 2,6-dimethyl) | −17.87 | | | |
| **18.** Proline (*Oi*Pr/EFB) | (D) | $\gamma$-CD(3-TFA | 20.59 | 1.20 (120) | (D < D | negative |
| | (L) | 2,6-dimethyl) | −3.81 | | | |
| **19.** $\delta$-decalactone | (*R*) | $\gamma$-CD(3-TFA | 183.42 | 1.06 (150) | *R* < *S* | positive |
| | (*S*) | 2,6-dimethyl) | 51.88 | | | |
| **20.** $\delta$-dodecalactone | (*R*) | $\gamma$-CD(3-TFA | −58.70 | 1.05 (150) | *R* < *S* | negative |
| | (*S*) | 2,6-dimethyl) | −43.30 | | | |

the GC system. When the conformational analysis on the guests showed small energy differences, docking computation was performed on more than one conformer. Guests showing conformers with very similar energies generally show lower GC separation factors on cyclodextrins, and host–guest molecular models give contradictory results even if refined by docking with SYBYL and BIOSYM packages. Thus the GC elution order of leucine and valine derivatives correlate with the energies recorded, while alanine and norleucine do not. However, amino acids have —NH groups responsible for stereospecific hydrogen bonding which can play a role in the chiral separation. $\delta$-Lactones tested on $\gamma$-cyclodextrins also give contradictory results; in this case the cavity of the cyclodextrin is larger and the energy of the complex is influenced by the orientation of the side chain of the lactone.

## 4. Conclusions

In general, the gas chromatographic elution order of chiral cyclic compounds such as proline derivatives and $\gamma$-lactones on 2,6-di-*O*-methyl-3-*O*-trifluoroacetyl-$\beta$-cyclodextrin stationary phases correlates positively with the energies of the guest–host complexes calculated by molecular modeling. Phenylethanol derivatives recorded on permethylated $\beta$-cyclodextrin behave similarly. Contradictory results are given by aliphatic amino acids bearing —NH groups.

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