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Application and chiral recognition of heptakis (2,6-di-O-methyl-3-O-trifluoroacetyl)-β-cyclodextrin as a stationary phase for the gas chromatographic separation of enantiomers

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ABSTRACT

The separation of a large number of enantiomeric pairs of various classes (bornanes, aromatics, etc.) with heptakis(2,6-di-O-methyl-3-O-trifluoroacetyl)-β-cyclodextrin, used as a stationary phase in capillary gas chromatography, widens the scope of the application of the latter. The analysis of the selectivity of this phase with respect to individual substances and the separation achieved made it possible to draw some conclusions on the steric disposition of the enantiomers.

INTRODUCTION

An important breakthrough has been achieved recently in the gas chromatographic separation of enantiomers by using modified α,β-cycoldextrins (CDs) as chiral stationary phases in high-resolution capillary columns [1–4]. Schurig and Nowotny were the first to use open-tubular columns coated with a solution of heptakis(2,6-di-O-methyl-3-O-trifluoroacetyl)-β-cyclodextrin in OV-1701 for the gas chromatographic separation of volatiles belonging to different classes of compounds, such as γ-lactones, oxiranes, ketones and spiroketals. No derivatization procedures were necessary for most of the resolved chiral molecules.

Trifluoroacetylation of 2,6-O-alkyl-CDs is useful because CF_3COO groups are able to form additional hydrogen bonds and to induce dipole–dipole interactions between them and the enantiomers. Later, 2,6-dipentyl-3-O-trifluoroacetyl-CDs (α, and γ) were synthesized and coated on fused-silica capillary columns [5]. These phases were used for the separation of alcohols, polyols, amines and other compounds after derivatization with trifluoroacetyl anhydride.

This paper extends the scope of enantiomeric separations on heptakis(2,6-di-O-trifluoroacetyl)-β-CD to compounds of different types (bornanes, aromatics, etc.). Some assumptions are made concerning the mechanism of chiral recognition and the steric disposition of the investigated compounds in the cavity of the CD. The steric disposition of enantiomers in the cavity is assessed by the steric interaction between the latter and the enantiomers. The larger the difference between the energies of formation of the diastereomeric complexes, the higher is the selectivity α of the modified CD used as a stationary phase in gas chromatographic separations. Hence, on the basis of selectivity it is possible to make assumptions regarding the chiral recognition, to model the modification of CDs, to evaluate the extent of modification, etc. In some instances the steric disposition of the molecules in a CD can be exactly determined by molecular modelling [6,7], but this is an expensive and mostly inaccessible method. Therefore, we decided to use the data for

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the chromatographic separation of enantiomeric pairs and the steric direction with modified CDs, which also offer the opportunities for different intermolecular interactions. For this purpose we used compounds of different classes with CDs modified to various extents.

EXPERIMENTAL

Synthesis of heptakis(2,6-di-O-methyl-3-O-trifluoroacetyl)-β-CD

This compound was synthesized as described previously [8] and purified by column chromatography. The purity was controlled by thin-layer chromatography (TLC) (R_F = 0.30) with toluene–ethanol (90:10, v/v). Trifluoroacetylation was performed similarly to a reported procedure [1,9]. The extent of acetylation was controlled by TLC and IR spectrometry. A completely trifluoroacetylated phase (I) and a partially trifluoroacetylated phase (II) (*ca.* 75% by IR spectrometry) were prepared.

Preparation of glass capillary columns

Pyrex glass tubing (Schott Ruhrglass, Mainz, or Jenaglass, Jena, Germany) were drawn out to capillaries of 0.26 mm I.D. using a Shimadzu GDM1 drawing machine. Acid leaching, rinsing and deactivation were performed as described [10,11]. The columns were coated with a 0.3% solution of the modified β-CD I and II and OV-1701 (Macherey, Nagel & Co., Düren, Germany) (12:88) in dichloromethane.

The capillary columns were tested with a Grob test mixture to assess their efficiency and inertness.

Gas chromatography

A Pye Unicam Model 304 or a Perkin-Elmer Sigma 300 instrument with split injection and flame ionization detectors were used. Nitrogen served as the carrier gas. Data were registered with a Shimadzu CR-1B electronic integrator.

Solutes

Compounds of different classes (bornanes, aromatics, etc.) were analysed.

RESULTS AND DISCUSSION

Bornanes (bicyclo[2.2.1]heptanes)

The selectivity of phase I with respect to bornan-2-one (**1**) is satisfactory, α = 1.05 (Table I). Compound **1** was analysed also with phase II (con-

TABLE I

GAS-CHROMATOGRAPHIC DATA ON THE SEPARATION OF ENANTIOMERS ON HEPTAKIS(2,6-DI-O-METHYL-3-O-TRIFLUOROACETYL)-β-CD (PHASE I) IN OV-1701

The separation was carried out with a capillary column (25 m × 0.26 mm I.D.) with nitrogen as the carrier gas at a flow-rate of 25–35 cm/s.

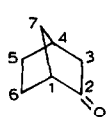
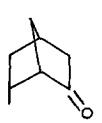
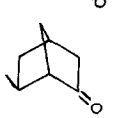
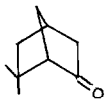
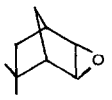
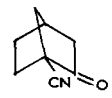
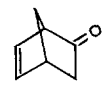
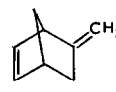
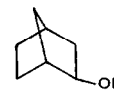
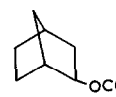
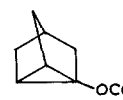
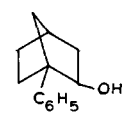
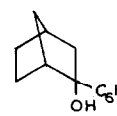
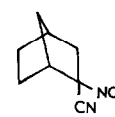
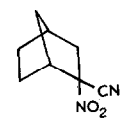
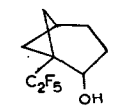
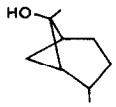
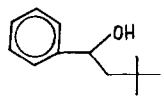
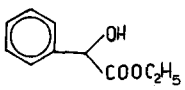
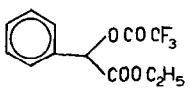
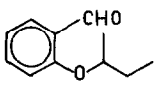
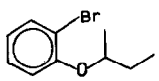
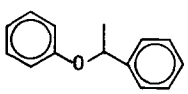
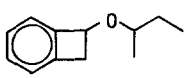
No.	Substance		Temperature (°C)	Selectivity (α)	Resolution (*R*)
1			80	1.05	0.6
2		*endo*	80	–	–
3		*exo*	80	1.19	3.9

TABLE I *(continued)*

No.	Substance		Temperature (°C)	Selectivity (α)	Resolution (R)
4			80	1.05	1.0
5		*exo*	100	1.11	2.2
6			140	1.14	3.5
7			80	1.05	1.5
8			80	1.05	1.5
9			80	1.05	1.5
10			70	1.06	1.6
11			80	–	–
12			120	1.04	1.7
13			120	–	–
14			120	1.07	0.5
15			120	–	–

(Continued on p. 260)

TABLE I *(continued)*

No.	Substance	Temperature (°C)	Selectivity (α)	Resolution (R)
16		80	1.04	0.7
17		80	–	–
18		100	1.03	1.2
19		100	1.07	2.0
20		100	1.03	1.4
21		110	–	–
22		80	–	–
23		140	1.02	1.4
24		90	1.06	1.8

taining hydroxyl groups). It was found that phase II shows the same selectivity for **1**, but the separation of the enantiomers was $R = 0.6$, on account of tailing due to hydrogen bonds. The same selectivity of phases I and II with respect to **1** reveals that it is determined predominantly by the interaction between the hydrophobic parts of the bornane skeleton and the cavity of the CD, after the penetration of **1** into the latter.

The addition of one or two methyl groups at C-6 of bornan-2-one (compounds **2–4**) has a considerable impact on the separation. When the methyl group is in an *endo* position (**2**) the enantiomeric pair cannot be separated. This pair is less strongly retained than **3** (Fig. 1). In **3** the methyl group is in an *exo* position and with respect to this pair phase I exhibits considerable selectivity ($\alpha = 1.19$). It should be emphasized that no tailing is observed in

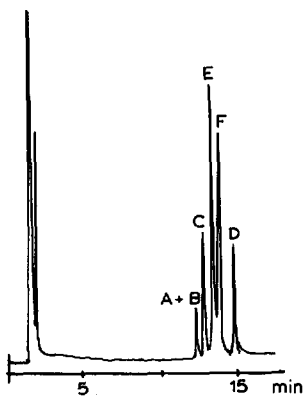


Fig. 1. Separation of racemic mixtures of **2** (A, B), **3** (C, D) and **4** (E, F) on a 25 m × 0.26 mm I.D. capillary column, coated with 0.2 μm of phase I. Oven temperature, 80°C; carrier gas, nitrogen; flow-rate, 30 cm/s.

the analysis of these compounds with phase II. Hence the inserted methyl group favours a disposition of **2** and **3** such that the carbonyl group cannot form hydrogen bonds with the hydroxyl groups at the C-2 of CD. As the methyl group cannot generate interaction forces with the cavity of CD other than dispersion forces, it is evident that in **2** the position of this group is such that the norbornane skeleton cannot fit to the hydrophobic internal surface of the CD cavity. With **3** the fitting is very close. This indicates that the methyl group in **2** prevents the bornane skeleton from fitting to the internal surface of the CD on the plane formed by the C-2, C-3, C-7 and C-5, C-6, C-7 atoms. When the methyl group is in an *exo* position it represents a prolongation of this plane, whereby it enhances the Van der Waals forces of attraction. These suppositions are corroborated by **4** containing *endo*- and *exo*-CH_3 groups at C-6. With respect to **4**, phases I and II exhibit an intermediate selectivity between **2** and **3**.

The substitution of the keto group of **4** by an epoxy ring in an *exo* position (**5**) considerably raises the selectivity, from 1.05 to 1.11, despite the fact that the chromatographic procedure was carried out at a temperature 30°C higher than that for **3**. Moreover, when the chromatographic procedure is performed with phase II the tailing diminishes, *i.e.*, the oxygen atom of the epoxy ring in **5** is disposed in such a way in the cavity that it cannot form hydrogen bonds with 2-OH groups of CD. The high selectivity of **5** can be explained by the fact that the epoxy ring enlarges the contact surface area, *i.e.*, it is now with the C-2, O, C-3, C-7 and C-5, C-6, C-7 atoms.

On synthesizing **6** from **1** the selectivity of phases I and II considerably increases and at the same time the tailing is reduced.

The double bond between C-5 and C-6 in the bornan-2-one skeleton of **7** and **8** has no effect on the selectivity of the stationary phase, *i.e.*, the selectivity is again determined by the bornane skeleton and not by the C=O or $=CH_2$ groups. From the fact that the selectivity and separation rates with **7** and **8** are identical follows that their behaviours towards the stationary phase are similar. In contrast to **1**, where C=O is at C-2 and tailing occurs, in **7** C = O is at C-3 and no tailing is observed. For the series **1–5** the bornane skeleton fits to the internal surface of CD on the planes formed by C-2, C-3, C-7 and C-5, C-6, C-7. In this position the C=O group is at the orifice of the CD cavity and may form hydrogen bonds with the 2-OH group of the CD. With **7** C=O is at C-3 and no tailing is observed with phase II. This presupposes the same disposition of the bornane skeleton in the CD as in **1–5**, but the C=O group is inside the cavity and not at the orifice.

The replacement of the keto group in **1** by a hydroxyl group in **9** again has no effect on the selectivity of the phase, but the separation is poor owing to hydrogen bonding. To prevent tailing, which is induced by the hydrogen bonds between the stationary phase and the solutes, Amstrong and coworkers [12,13], who used a polar CD-derivative phase containing OH groups, acetylated solutes with trifluoroacetic anhydride. Li *et al.* [5] also acetylated solutes, although they separated them with perdi-O-methyl-3-O-trifluoroacetyl)-α-, -β- or -γ-CDs. Further, Schurig *et al.* [14] separated menthols, alcohols and diols by permethylated 2,3,6-β-CD, but not with 2,6-dimethyl-3-trifluoroacetyl-β-CD, perhaps because of the incomplete acetylation or hydrolysis of the latter.

Compound **11** can be seen as a derivative of **10** by linking the C-2 and C-5 atoms, whereby the bornane skeleton is altered and thus cannot fit to the internal surface of the cavity.

The addition of a benzene ring to the bornane skeleton offers a new opportunity for interaction

between the solute and the CD. It becomes possible for the bornane skeleton to fit in the cavity and the benzene ring to remain outside and *vice versa*. No supposition can be made regarding the disposition of **12** and **13** in the cavity.

On accumulation of negative electric charges on the CN and NO_2 groups on C-2 in the bornane skeleton (**14**), there is nevertheless a separation of the enantiomeric pairs (Fig. 2a, b). If the values of R and α for **14** are compared with those of **1** and **9**, it can be concluded that the CN group in an *endo* position at C-2 has no effect on selectivity, whereas NO_2 forms hydrogen bonds with 2-OH groups of phase II. No tailing was observed when I was used for chromatographic analysis. It can be stated that the hydrogen bonds are stronger than those of **1** and **9** as the tailing is stronger and, despite the higher selectivity, the separation is poorer. After rearrangement of the groups in **15** the phase does not exhibit any selectivity. From this it follows that the *endo*-CN group at C-2, through interaction with the CF_3CO groups, guides the bornane skeleton to adhere to the cavity of β-CD similarly to **6**.

Both phases I and II exhibit almost the same selectivity with respect to **16** (1-perfluoroethyl-2-hydroxybicyclo[1.1.3]heptane) as **1**, **4** and **9** (Fig. 2c). The unsatisfactory separation is due again to the hydrogen bonds. The phases, however, do not exhibit selectivity if the OH group is introduced at the bridge of the skeleton, compound **17** (7-methyl-2,7-dihydroxybicyclo[1.1.3]heptane). This indicates that the substitution at C-7 hinders the adherence of the skeleton to the cavity of the β-CD.

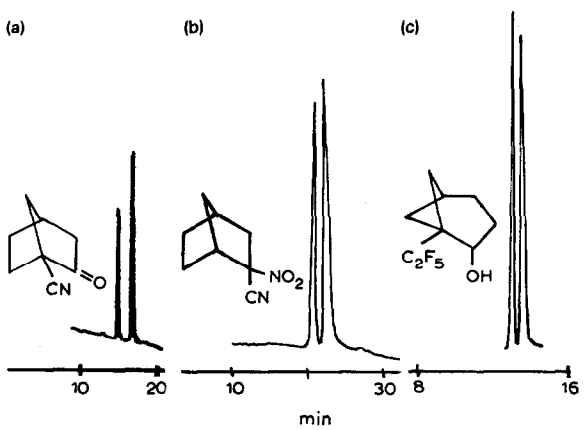


Fig. 2. Separation of enantiomers of some bornane derivatives on a 25 m × 0.26 mm I.D. capillary column coated with 0.15 μm of phase I at a linear velocity 30 cm/s of nitrogen.

Benzene derivatives

In these compounds one of the substituents at the chiral centre is a benzene ring and the other is a hydrogen atom. The two remaining valences of the chiral centre are linked with groups having various polarities and volumes.

The role of the volume of the substituent on the separation in the case of **18** is noteworthy. With **19** the separation is good because of the well expressed selectivity, $\alpha = 1.07$, *i.e.*, the C_2H_5COO group exhibits a considerable impact by interaction with the CF_3COO groups of the stationary phase. The acetylation of the hydroxyl group giving **20** reduces tailing but also decreases the selectivity as well. Hence, with these compounds the benzene ring has a marked effect on the selectivity in addition to the hydroxyl group.

The role of the substituent with respect to the selectivity of **21**–**24** is interesting. No separation of the enantiomers takes place with **21**. No separation occurs even if the electronegative bromine is substituted for the aldehyde group (**22**). However, if a benzene ring is substituted for the methyl group at the chiral centre of **21** (**23**) the enantiomeric pair can be separated.

Compound **24** has two chiral centres, but on account of the consideration on the effect of the substituents presented above, it was possible to separate an enantiomeric pair with a chiral centre at the four-membered ring. This pair was synthesized by stereoselective synthesis especially to confirm this prediction.

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