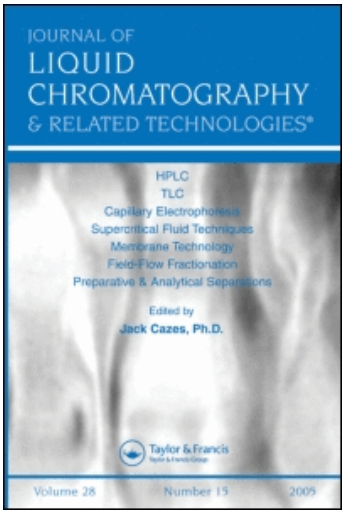

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SEPARATION OF ENANTIOMERS USING A γ-CYCLODEXTRIN LIQUID CHROMATOGRAPHIC BONDED PHASE

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ABSTRACT

A γ-cyclodextrin bonded phase was used for the enantiomeric separation of various compounds including several chiral crown ethers and dansylated amino acids. Methanol/buffer solutions were used as the mobile phase. Earlier studies of enantiomeric separations on β-cyclodextrin bonded phases have shown that enantiomers in which the chiral center was the α substituent on a naphthyl ring were unresolved on the β-cyclodextrin bonded phase. In contrast, several enantiomeric pairs of this type were readily resolved on the γ-cyclodextrin bonded phase. In addition, the γ-cyclodextrin bonded phase was able to resolve several enantiomeric pairs in which the optical activity was the result of an axis of dysymmetry rather than a stereogenic atom. Differences in the previously reported selectivity on β-cyclodextrin and the results reported here for the γ-cyclodextrin bonded phase are examined and discussed in terms of the overall retention mechanism.

INTRODUCTION

Cyclodextrins (CD) are cyclic oligomers of D-glucose bonded through 1,4-α linkages. The three most widely used CD are α-, β- and γ-CD which differ by the number of glucose units incorporated in the ring (α=6, β=7 and γ=7). Of these three, β-CD and its derivatives have been the most widely used as chiral bonded stationary phases (CBP) for high performance liquid chromatography (HPLC) [1,2], as chiral mobile phase additives (CMA) for thin layer chromatography (TLC) [3,4] and most recently, as stationary phases in capillary gas chromatography (GC) [5,6].

In reversed-phase LC applications, the operative mechanism for chiral recognition by CD seems to be the formation of inclusion complexes. Enantiomeric separation or enrichment most often occurs when there is a good match between the size of the CD cavity and the hydrophobic portion of the solute. For instance, α-CD was found to be useful as a CBP in LC for the separation of underivatized aromatic amino acids [7]. β-CD CBP have been more successful at resolving larger optical isomers such as derivatized amino acids or enantiomers which have substituted benzene, naphthyl or biphenyl moieties or have more than one aromatic or cyclic substituent from the stereogenic center [8,9]. Previous experience with α- and β-CD CBP leads us to expect that the optimal candidates for chiral recognition on γ-CD are solutes containing fused ring substituents off the chiral center which can form fairly tight inclusion complexes with γ-CD. Although γ-CD CBP has been

proposed as an alternative to conventional reversed-phase stationary phases for the separation of polycyclic aromatic hydrocarbons (PAH) [10,11] and also has been used for the normal phase and reversed phase separation of structural isomers of substituted anilines [12] and phenols [13], relatively little work has been done to investigate the ability of γ-CD bonded phases to distinguish between enantiomers or other types of isomers. To our knowledge, the only reported previous application to the separation of isomers other than structural or geometrical on a γ-CD CBP was the separation of (±)-norgestrel [14]. This neglect may, in part, be a consequence of the fact that the CD coverage for the γ-CD phases is generally less than that obtained on the β-CD phases due the greater bulkiness of γ-CD relative to β-CD. The purpose of this study was to explore the potential of γ-CD CBP for enantiomeric separations, to determine which types of compounds might make likely candidates for chiral separations and to compare the results, when possible, with previously published results obtained on β-CD CBP.

EXPERIMENTAL

The chromatographic experiments were performed on a Shimadzu LC-6A Liquid Chromatograph interfaced with a C-R3A Chromatopac Data System. Detection was accomplished using a variable wavelength detector. The chromatographic column (received from Advanced Separations Technology, Inc., Whippany, NJ) was 250 x 4.6 mm i.d. stainless steel packed

with 5 μm Cyclobond II (γ-CD). The 2,2′-binaphthyldiyl crown ethers were synthesized as reported previously [15]. The other solutes were obtained from various sources and used as received. HPLC grade methanol (MeOH) was obtained from Fisher Scientific (St. Louis, MO). Water was distilled, subsequently deionized using a Barnstead Cartridge, filtered and used without further purification.

RESULTS AND DISCUSSION

The chromatographic data is summarized in Table I. A typical chromatogram demonstrating the enantiomeric separation for several of the solutes studied is shown in Figure 1. All of the solutes used in this study have two fused rings.

The use of β-CD CBP to resolve chiral 2,2′-naphthyldiyl crown ethers has been previously reported [15]. The concentration and type (MeOH) of organic modifier in the optimized mobile phases used in this study and in the previous work [15] was roughly the same. In general, it appears that retention of the chiral crown ethers used in this study was greater on the γ-CD column than on the β-CD column although buffers were not used in the previous work. It should also be noted that many of the separations in the previous work used a 25 cm column and a 10 cm β-CD column in series. This may account for some of the greater resolution obtained for compounds **4-6** and **8** on the β-CD CBP relative to that reported here on the γ-CD CBP.

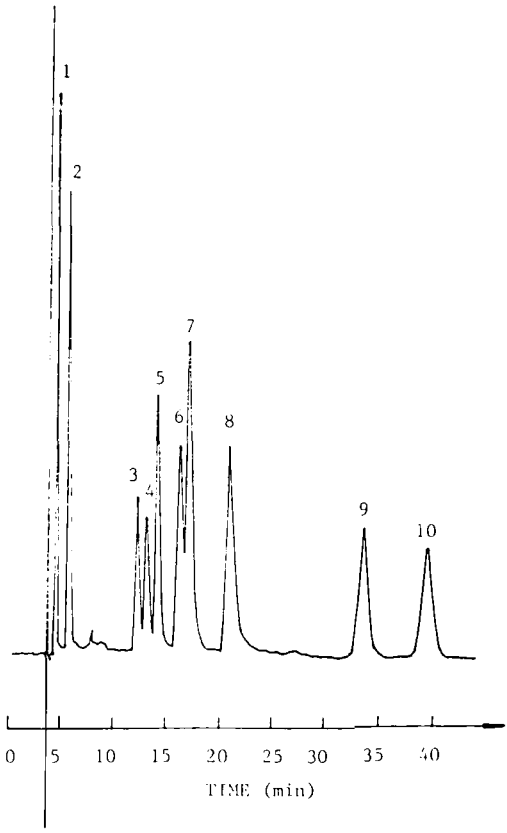
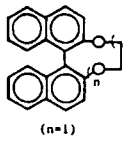
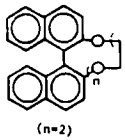
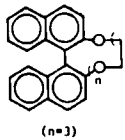
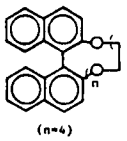
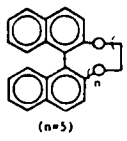
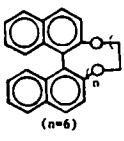
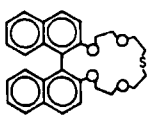


Figure 1. Chromatograph of the separation of enantiomers on a 25 x .46 cm γ-CD column. Mobile phase: 38% MeOH/1% TEAA pH 4.2; flowrate: 1 mL/min. Detector: UV @ 254 nm.
peaks: 1,2: **11**; 3,4: **3**; 5,6: **2**; 7,8: **1**; 9,10: **14**. (See Table I for further information.)

Earlier workers found that enantioselectivity (α) on a β-CD column for chiral 2,2′-naphthyldiyl crown ethers increased as the size of the crown increased up to crown-4 and then decreased [15]. In contrast, enantioselectivity was found to decrease with increasing crown size on the γ-CD column.

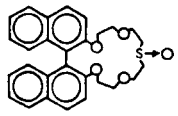
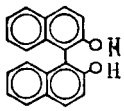
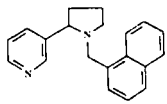
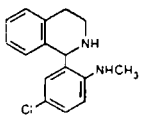
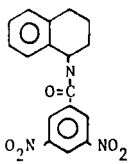
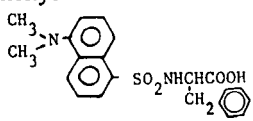
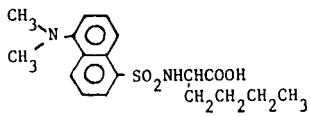
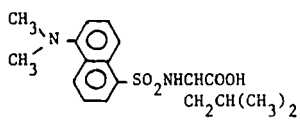
TABLE I.

SEPARATION DATA FOR RACEMATES ON γ-CYCLODEXTRIN BONDED PHASE

Compound	Structure	k'^{1}	α	R_s	Mobile phase
1.	 (n=1)	6.88	1.33	3.3	40% MeOH/Buffer[2]
2.	 (n=2)	4.84	1.23	2.21	40% MeOH/Buffer[2]
3.	 (n=3)	3.78	1.12	1.20	40% MeOH/Buffer[2]
4.	 (n=4)	8.83	1.05	0.7	35% MeOH/Buffer[2]
5.	 (n=5)	9.21	1.04	0.7	35% MeOH/Buffer[2]
6.	 (n=6)	8.98	1.04	0.6	35% MeOH/Buffer[2]
7.		12.42	1.05	0.7	25% MeOH/Buffer[2]

[1] Capacity factor of the first eluted enantiomer.

[2] 1% triethylamine acetate, pH 4.2.

8.		7.67	1.05	0.7	35% MeOH/Buffer[3]
9.	(±)-1,1′-bi-2-naphthol 	7.92	1.09	1.15	30% MeOH/Buffer[3]
10.	N-(α-naphthyl-methyl)nornicotine 	8.13	1.05	0.8	40% MeOH/Buffer[3]
11.	DL-1-(5-chloro-2-(methylamino)-phenyl)-1,2,3,4-tetrahydroisoquinoline 	1.71	1.36	1.25	40% MeOH/Buffer[3]
12.	acenaphthenol 	4.41	1.04	0.9	25% MeOH/Buffer[3]
13.	N-(3,5-dinitro-benzoyl)-1,2,3,4-tetrahydronaphthyl-1-amine 	5.48	1.05	0.8	20% MeOH/Buffer[3]
14.	dansyl-DL-phenylalanine 	8.93	1.21	4.36	40% MeOH/Buffer[3]
15.	dansyl-DL-leucine 	6.42	1.09	1.26	35% MeOH/Buffer[3]
16.	dansyl-DL-norleucine 	10.95	1.07	1.03	30% MeOH/Buffer[3]

[3] 1% triethylamine acetate, pH 6.9.

The elution order obtained on the γ-CD roughly follows that obtained on the β-CD (e.g., decreased retention with increasing crown size). Also, successful separation of the enantiomers on the β-CD column in the previous work required cyclization of the substituents on the naphthyls, thereby resulting in a more rigid molecule. In comparing the stereoselectivity of compound **1** and compound **9**, it is evident that although cyclization does confer enhanced enantioselectivity on the γ-CD phase, it may not be as rigid a requirement for enantioselectivity as on the β-CD column. Compound **9** was reported as unresolved in the earlier work. Earlier workers also found that substituting one of the crown oxygens with sulfur (**7**) or sulfoxide (**8**) affected the retention to a much greater extent than the separation (α) or resolution (R_s) [15]. This is consistent with the results reported here.

Previously, it has been found that α substituted naphthalenes elute before β substituted naphthalenes from β-CD bonded phases [16]. This can be explained if one assumes that, in the case of inclusion complex formation with β-CD, the most favorable orientation of solutes containing two fused rings is for longest axis of the fused rings of the molecule to be oriented perpendicular to the mouth of the β-CD cavity. The α substituent sterically restricts full penetration of the molecule into the β-CD cavity and is therefore less retained than the β substituted isomer. (Figure 2) Earlier workers were unable to resolve N-(α-naphthylmethyl)nornicotine but were able to resolve N-(β-naphthylmethyl)nornicotine on a β-CD bonded phase [17]. The larger diameter of the γ-CD cavity, however, permits solute orientations within the

Figure 2. Schematic diagram of the inclusion complexes formed between β-cyclodextrin and a) α- and b) β-substituted naphthalenes.

cavity in which the longest axis of the fused rings is more parallel to the mouth of the γ-CD cavity. This model not only accounts for the fact that stereoselectivity was observed on the γ-CD bonded phase for N-(α-naphthylmethyl)nornicotine but also for acenaphthenol and the 3,5-dinitrobenzoyl derivative of 1,2,3,4-tetrahydronaphthylamine as well. Although DL-1-(5-chloro-2-(methylamino)-phenyl)-1,2,3,4-tetrahydroisoquinoline was separated on the γ-CD column, the stereoselectivity obtained was less than that reported earlier [11]. In this case, orientation of the fused rings of the solute parallel to the mouth of the CD cavity could reduce chiral selectivity by positioning the amines too far from the hydroxyls that line the mouth of the CD cavity for effective hydrogen bonding. In addition, the presence of the heterocyclic amine may diminish the affinity of the solute for the hydrophobic interior of the CD cavity. It also should be noted that the earlier chromatographic data for DL-1-(5-chloro-2-(methylamino)-phenyl)-1,2,3,4-tetrahydroisoquinoline was generated using two 25 cm β-CD columns in series and a slightly different buffer although the amount and type of organic modifier used was the same.

The separation of dansyl amino acids have also been studied on β-CD bonded phases [18]. In general, better separations are obtained on the β-CD

phase for the solutes reported here despite the fact that the k´s are larger on the γ-CD phase. It has been found for β-CD phases that enantioselectivity requires that there be a relatively tight fit between the included solute and the CD cavity and that this selectivity is enhanced if one of the substituents on the chiral center is aromatic. The enhancement due to the presence of the aromatic substituent diminishes, however, with greater distance from the chiral center. If the most favorable orientation positions the molecule so that the dimethylamine portion of the molecule can interact with the primary hydroxyls, the chiral portion of the molecule may be too distant from the secondary hydroxyls which line the mouth of the γ-CD cavity for enantioselectively-productive hydrogen bonding.

The large cavity of the γ-CD can also accomodate larger solutes such as 2,2,2-trifluoro-1-(9-anthryl)ethanol. In this case, the longest axis of the fused rings is probably oriented diagonally with respect to the mouth of the CD cavity, thereby allowing the hydroxyl on the chiral center of the solute to interact with the CD hydroxyls thereby effecting chiral separation.

In conclusion, it can be seen from the results presented here that γ-CD bonded phases are an additional CBP available for the separation of optical isomers. γ-CD CBP offers unique selectivity and seems to be suited for the resolution of enantiomers containing fused rings such as naphthalene or multiple fused-ring moieties. For these types of compounds, retention seems to be greater on the γ-CD CBP relative to retention on the β-CD CBP and is indicative of the sterically favored formation of an inclusion complex between

these solutes and the larger γ-CD. The γ-CD phase seems to be particularly adept at distinguishing between enantiomers in which the chiral center is the α substituent on a naphthalene or two fused-ring moiety, compounds which are generally unresolvable on the β-CD phase.

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