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Structural factors affecting the enantiomeric separation of barbiturates and thiobarbiturates with a chiral side-chain by various β-cyclodextrin supports. Effects of the presence of hydroxypropyl substituents on the chiral selector

N. Thuaud*, B. Sebille

Laboratoire de Physicochimie des Biopolymères, UM 27 CNRS, Université Paris XII, 2 Rue H. Dunant, 94320 Thiais, France

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Abstract

The chromatographic separation of barbiturate and thiobarbiturate enantiomers on β-cyclodextrin (β-CD) HPLC columns was studied. The effect of the nature of the side-chain bearing the chiral centre was examined. The stationary phases used were of two types, commercial with native β-CD or hydroxypropyl-β-CD residues linked to silica and laboratory-made with β-CD polymers adsorbed on silica. The chiral recognition of the studied compounds is mainly dependent on the presence of hydroxypropyl substituents in the β-CD cavity.

1. Introduction

In recent years, various chiral cyclodextrin stationary phases (CDSP) have been developed. Two types of packing have been synthesized.

The first relies on the covalent bonding of cyclodextrins (CD) or derivatives to silica via different spacers. Columns packed with this type of support are commercially available: Cyclobond (Astec) columns containing an ether arm through which α-, β- and γ-native CD [1] or some derivatized CDs are linked to 5-μm silica [2,3], and the Chiradex column (Merck) with native β-CD bonded to 5-μm LiChrospher via a carbamate-type spacer. These supports exhibit excellent properties allowing the enantiomeric resolution of numerous compounds.

The second type of packing was obtained by absorption of β-CD polymers on silica. Two polymers have been prepared, either by condensation of β-CD molecules with a bifunctional reagent (EP–β-CD–N^+ polymer) [4], or by grafting a monosubstituted β-CD derivative on to a linear polymer (polyvinylimidazole, PVI) [5]. The ability of these stationary phases to resolve racemic mixtures usually separated on commercial supports has been demonstrated. Moreover, we have shown that the chemical microenvironment of the β-CD influences the selectivity of the chromatographic phases. Thus, pendant β-CD residues linked to the PVI chain by a single spacer arm exhibit a chemical environment different to that observed with β-CD

* Corresponding author.

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cavities bearing 6–7 hydroxypropyl groups in the EP–β-CD–N^+ polymer. The overcrowding resulting from this structure generates steric hindrance for the penetration of guest molecules into the cavities and/or additional interactions [5].

Preliminary results on the discrimination of chiral barbiturates by β-CD complexation have been obtained both on β-CD chiral columns and on reversed-phase achiral columns with β-CD in the mobile phase. The most commonly studied barbiturates, such as hexobarbital and mephobarbital, which have their chiral centre at the C-5 position on the pyrimidine ring, have been reported to be resolved on CDSP [4,6] or with β-CD complexation in the mobile phase [7–9]. In contrast, it has been reported that it is more difficult to resolve barbiturates bearing the chiral centre outside the heterocyclic ring. Little information is available on the resolution of this type of compound by β-CD complexation. Cyclobond columns failed to resolve butabarbital and pentobarbital [10]. This latter racemate could not be resolved either on PVI–β-CD polymer [5] or with β-CD in the eluent [7]. With this HPLC mode, secobarbital [7,9], thiopental [7] and butabarbital [9] enantiomers also were not separated. To our knowledge we have reported the only example of the resolution of a barbiturate of this type, pentobarbital, using a CDSP, the EP–β-CD–N^+ support [5]. Chiral recognition of thiamylal enantiomers have also been reported using an achiral reversed-phase column and addition of β-CD to the mobile phase [8].

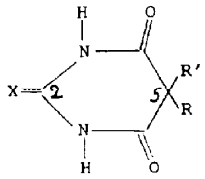
This paper describes the chiral separation of a series of barbiturates (BA) and thiobarbiturates (TBA), bearing the chiral centre on a substituent at the C-5 position, by employing several CDSPs, commercial with β-CD residues covalently linked to silica and laboratory-made with β-CD polymers adsorbed on silica. It has been pointed out that the structure of the guest, i.e., the nature of the heteroatom at C-2 and that of the substituents at C-5, greatly influences the chiral recognition of the racemates. Stationary phases with β-CD hydroxypropyl substituents allow some separations of BA that cannot be resolved with native β-CD.

2. Experimental

2.1. Chemicals

The structures of the optically active BA and TBA studied are shown in Table 1. These racemic compounds were kindly provided by

Table 1
Structural formulae of the investigated TBA and BA



Compound	Name	X	R	R'
1	Butabarbital	O	Ethyl	*sec.*-Butyl
2	Talbutal	O	Allyl	*sec.*-Butyl
3	Pentobarbital	O	Ethyl	1-Methylbutyl
4	Secobarbital	O	Allyl	1-Methylbutyl
5	Cyclopental	O	Allyl	2-Cyclopentenyl
6	Brevital	O	Allyl	1-Methyl-2-pentenyl
7	Thiopental	S	Ethyl	1-Methylbutyl
8	Thiamylal	S	Allyl	1-Methylbutyl

Professor Dr. J. Bojarski, Department of Organic Chemistry, N. Copernicus Academy of Medicine, Krakow, Poland. An examination of these structures revealed that, owing to the absence of a substituent at the N-3 position of the pyrimidine ring, all these compounds are not chiral at C-5, but do possess chiral side-chains 5-R'.

2.2. HPLC chiral columns

Polymeric HPLC supports, EP–β-CD–N^+ and PVI–β-CD, were prepared by absorption on 5-μm silica of the corresponding polymers, as described previously [5], and were packed by a slurry packing technique in 25 cm × 4.6 mm I.D. stainless-steel columns. These supports were characterized from the amount of carbon determined by elemental analysis (Table 2). Compared with the procedures reported previously [5], in order to obtain similar loadings for both supports (in terms of the number of β-CD cavities per gram of silica), a more concentrated aqueous solution (20%, w/w) was used for the adsorption of the EP–β-CD–N^+ polymer.

Three commercially available HPLC columns (25 cm × 4.6 mm I.D.) were used: Cyclobond I and Cyclobond I RSP columns were obtained from Astec (Whippany, NY, USA) and a Chiradex LiChroCART cartridge was obtained from Merck (Darmstadt, Germany).

2.3. Chromatographic experiments

Sodium phosphate solutions (0.1 *M*) adjusted to pH 4 were used as eluents. The organic modifier was methanol. The flow-rate was 1.0 ml/min.

3. Results and discussion

3.1. Influence of the nature of the heteroatom at the C-2 position on the resolution of the racemates

The data in Tables 3 and 4 demonstrate the influence of the presence of a surfur or an oxygen atom at the C-2 position of the ring, comparing the results for TBA compounds (**7** and **8**) with those of BA compounds (**3** and **4**). It can be seen that the pairs **7**–**3** and **8**–**4** have similar 5-R and 5-R' substituents.

Both TBA were resolved, at least partially, on each CDSP, whereas the enantiomers of **3** and **4** were not discriminated on Cyclobond I, Chiradex and PVI–β-CD columns. An enantioselectivity for **3** and **4** racemates was observed on EP–β-CD–N^+ was Cyclobond I RSP columns for **3** and **4** BA racemates but the resolution was lower than for the **7** and **8** TBA enantiomers. Typical chromatograms obtained on these both supports are shown in Fig. 1.

Hence the presence of the sulfur atom at the C-2 position in the guest molecules promotes considerably their chiral recognition by CDSP. Moreover, the nature of the stationary phase modifies the selectivity resulting from the environment of the β-CD residues.

Some comments can also be made based on the examination of the retention data of these compounds. On all supports the order of elution is the same for TBA and BA compounds. Moreover, it is observed that the enantioselectivities increase as the retentions increase. The observed greater TBA affinity for the CDSP can be related to previous studies on β-CD complexes in solution. The stability constant of the

Table 2
β-CD–polymer HPLC supports

Polymer	Support	
	Carbon content (%)	β-CD moieties (μmol/g)
PVI–β-CD	7.5	87
EP–β-CD–N^+	7.4	94

Table 3
Comparison of capacity factors (k'), selectivity factors (α) and resolutions (R_s) of BA and TBA obtained on the CDSP

Compound	Mobile phase[a]	Cyclobond I			Chiradex			PVI–β-CD		
		k'^{b}	α	R_s	k'^{b}	α	R_s	k'^{b}	α	R_s
1	15:85	14.6	1.0	0	12.3	1.0	0	5.2	1.0	0
2	15:85	14.1	1.0	0	16.3	1.0	0	6.8	1.0	0
3	30:70	4.9	1.0	0	5.4	1.0	0	2.0	1.0	0
	15:85	18.3	1.0	0	17.3	1.0	0	7.1	1.0	0
4	30:70	7.2	1.0	0	6.5	1.0	0	2.5	1.0	0
	20:80	13.3	1.0	0	17.1	1.0	0	7.0	1.0	0
5	30:70	7.7	1.07	0.9	6.9	1.03	NC[c]	3.0	1.0	0
	15:85							11.1	1.05	0.5
6	30:70	0.9	1.15	NC	5.1	1.57	2.9	1.0	1.52	1.8
	15:85	3.0	1.15	0.9				10.7	1.39	2.4
7	30:70	10.4	1.05	0.5	6.1	1.05	NC	2.8	1.04	NC
	15:85							10.9	1.05	0.4
8	30:70	14.1	1.07	1.0	9.3	1.08	0.9	3.3	1.07	0.5
	15:85							15.4	1.07	0.8

[a] Methanol–0.1 *M* phosphate buffer (v/v).
[b] Capacity factors for the first-eluted peaks.
[c] NC = the resolution could not be calculated.

thiopental–β-CD complex has been reported to be twice that of the pentobarbital–β-CD complex, as measured by UV spectrophotometry [11]. Similar results were obtained by ion-exchange HPLC with addition of β-CD to the mobile phase [12].

Moreover, from the different patterns of the induced Cotton effect previously observed for TBA– and BA–β-CD complexes, circular dichroism experiments suggested that the configuration of TBA within the cavity of β-CD is different from that of BA [11]. The difference

Table 4
Comparison of capacity factors (k'), selectivity factors (α) and resolution (R_s) of BA and TBA obtained on the CDSP

Compound	Mobile phase[a]	EP–β-CD–N^+			Cyclobond I RSP		
		k'^{b}	α	R_s	k'^{b}	α	R_s
1	30:70	4.0	1.0	0	3.0	1.0	0
2	30:70	7.0	1.0	0	4.0	1.0	0
3	30:70	6.2	1.07	0.7	3.5	1.08	0.94
4	30:70	7.8	1.08	0.70	5.13	1.09	1.02
5	30:70	11.2	1.08	0.70	8.8	1.08	0.74
6	30:70	0.6	1.22	0.54	1.47	1.43	3.6
	15:85	1.2	1.28	1.22			
7	30:70	11.7	1.14	1.25	9.17	1.17	2.04
8	30:70	18.0	1.15	1.20	11.37	1.19	1.8

[a,b] See Table 3.

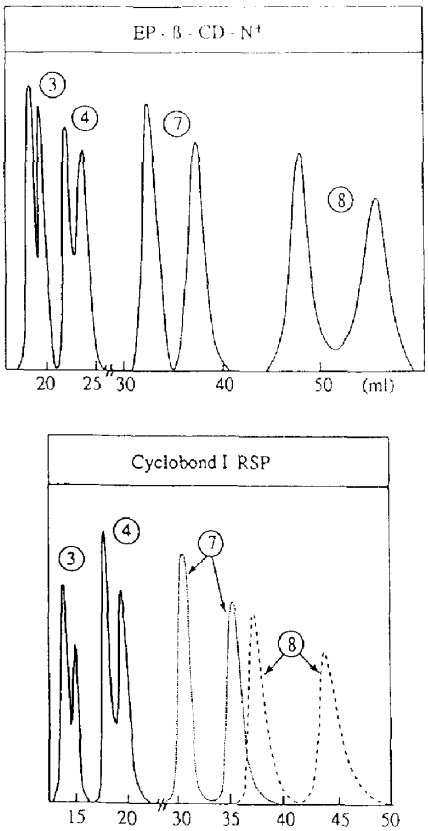


Fig. 1. Elution profiles of compounds **3**, **4**, **7** and **8** on EP–β-CD–N^+ and Cyclobond I RSP columns. Eluent: methanol–0.1 *M* phosphate solution (pH 4) (30:70).

resulting from fitting these two types of compounds into the β-CD cavity could explain the different chiral discrimination observed by the CDSP, with a TBA arrangement more favourable to chiral recognition.

On the other hand, it has been reported from thermodynamic studies that the inclusion of BA or TBA in β-CD involves forces other than hydrophobic forces such as hydrogen bonding and dipole–dipole interactions [11].

Similar observations have recently been made with an enhanced interaction with CDs in the presence of a thiocarbonyl function compared with that observed in presence of a carbonyl group. By HPLC, using a CD as a mobile phase additive, it was found that N-arylthiazoline-2-thiones have higher association constants than N-arylthiazoline-2-ones with γ-CD, leading to better chiral recognition of their atropisomers [13]. Dipole–dipole interactions rather than hydrogen bonding has been suggested to be at the origin of the large association constants of thiocarbonyl derivatives with CDs as these compounds have a smaller proton-accepting ability than their oxygen analogues [13].

In summary, the sulfur atom at the C-2 position of the pyrimidine ring enhances the affinity of the solutes with the CDSP, and the occurrence of a polar interaction involving this atom leads to a better fitting of TBA with the β-CD cavities, favouring their chiral recognition.

3.2. Influence of the nature of the C-5 TBA substituents

Changing the substituent at the C-5 position from ethyl to allyl (comparing compounds **3** with **4** and **7** with **8**) leads to only a slight change in the chiral recognition by β-CD (α-values from Tables 3 and Table 4). Hence there is a small increase in enantioselectivity in the presence of the longer allyl substituent, when nevertheless a chiral discrimination is observed, i.e., for BA compounds (**3** and **4**) on EP–β-CD–N^+ and Cyclobond I RSP supports only, and for TBA compounds (**7** and **8**) on each column.

However, changing the other C-5 substituent from *sec.*-butyl to 1-methylbutyl, 2-cyclopentenyl or 1-methyl-2-pentenyl greatly influences the enantioselectivity observed. Thus, compounds **1** and **2**, with the shortest 5-R′ substituent, are not resolved by the CDSP, the presence of a *sec.*-butyl group together with an allyl or an ethyl group precluding the resolution of the barbiturates on all the columns. However, the presence of a 1-methylbutyl group allows the recognition of **3** and **4** by EP–β-CD–N^+ and Cyclobond I RSP stationary phases only. This substituent is not effective for chiral recognition on the other columns. The presence of more bulky groups such as 1-methyl-2-pentenyl and 2-cyclopentenyl (**5** and **6**) is more favourable, with preference for 1-methyl-2-pentenyl, which provides the greatest enantioselectivity on all the columns.

The capacity factors in Tables 3 and 4 (com-

paring compounds **1** with **2**, **3** with **4** and **7** with **8**) show that higher retention times are obtained in the presence of an allyl than with an ethyl substituent. Increased retentions are observed also on passing from a *sec.*-butyl (BA **1** or **2**) to a 1-methylbutyl group (BA **3** and **4**), and then to a 2-cyclopentenyl group (BA **5**). A 1-methyl-2-pentenyl substituent reduces considerably the retention of the guest (**6**), except on Chiradex. The decrease in affinity for the other four stationary phases does not affect their chiral recognition as this BA has the best enantioselectivity values on all the columns.

Previous ^{13}C NMR experiments indicated that the cyclic moiety of BA is at least partly accommodated in the hydrophobic cavity of β-CD with the 5-substituents participating in inclusion formation and that their size affects the equilibrium constants of the β-CD complexes, with lower constants for smaller or too bulky substituents [11,14]. These results are in good agreement with our capacity factor results.

3.3. Influence of the β-CD stationary phase structure

Among the stationary phases used, only EP-β-CD–N^+ and Cyclobond I RSP can separate the enantiomers of pentobarbital and secobarbital. We conclude that the overcrowding due to the presence of substituents on the β-CD cavity edges of these supports allows a better adjustment of these guest molecules, resulting in a more favourable position of their chiral centre relative to the host for enantiomeric discrimination.

The presence of more bulky substituents than the 1-methylbutyl group on BA at the C-5 position of the pyrimidine ring is needed for chiral recognition when using the native β-CD supports PVI–β-CD, Cyclobond I and Chiradex.

Better enantioselectivities are also observed for TBA on columns with β-CD hydroxypropyl substituents. Nevertheless, the supports bearing native β-CD residues can discriminate both TBA enantiomers (with a 1-methylbutyl group at the C-5 position).

It has been reported that the presence of hydroxypropyl substituents on the Cyclobond I RSP support allows the resolution of several enantiomers that could not be separated on native β-CD phases [3,15], but to our knowledge, experiments dealing with a chiral centre located on the side-chain of BA have not been reported for this type of support.

In conclusion, the chiral recognition of the studied compounds is highest when they have a thiocarbonyl instead of a carbonyl group and when their substituents at the C-5 position have an adequate size.

The presence of β-CD hydroxypropyl substituents on CDSP favours the enantiomeric discrimination of the BA and TBA. It is emphasized that these supports resulting from different synthetic routes lead to very similar results.

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