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Note

Structural factors affecting chiral recognition and separation on β-cyclodextrin bonded phases

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Cyclodextrins (CDs) have been used as a bonded phase and as mobile phase additives for enantiomeric separations. In both cases the separation of enantiomers using CD depends on the formation of an inclusion complex which is due to the hydrophobic effect, hydrogen bonding interactions between the guest molecule and the secondary hydroxyl groups of the CD, decrease of the strain energy in the ring frame system and release of high-energy CD cavity water molecules[1,2].

Previously, we developed a stable, high-efficiency CD bonded phase and separated many compounds which had two to four aromatic rings[3–10]. We also studied the mechanism of chiral recognition in chromatographic processes that utilize CD. Generally, there must be a relatively tight fit between the complexed guest molecule and the CD host. Also the guest should interact with the 2- or 3-hydroxyl groups at the mouth of the CD cavity in order to maximize the chiral recognition.

Most of the separations on β-CD bonded phase columns have been done with compounds containing two to four ring moieties of which at least one was aromatic. Since the diameter of β-CD cavity is similar to that of naphthalene, compounds containing two ring moieties (*e.g.*, naphthalene or biphenyl-like) usually show better enantiomeric separation than aromatic single-ring systems. Recently, however, it was demonstrated that a few single-ring containing enantiomers of specific structural types are readily resolved as well[11].

Sybilska and co-workers[12–14] separated some enantiomers by using CDs as mobile phase additives. Relatively fewer compounds have been separated by this approach possibly due to the limited solubility of CDs, particularly in the case of β-CD. However, upon addition of urea, the solubility of β-CD increases dramatically. It has been demonstrated that these solutions have significantly greater enantiomeric resolving power in some cases[15].

In this study 43 multi-ring compounds were examined. Sixteen of these compounds, possessing two- to three-rings, were separated on β-CD bonded phase columns. Good resolution ($R_s > 1.0$) was obtained for eleven of the sixteen compounds. All racemates were examined for common structural features that might account for the observed enantioselectivity.

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EXPERIMENTAL

β-CD bonded phase columns (250 × 4.6 mm I.D.) were obtained from Advanced Separation Technologies (Whippany, NJ, U.S.A.). A Shimadzu Model LC-6A liquid chromatograph (Columbia, MD, U.S.A.) with a variable-wavelength detector was used in the constant flow mode (1.0 ml/min). A Rheodyne model 7125 sample injection valve with a 20-μl loop was used.

5-(4-Methylphenyl)-5-phenylhydantoin; 5-(4-hydroxyphenyl)-5-phenylhydantoin; 5-methyl-5-phenylhydantoin; α-methyl-α-phenylsuccinimide; α-methyltryptamine; 9-methyl-$\Delta^{5(10)}$-octaline-1,6-dione; (±)-1-[5-chloro-2-(methylamino)phenyl]-1,2,3,4-tetrahydroisoquinoline; N-ethyl-3-phenyl-2-norbornanamine hydrochloride; 5-ethyl-5-(*p*-tolyl)-2-thiobarbituric acid; (±)-4-benzyl-2-oxazolidinone; (±)-2-phenylbutyrophenone; D,L-chloro-2-(α-methylbenzyl)phenol; indole-3-acetaldehyde; D,L-laudanosine; D,L-laudanosoline hydrobromide trihydrate; (1*S*,2*R*,5*S*)-(+)-menthyl-(*R*)-*p*-toluenesulfinate; (1*R*,2*S*,5*R*)-(−)-menthyl-(*S*)-*p*-toluenesulfinate; 4-maleimido-2,2,6,6-tetramethyl-1-piperidinyloxy; D,L-α-methyltryptamine; D,L-aminoglutethimide; 1-adamantanamine; tioxolone; 1-methyl-4-piperidinyl-3-methyl-2-phenylvalerate; α-ethyltryptamine; 2-amino-2-norbornane-carboxylic acid; catechin hydrate; 4-hydroxy-4-phenylpiperidine; (2*S*,3*S*)-(−)-2-methyl-3-phenylglycidol; (2*R*,3*R*)-(+)-2-methyl-3-phenylglycidol; N,N′-dibenzyl-D-tartramide and N,N′-dibenzyl-L-tartramide were obtained from Aldrich (Milwaukee, WI, U.S.A.). Brompheniramine, disopyramide, promethazine, carbinoxamine, doxylamine, pentazocine, chlorotrianisene; propiomazine, pindolol, gossypol, warfarin, labetalol, primaquine, ketamine, benzoin and mandalic acid benzyl ester were obtained from Sigma (St. Louis, MO, U.S.A.).

HPLC-grade water, acetonitrile and methanol were from Fisher Scientific (St. Louis, MO, U.S.A.). The mobile phase was filtered through a 0.45-μm frit before use. The mobile phases used were buffer, mixtures of methanol and buffer, and acetonitrile–buffer. The aqueous portion of the mobile phases consisted of 1% triethyl ammonium acetate (pH 4.1 and 7.1) and all samples were dissolved in methanol or water before injection and all separations of the racemic compounds were done at room temperature.

RESULTS AND DISCUSSION

In this study on the enantiomeric resolution of racemates containing two- to three-rings moieties, all samples could be catagorized into two main groups: those with the chiral center in an acyclic portion of the molecule and those with the chiral center as a part of a ring. The latter group could be catagorized into two subgroups: those where there are two ring-substitutents directly attached at the chiral center and those with only one ring-substituent. Enantiomeric separation data for those compounds with the chiral center as a part of a ring are given in Table I. As one can see, compounds with two ring-substituents attached to the chiral carbon [*i.e.*, 5-(4-methylphenyl)-5-phenylhydantoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin] had larger separation factors (α) and better R_s values compared to compounds with a single ring substituent (for example, 5-methyl-5-phenylhydantoin). When a phenyl group was replaced with a phenol group or a methylphenyl group, the capacity fac-

TABLE I

ENANTIOMERIC SEPARATION OF RACEMATES IN WHICH THE CHIRAL CENTER IS A PART OF A RING

This data was generated using two 25-cm β-cyclodextrin columns in series.

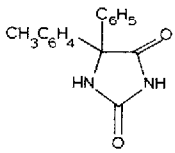
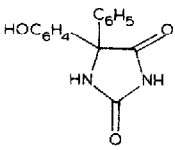
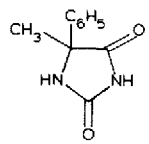
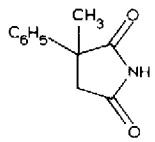
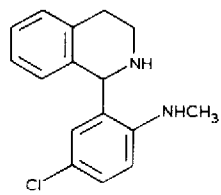
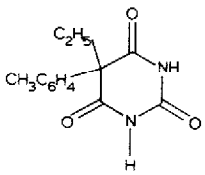
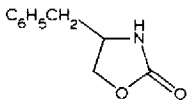
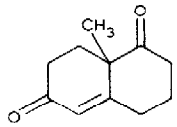
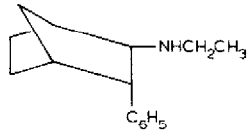
Compound	*Structure*	k'*	α	R_s	*Mobile phase*
5-(4-Methylphenyl)-5-phenylhydantoin		10.17	1.12	2.0	Methanol–buffer** (30:70)
5-(4-Hydroxyphenyl)-5-phenylhydantoin		2.96	1.35	2.0	Methanol–buffer*** (20:80)
5-Methyl-5-phenyl-hydantoin		1.20	1.07	1.1	Methanol–buffer** (15:85)
α-Methyl-α-phenyl-succinimide		6.0	1.07	2.0	Methanol–buffer** (30:70)
(±)-1-[5-Chloro-2-(methylamino)phenyl-1,2,3,4-tetrahydro-isoquinoline		0.32	2.34	3.0	Methanol–buffer** (40:60)
5-Ethyl-5-(*p*-tolyl)-2-thio-barbituric acid		4.66	1.03	0.5	Methanol–buffer*** (50.50)
(±)-4-Benzyl-2-oxazolidinone		2.08	1.07	2.0	Methanol–buffer** (4:96)

TABLE I (*continued*)

Compound	*Structure*	*k'**	*α*	*R_s*	*Mobile phase*
9-Methyl-$\Delta^{5(10)}$-octaline-1,6-dione		4.51	1.04	1.0	Methanol–buffer*** (20:80)
N-Ethyl 3-phenyl-2-norbornanamine		0.91	1.06	0.7	Methanol–buffer** (30:70)

* Capacity factor of the first eluted enantiomer.
** 1% Triethylamine acetate, pH 4.1.
*** 1% Triethylamine acetate, pH 7.1.

tors (k') increased, with the methylphenyl derivative having the greatest retention time in this series. Enantiomers of 5-methyl-5-phenylhydantoin; 5-(4-hydroxyphenyl)-5-phenylhydantoin and urinary phenolic metabolites of phenytoin also were separated using a β-CD bonded phase column developed by Maguire and co-workers[16,17]. The added buffer in the present system did not seem to change the enantioselectivity significantly; however, the capacity factors of (±)-5-methyl-5-phenylhydantoin were greater when neat water was used in the mobile phase mixtures.

It is apparent that (±)-1-[5-chloro-2-(methylamino)phenyl]-1,2,3,4-tetrahydroisoquinoline shows greater enantioselectivity than any of the other racemates studied. This is probably because the chiral center is part of a fused two ring system (*i.e.*, the tetrahydroisoquinoline). This part of the molecule is most likely included in the hydrophobic cavity of β-CD with the 5-chloro-2-(methylamine)phenyl ring projecting from the mouth. The orientation of this ring would be very different for each enantiomer. As the projecting ring also has a hydrogen bonding group, the opportunity exists for an appreciable free energy difference in the two complexes.

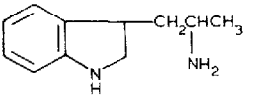
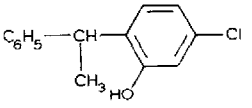
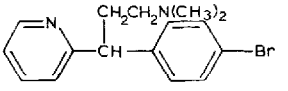
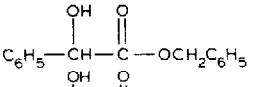
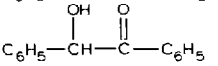
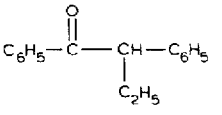
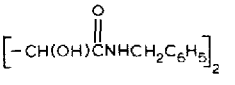
Table II shows two- and three-ring compounds in which the chiral center is part of the acyclic portion of the molecule. Included in this list is mandelic acid benzyl ester. The first reported LC separation of mandelic acid and derivatives on a β-CD column was by Feitsma *et al.*[18,19] and Fujimura *et al.*[20]. In their studies, the CD bonded stationary phase contained a nitrogen linkage. Subsequently we obtained partial separation of these compounds on a non-nitrogen containing β-CD bonded phase[11]. Fujimura *et al.*[20] reported that mandelic acid benzyl ester did not elute with a CD-carbamate bonded phase. However, on our epoxy-linked β-CD bonded phases, enantiomers of mandelic acid benzyl ester could be eluted. Although the resolution was not baseline ($R_s \approx 0.6$), the elution time was reasonable ($k' \approx 3.05$). Fig. 1 shows the best and worst enantiomeric separation obtained in this study.

Interestingly, for the racemates in Table II, the chiral center was always between two π systems with the exception of one compound (α-methyl-α-tryptamine). In most cases, one of the π systems was an aromatic ring while the second π-system

TABLE II

ENANTIOMERIC SEPARATION OF RACEMATES IN WHICH THE CHIRAL CENTER IS IN AN ACYCLIC PORTION OF THE MOLECULE

This data was generated using two 25-cm β-cyclodextrin columns in series.

Compound	*Structure*	*k'**	α	R_s	*Mobile phase*
α-Methyl-tryptamine		1.60	1.06	1.0	Methanol–buffer** (5:95)
4-Chloro-2-(α-methylbenzyl)-phenol		9.44	1.06	1.3	Methanol–buffer** (40:60)
Brompheniramine		2.70	1.10	0.8	Methanol–buffer** (30:70)
Mandelic acid-benzyl ester		3.05	1.02	0.6	Methanol–buffer** (30:70)
Benzoin		3.17	1.08	1.0	Methanol–buffer** (30:70)
(±)-2-Phenyl-butyrophenone		2.38	1.16	1.2	Methanol–buffer** (50:50)
N,N'-Dibenzyl-D,L-tartramide		1.95	1.04	0.6	Methanol–buffer** (30:70)

* Capacity factor of the first eluted enantiomer.
** 1% Triethylamine acetate pH 4.1.

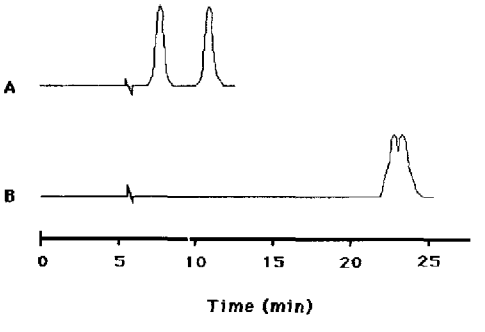


Fig. 1. Chromatogram showing the liquid chromatographic (LC) separation of (±)-1-[5-chloro-2-(methylamino)phenyl]-1,2,3,4-tetrahydroisoquinoline (A) and mandelic acid-benzyl ester (B) on two β-cyclodextrin bonded phase LC columns. Chromatographic conditions are given in Tables I and II.

was either a carbonyl group or a second aromatic ring. It is apparent that enantioselectivity is enhanced when the chiral center is sandwiched between two π systems, thereby resulting in a chiral molecule of some rigidity. Furthermore, the chiral center often seems to be the main point at which rotation can occur between the two π systems.

In summary, chiral recognition and enantiomeric separation for two- to three-ring compounds seem to be enhanced if: (1) the compounds have at least two ring moieties of which at least one is aromatic, (2) the chiral center is between two aromatic rings or a single aromatic ring and a carbonyl group. This experimental evidence as to the type of structural features required for chiral recognition using β-CD should be of interest to other researchers in this field.

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