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USE OF HYDROXYPROPYL- AND HYDROXYETHYL-DERIVATIZED β-CYCLODEXTRINS FOR THE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS AND DIASTEREOMERS

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SUMMARY

Partially substituted hydroxypropyl-β-cyclodextrin and hydroxyethyl-β-cyclodextrin proved to be effective chiral mobile phase additives (CMAs) for the thin-layer chromatographic (TLC) resolution of racemic benzyl-2-oxazolidinone, 5-(4-methylphenyl)-5-phenylhydantoin, mephenytoin and several dansyl and β-naphthylamide amino acids. Several diastereomeric compounds including steroid epimers and alkaloids were separated as well. The derivatized β-cyclodextrins tended to be much more soluble in water and hydro–organic solvents than native β-cyclodextrin. Their chromatographic selectivity also was somewhat different. The use of CMAs in TLC is a potentially useful and powerful method that has not been considered adequately. The relative lack of chiral stationary phases available in planar format makes the use of CMAs particularly attractive.

INTRODUCTION

Cyclodextrins (CDs) have been used effectively in chromatography for the separation of enantiomers, diastereomers, structural isomers and routine compounds. Most of these separations have been done via high-performance liquid chromatography (HPLC) with the CD bonded phase[1–18]. However, there has been some success with CD mobile phase additives[19–25]. Two things that limit the use of CDs as mobile phase additives are solubility and cost. β-CD is the least expensive and most widely used homologue. However, its solubility in water is only about 0.017 *M* at room temperature. This value can decrease significantly when organic modifiers are added. The solubility of β-CD in water can be increased by going to very high pH values or by adding large amounts of urea[26]. Another alternative is to synthetically modify the CD so as to increase its water solubility. In fact, hydroxypropyl- and hydroxyethyl-substituted β-CDs can be made relatively inexpensively and are much more soluble in water than native β-CD (see Experimental). In fact, their solubility increases as the degree of substitution increases. In chromatography, the concentration of the chiral mobile phase additive (CMA) frequently is the critical parameter for obtaining enantiomeric separations. In the specific case of CD additives, enantioselectivity generally increases with increasing CD concentrations in the mobile phase[24,26].

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While a large number of chiral stationary phases (CSPs) have been introduced for HPLC[27], relatively few CSPs are available, commercially, in TLC (thin-layer chromatography) format. Consequently, the use of CMAs will continue to play an important role in the TLC separation of optical isomers. In fact, there are a number of advantages to using CMAs that complement the TLC approach. For example, there is a wider variety of CMAs available than there are CSPs. The use of CMAs permits enantiomeric separations to be done on less expensive and often more durable achiral stationary phases. The selectivities of CMA methods are often different from those of analogous CSP techniques[19–26]. Also, much less of a CMA is needed in TLC than in the equivalent HPLC method. This can be important if the CMA is costly.

EXPERIMENTAL

Chemically bonded octadecylsilane reversed-phase TLC plates, KC 18F (200 μm layer thickness, 5 × 20 cm and 20 × 20 cm) were obtained from Whatman Chemical Separation Division (Clifton, NJ, U.S.A.). All dansyl amino acids, cinchonine, cinchonidine, quinine, quinidine, 17α,20α-dihydroxy-4-pregnen-3-one, 17α,20β-dihydroxy-4-pregnen-3-one, 20α-hydroxy-4-pregnen-3-one, 20β-hydroxy-4-pregnen-3-one, 17α,20α,21-trihydroxy-4-pregnene-3,11-dione, 17α,20β,21-trihydroxy-4-pregnene-3,11-dione, 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one, 11β,17α,20β,21-tetrahydroxy-4-pregnen-3-one and sodium chloride were obtained from Sigma (St. Louis, MO, U.S.A.). DL-Alanine-β-naphthylamide, DL-methionine-β-naphthylamide, 5-(4-methylphenyl)-5-phenylhydantoin, *R*-(+)benzyl-2-oxazolidinone and *S*-(−)-benzyl-2-oxazolidinone were obtained from Aldrich (Milwaukee, WI, U.S.A.). Mephenytoin was obtained from Dr. R. D. Armstrong of the La Jolla Cancer Research Foundation. Hydroxypropyl- and hydroxyethyl-β-CDs were obtained from Dr. Otto Huber of the Consortium für Elektrochemische Industrie (Munich, F.R.G.). Relevant information on these compounds is given in Table I. The basic structure of these compounds is shown in Fig. 1. N′-(Menthoxycarbonyl)anabasine and N′-

TABLE I

PROPERTIES OF SUBSTITUTED β-CYCLODEXTRINS

Data obtained from the Consortium für Elektrochemische Industrie GMBH technical literature. The following solvents also will dissolve the substituted CD to some extent: methanol, ethanol, dimethylformamide, dimethyl sulfoxide and pyridine.

Compound	*Average molar substitution**	*Average molecular mass***	*Solubility in water at 25°C (g/100 ml)*
Hydroxypropyl-β-CD	0.6 ± 0.1	1379 ± 40	96
Hydroxypropyl-β-CD	0.9 ± 0.1	1501 ± 40	>100
Hydroxyethyl-β-CD	1.0 ± 0.2	1443 ± 62	>200
Hydroxyethyl-β-CD	1.6 ± 0.2	1628 ± 62	>200

* Average molar substitution is defined as the average number of hydroxypropyl or hydroxethyl groups per anhydroglucose unit.

** Each sample contained a narrow distribution of homologues. For example, the molar substitution of the first compound in this table varied from 0.5 to 0.7 for an average of 0.6. Consequently, the molecular mass varied from 1338 to 1419 for an average of 1379.

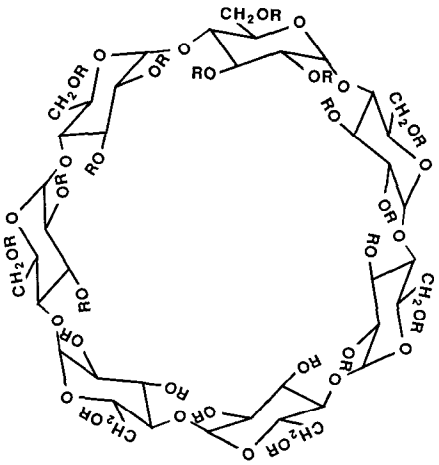

Fig. 1. Basic structure of derivatized CD. For hydroxyethyl-β-CD, R = $(CH_2–CH_2–O)_nH$. For hydroxypropyl-β-CD, R = $(CH_2–C(CH_3)H–O)_nH$. The cyclodextrin is randomly substituted and side-chains with $n > 1$ are possible. The AMS of each derivative (*i.e.*, 0.6, 0.9, 1.0 and 1.6 in Table I) refers to the number of hydroxyalkyl groups per anhydroglucose unit.

(menthoxycarbonyl)3-pyridyl-1-aminoethane were produced as previously reported[26]. HPLC-grade water, acetonitrile, methanol and triethylamine were obtained from Fisher Scientific (St. Louis, MO, U.S.A.). All chemicals were used without purification.

All developments were done at room temperature. Cylindrical glass developing chambers (23 × 6 cm I.D.) and rectangular glass chambers (28.5 × 9.5 × 27.0 cm) were used. Spot visualization was accomplished using a fixed-wavelength (254 nm) UV lamp.

RESULTS AND DISCUSSION

Past work on the use of native CDs as CMAs (in both HPLC and TLC) indicated that some minimum concentration was needed to obtain an enantiomeric separation. Increasing the CD concentration generally improved the separation. Eventually a point is reached were no further CD can be solubilized or where further increases in the CD concentration do not improve the separation[26]. In the case of β-CD, often the solubility limit was reached before any enantioselectivity was observed. The solubility of hydroxypropyl- and hydroxyethyl-β-CDs in water is much greater than that of underivatized β-CD (Table I). Solutions exceeding 0.4 *M* can be made without including additives to enhance solubility. While greater solubility is desirable, there are a number of other ramifications and "trade-offs" that must be considered. For example, the viscosity of derivatized β-CD solutions also increases with concentration. Consequently TLC development times increase substantially. This is shown in Fig. 2. Indeed, the time of development can become prohibitively long at the higher derivatized β-CD concentrations.

It appears (Table I) that the solubility of the derivatized β-CD increases as the

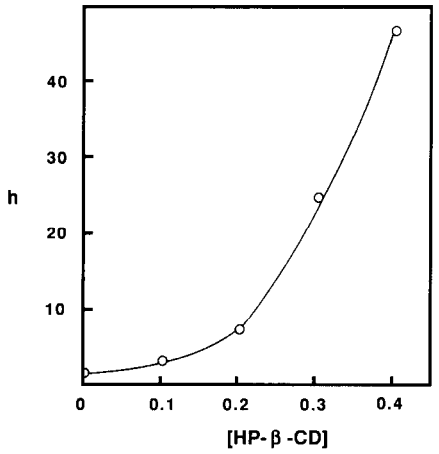

Fig. 2. Plot of the TLC development time (h) *versus* the molar concentration of 0.6 AMS hydroxypropyl-β-CD in the mobile phase. The solute used was dansyl DL-leucine. The mobile phase was acetonitrile–water (30:70, v/v). The separation was done on a 5 × 20 cm reversed-phase TLC plate.

degree of substitution increases. Also, the hydroxyethyl-β-CD may be more soluble than an analogous hydroxypropyl-β-CD. Unfortunately, little is known about the effect of derivatization on enantioselectivity and inclusion complex formation. Since hydrogen bonding to the secondary hydroxyl groups at the mouth of the CD cavity is known to be important for chiral recognition[13,15], it is likely that derivatization will induce changes in chromatographic behavior. In fact, it was found that some optical isomers were better resolved with the derivatized β-CDs while others separated better with native β-CD. As expected, there were substantial differences in the performance of the various β-CD derivatives. This is shown for dansyl DL-leucine in Fig. 3. Several

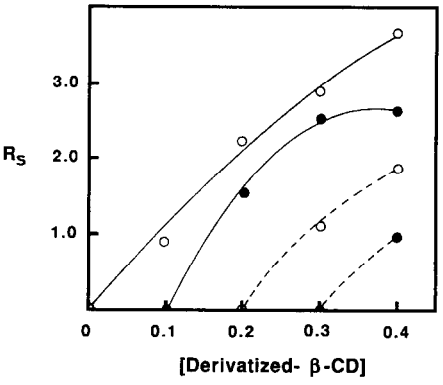

Fig. 3. Plots of enantiomeric resolution (R_s) of dansyl DL-leucine *versus* concentration of four different hydroxyalkyl-β-CDs. The curves, from left to right, are: (—○—) 0.6 AMS hydroxypropyl-β-CD; (—●—) 0.9 AMS hydroxypropyl-β-CD; (--○--) 1.0 AMS hydroxyethyl-β-CD; and (--●--) 1.6 AMS hydroxyethyl-β-CD.

TABLE II

SEPARATION DATA FOR ENANTIOMERS

Compound	R_F*	R_s	*Mobile phase***
(1) Dansyl DL-leucine**	0.44	3.6	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(2) Dansyl DL-valine***	0.37	1.3	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(3) Dansyl DL-methionine***	0.37	1.6	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(4) Dansyl DL-threonine***	0.58	0.9	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(5) Dansyl DL-phenylalanine***	0.36	1.3	0.4 *M* HP-β-CD acetonitrile–water (35:65)
(6) Dansyl DL-norleucine***	0.20	1.8	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(7) DL-Methionine-β-naphthylamide	0.60	1.8	0.3 *M* HP-β-CD acetonitrile–water (35:65)
(8) DL-Alanine-β-naphthylamide	0.66	1.0	0.3 *M* HP-β-CD acetonitrile–water (25:75)
(9) Mephenytoin	0.39	2.0	0.4 *M* HP-β-CD acetonitrile–water (30:70)
(10) 5-(4-Methylphenyl)-5-phenylhydantoin	0.24	0.8	0.3 *M* HP-β-CD acetonitrile–water (35:65)
(11) *R*-(+)Benzyl-2-oxazolidinone *S*-(−)Benzyl-2-oxazolidinone	0.54	0.9	0.3 *M* HP-β-CD acetonitrile–water (35:65)

* This is the R_F of the enantiomer that moved nearest the solvent front.
** HP-β-CD stands for the 0.6 AMS hydroxypropyl-β-CD (Table I).
*** D-isomer was eluted first.

things are apparent from this figure. First, as the average molar substitution (AMS) of the CD is increased, the amount of the CMA needed to achieve an enantiomeric resolution increases. Consequently, a balance must be reached in which the degree of derivatization is sufficient to increase solubility and perhaps alter selectivity, but not high enough to interfere with complexation or chiral recognition. For the compounds in this study, the 0.6 AMS hydroxypropyl-β-CD seemed to be the most effective CMA. Solutions of the higher AMS β-CDs had to be two to ten times higher in concentration than the 0.6 AMS β-CD in order to obtain equivalent or often inferior separations.

Table II gives the optimum separation conditions and data for eleven racemates. Table III gives analogous information for eight pairs of diastereomers. Note that in all cases the mobile phase consists of an acetonitrile–water mixture plus the substituted CDCMA. Organic modifier concentrations between 20 and 40% (v/v) are optimal for this technique. When less than 20% acetonitrile was added, some streaking occurred. If the acetonitrile concentration exceeded 40%, it became difficult to dissolve a sufficient concentration of the CMAs. When methanol was used in place of acetonitrile, streaking was more common.

Fig. 4 shows the effect of hydroxypropyl-β-CD concentration on the retention of racemic dansyl leucine and dansyl valine. Note that the dansyl leucine (circles) begins to resolve at a lower CMA concentration that the dansyl valine (triangles). The enantioselectivity increases up to about 0.3 *M* CMA for dansyl DL-leucine and 0.4

TABLE III

SEPARATION DATA FOR DIASTEREOMERS

Compound	R_F	R_s	*Mobile phase**
(1) Cinchonine	0.40	4.2	0.3 *M* HP-β-CD
Cinchonidine	0.23		acetonitrile–water (35:65)
(2) Quinidine	0.29	4.3	0.3 *M* HP-β-CD
Quinine	0.15		acetonitrile–water (35:65)
(3) 17α,20α-Dihydroxy-4-pregnen-3-one	0.54	4.0	0.3 *M* HP-β-CD
17α,20β-Dihydroxy-4-pregnen-3-one	0.40		acetonitrile–water (35:65)
(4) 20α-Hydroxy-4-pregnen-3-one	0.37	3.3	0.3 *M* HP-β-CD
20β-Hydroxy-4-pregnen-3-one	0.16		acetonitrile–water (35:65)
(5) 17α,20α,21-Trihydroxy-4-pregnene-3,11-dione	0.69	2.2	0.3 *M* HP-β-CD acetonitrile–water (30:70)
17α,20β,21-Trihydroxy-4-pregnene-3,11-dione	0.61		
(6) 11β,17α,20α,21-Tetrahydroxy-4-pregnen-3-one	0.63	0.8	0.3 *M* HP-β-CD acetonitrile–water (35:65)
11β,17α,20β,21-Tetrahydroxy-4-pregnen-3-one	0.0		
(7) N′-(Menthoxycarbonyl)	0.02	0.8	0.3 *M* HP-β-CD
anabasine	0.04		acetonitrile–water (35:65)
(8) N′-(Menthoxycarbonyl)	0.11	2.2	0.3 *M* HP-β-CD
3-pyridyl-1-aminoethane	0.18		acetonitrile–water (35:65)

* HP-β-CD stands for the 0.6 AMS hydroxypropyl-β-CD (Table I).
** 1% Aqueous triethyl ammonium acetate (pH 7.1).

M for dansyl DL-valine (Fig. 4). It would appear from Fig. 4 that the resolution of dansyl DL-leucine may level off at higher CMA concentrations. As shown in Fig. 5, this is not necessarily the case. The enantiomeric resolution of dansyl DL-leucine increases at high CMA concentrations even though the enantioselectivity (*i.e.*, α) remains approximately constant. The reason for this is that the spots are significantly smaller (*i.e.*, better efficiency) at the higher CMA concentrations. Dansyl DL-valine does not show as pronounced an improvement in resolution at 0.4 *M* CMA.

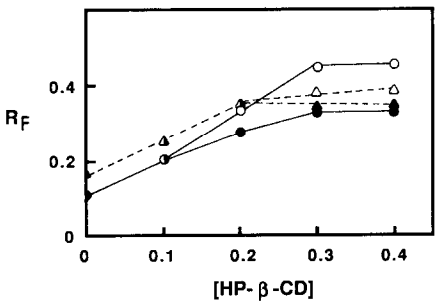

Fig. 4. Plots showing the change in R_F *versus* concentration of 0.6 AMS hydroxypropyl-β-CD for dansyl DL-leucine (circles) and dansyl DL-valine (triangles).

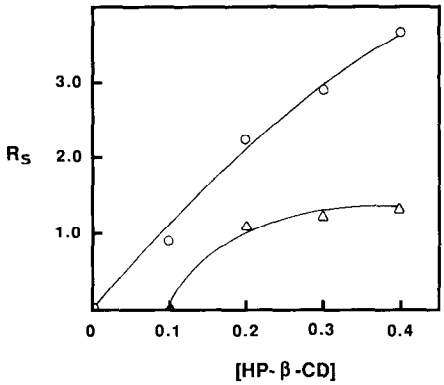

Fig. 5. Plots of resolution (R_s) *versus* concentration of 0.6 AMS hydroxypropyl-β-CD for dansyl DL-leucine (○) and dansyl DL-valine (△).

CONCLUSIONS

Hydroxypropyl- and hydroxyethyl-β-CDs can be useful CMAs for the TLC separation of enantiomers. Their increased solubility in water and hydro–organic solvents (compared to β-CD) makes them particularly convenient and useful CMAs. The 0.6 *M* substituted β-CD seemed to be the most effective CMA. Higher degrees of substitution increased the solubility of the base molecule, but also seemed to interfere with complexation or enantioselectivity. The use of CMAs in high-performance TLC is an area of great potential that has not been investigated adequately.

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