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PLANAR CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS AND DIASTEREOMERS WITH CYCLODEXTRIN MOBILE PHASE ADDITIVES

DANIEL W. ARMSTRONG*, FENG-YING HE and SOON M. HAN
Department of Chemistry, University of Missouri-Rolla, Rolla, MO 65401-0249 (U.S.A.)
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SUMMARY

A variety of racemic compounds were resolved using reversed-phase thin-layer chromatography (TLC) with mobile phases containing highly concentrated solutions of β-cyclodextrin (β-CD). These include the drugs labetalol and mephenytoin, metallocenes, crown ethers, methyl-*p*-toluenesulfinate, nornicotine derivatives and several dansyl and β-naphthylamide substituted amino acids. It was possible to resolve some racemates that could not be separated on β-CD bonded phase liquid chromatography (LC) columns with this technique. Likewise there were some compounds that could be resolved with the LC approach that failed to separate with the present TLC method. In cases of racemates that could be resolved by either approach, it was found that the retention order was exactly opposite for the two methods. Enantiomeric resolution is highly dependent on mobile phase composition. In particular, the type and amount of organic modifier as well as the concentration of β-CD affect the observed resolution. Possible reasons for the chromatographic behavior are discussed. Several diastereoisomeric compounds were separated as well, including steroid epimers and pharmaceutical compounds.

INTRODUCTION

Reports on the liquid chromatographic (LC) separation of enantiomers have increased substantially in the last few years. Several new chiral stationary phases (CSPs) have been proposed and evaluated[1–16]. In addition, a wide variety of chiral mobile phase additives have been shown to resolve certain racemates[17–24]. Unfortunately planar chromatographic methods [such as thin-layer chromatography (TLC) and paper chromatography] have lagged far behind their LC counterparts. There have been a few isolated reports on the TLC of a limited number of compounds. For example, Yuasa *et al.*[25] reported the partial separation of DL-tryptophan on a crystalline cellulose coated plate. Wainer *et al.*[26] separated racemic 2,2,2-trifluoro-1-(9-anthryl) ethanol on a chiral dinitrobenzoylphenylglycine bonded phase. Weinstein[27], Grinberg and Weinstein[28], and Gunther *et al.*[29] separated several racemic dansyl amino acids on reversed-phase plates impregnated with copper(II) complexes of chiral alkyl α-amino acid derivatives. Alak and Armstrong[30] reported

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the separation of several racemic amino acid and ferrocene derivatives on β-cyclodextrin (β-CD) bonded phase TLC plates. With the possible exception of the ternary complex–ligand exchange plates, none of the aforementioned CSPs are available commercially in a planar format. Consequently, the most readily available approach for the TLC separation of different enantiomers remains the use of chiral mobile phase additives. Unfortunately even less has been published on this subject than on TLC with CSPs.

Cyclodextrins were first used as mobile phase additives for chromatography in 1980 to separate a series of structural isomers[31,32]. In 1982, Sybilska and co-workers[20,22] used cyclodextrins as mobile phase additives in high-performance liquid chromatography (HPLC) to effect the resolution of racemic mandelic acid and its derivatives. Since this time, there have been a few additional reports on the use of cyclodextrin mobile phase additives in HPLC[23,24]. However, no racemate has been resolved by TLC using a cyclodextrin mobile phase additive to our knowledge. One of the reasons for this is the limited solubility of cyclodextrins (particularly β-CD) in hydro-organic solvents. Indeed, a saturated solution of β-CD in pure water is approximately 0.017 *M*, which is insufficient for the TLC separation of most enantiomers.

In this work we report the resolution of 21 racemates by reversed-phase TLC using a β-CD mobile phase additive. Resolution is achieved only when the concentration of β-CD is increased to levels exceeding its solubility in pure water. Types of racemates resolved include drugs, nicotinoids, amino acid derivatives, sulfinates, metallocenes and crown ethers. The TLC resolutions (R_s) of some of the racemates was equivalent to or better than analogous HPLC separations on CSPs. As expected, this planar method easily separated a number of diastereomeric compounds as well.

EXPERIMENTAL

Materials

Chemically bonded octadecylsilane reversed phase TLC plates, KC18F (200 μm layer thickness, 5 × 20 cm and 20 × 20 cm) were obtained from Whatman (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; 17α,20α,21-trihydroxy-4-pregnene-3,11-dione; 17α,20β,21-trihydroxy-4-pregnene-3,11-dione; 20-hydroxy-4-pregnen-3-one and 20β-hydroxy-4-pregnen-3-one were obtained from Sigma (St. Louis, MO, U.S.A.). (±)2-Chloro-2-phenylacetyl chloride, DL-alanine-2-naphthylamide hydrochloride, (1*R*,2*S*,5*R*)-(−)-menthyl-(*S*)-*p*-toluenesulfonate, (1*S*, 2*R*,5*S*)-(+)-menthyl-(*R*)-*p*-toluenesulfinate and α-ethyltryptamine acetate were obtained from Aldrich (Milwaukee, WI, U.S.A.). Urea and sodium chloride were obtained from MCB (Cincinnati, OH, U.S.A.). β-Cyclodextrin was obtained from Advanced Separation Technologies (Whippany, NJ, U.S.A.) and Ensuiko Sugar Refining. HPLC-grade water, acetonitrile, triethylamine, hydrochloric acid and methanol were obtained from Fisher Scientific (Plano, TX, U.S.A.). The (±)2-chloro-2-phenylacetyl chloride was hydrolyzed to the free acid before use. All other chemicals were used as received.

Ferrocene enantiomers [(±)*S*-(1-ferrocenyl-2-methylpropyl)thioethanol, (±)*S*-

(1-ferrocenylethyl)thiophenol], nicotine enantiomers [N′-benzylnornicotine, N′-(2-naphthylmethyl)nornicotine], N′-(methoxycarbonyl)-anabasine, N′-(menthoxycarbonyl)-3-pyridyl-1-aminoethane and crown ether enantiomers [(±)2,2-binaphthyldiyl-N-benzyl-monoaza-16-crown-5] were produced as previously reported[24,33–35]. Mephenytoin and labetalol were obtained from R. D. Armstrong of the La Jolla Cancer Research Foundation.

Methods

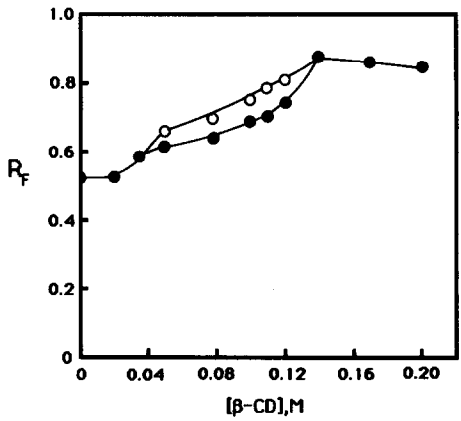
The solubility of β-CD in water is $1.67 \cdot 10^{-2}$ *M* at 25°C; however, when urea is added, one can increase the solubility of β-CD. In this study, saturated solutions of urea were used. 0.6 *M* Sodium chloride also was added to the mobile phase to stabilize the binder of the reversed-phase plates. Without this salt, mobile phases containing more than 50% water tend to dissolve the binder of Whatman reversed-phase plates, thereby resulting in the separation of the stationary phase from the glass support during development.

It took approximately 6–8 h to develop completely a 20 × 20 cm and a 5 × 20 cm TLC plate with a cyclodextrin mobile phase. All developments were done at room temperature (20°C) in 23 × 6 cm I.D. cylindrical glass chambers and a 28.5 × 9.5 × 27.0 cm glass chamber.

Spot visualization was done by use of a fixed-wavelength (254 nm) UV lamp. A Shimadzu dual-wavelength TLC scanner (CS-910) was used to measure resolution. A single-wavelength, reflection mode and linear scanning were used. The wavelength selected corresponded to that of maximum absorbance for each compound.

RESULTS AND DISCUSSION

The solubility of β-CD in neat water and hydro-organic solvent mixtures can be



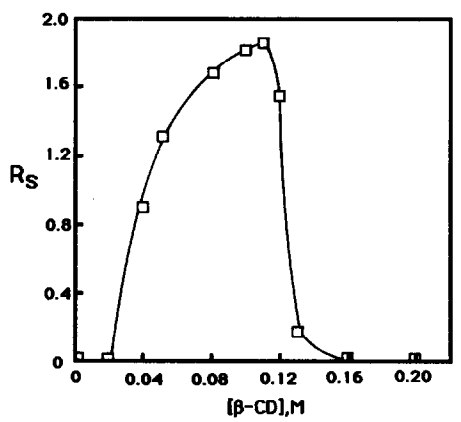


Fig. 1. Plot showing the effect of β-cyclodextrin concentration in the mobile phase on the R_F values of dansyl-D-glutamic acid (○) and dansyl-L-glutamic acid (●). In addition to the indicated levels of β-CD the mobile phase consisted of acetonitrile–water (30:70) (saturated with urea).

Fig. 2. Plot showing the effect β-cyclodextrin concentration on the TLC resolution (R_s) of dansyl-DL-glutamic acid. Other conditions are the same as in Fig. 1.

increased by over an order of magnitude using various additives. Both urea and sodium hydroxide tend to enhance the solubility of β-CD in the aforementioned solvents. In this study, aqueous solutions saturated with urea (see Experimental section) proved to be most effective. The significance of this in planar chromatography is that one can resolve many racemates by reversed-phase TLC using these "enhanced concentration" cyclodextrin solutions. This is illustrated in Fig. 1. Significant resolution of dansyl DL-glutamic acid occurs when the mobile phase contains more than 0.04 *M* β-CD. Optimum enantiomeric resolution occurs between approximately 0.08 and 0.12 *M* β-CD (Fig. 2). This range varies slightly with the compound studied and more substantially with the amount of organic modifier present. The resolution deteriorates at very high β-CD concentrations as the spots blend together near the solvent front (Fig. 2).

Both the concentration and type of organic modifier affect enantiomeric resolution in this technique. The results illustrated in Fig. 3 are typical for all of the solutes in this study. Enantiomeric resolution occurs over a narrow range of organic modifier concentrations but not outside that range. Optimum resolution occurs over a range of 10 to 15% modifier and the R_F values of solutes tend not to change appreciably in this region (see Fig. 3 between 20 and 30% acetonitrile). An analogous curve to that shown in Fig. 3 was generated using methanol as the modifier. The only difference was that the "plateau of optimum resolution" was shifted to 10% higher concentration of modifier (*i.e.*, to 30–40% methanol) and to slightly higher R_F values (*i.e.*, 0.5–0.6). The general effect of organic modifier type on enantiomeric resolution is shown in Fig. 4. Again it is apparent that resolution occurs over a relatively narrow range of mobile phase compositions. Also, the range for methanol is slightly greater than that for acetonitrile. The point of maximum resolution occurs at a lower modifier percentage in the acetonitrile case (Fig. 4). This was true for all of the solutes in this

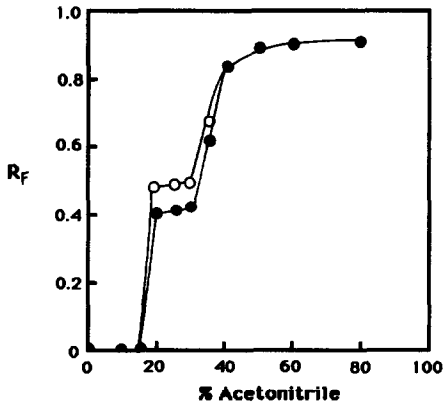


Fig. 3. Plot showing the effect of %acetonitrile in the mobile phase on the TLC separation of dansyl-D-serine (○) from dansyl-L-serine (●). The concentration of β-cyclodextrin is 0.106 *M*.

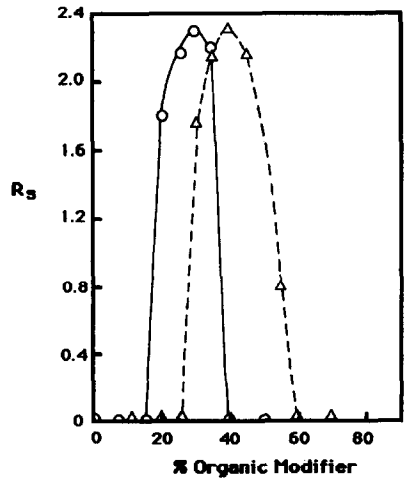


Fig. 4. Plots showing the difference in acetonitrile (○) *versus* methanol (△) phase modifiers in the TLC resolution of dansyl-DL-threonine with β-CD additives. The concentration of β-cyclodextrin is 0.106 *M*.

study. Although Fig. 4 shows that the maximum obtainable resolution was the same for both acetonitrile and methanol modifiers ($R_s \approx 2.3$) this was not true for all solutes in this study. Comparison plots, such as those in Fig. 4, could vary as to the height of their respective maxima as well as baseline range.

Table I gives the separation data and conditions for the resolution of twelve dansyl amino acids. Table II gives equivalent data for ten other racemates that are not related structually to the dansyl amino acids or to one another. In all cases the solubility of the β-CD mobile phase modifier had to be enhanced with urea before enantiomeric resolution was possible. Fig. 5 is a TLC chromatogram showing the baseline resolution of several dansyl amino acid racemates. Note that the D-enantiomer always elutes ahead of the L isomer. This retention behavior is opposite to that observed for the β-CD bonded phase.

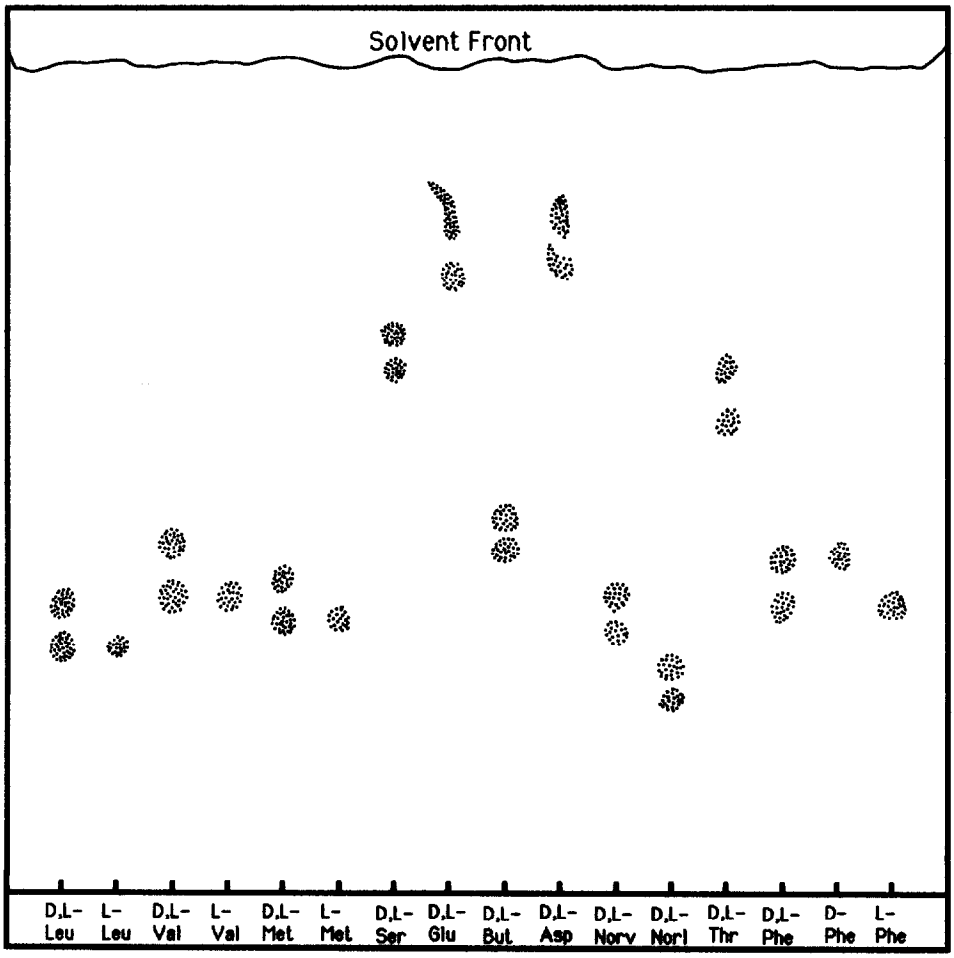


Fig. 5. RP-TLC chromatogram showing the resolution of the racemates: dansyl-DL-leucine; dansyl-DL-valine; dansyl-DL-methionine; dansyl-DL-serine; dansyl-DL-glutamic acid; dansyl-DL-α-amino-*n*-butyric acid; dansyl-DL-aspartic acid; dansyl-DL-norvaline; dansyl-DL-threonine and dansyl-DL-phenylalanine. The mobile phase consisted of acetonitrile–0.10 *M* β-CD (aq.) (30:70, v/v) (see Experimental section).

TABLE I

SEPARATION DATA FOR DANSYL AMINO ACIDS

A UV lamp (254 nm) was used for detection of fluorescence spots.

Compounds	R_{F_1}*	R_{F_2}	α	R_s	*Mobile phase***
Dansyl-DL-leucine	0.30	0.35	1.17	2.0	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-valine	0.36	0.43	1.19	2.5	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-methionine	0.34	0.38	1.12	2.1	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-glutamic acid	0.65	0.72	1.11	2.0	Methanol–0.163 *M* β-CD (35:65)
Dansyl-DL-α-amino-*n*-butyric acid	0.42	0.47	1.12	1.5	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-norvaline	0.32	0.34	1.06	1.4	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-norleucine	0.24	0.28	1.17	1.6	Acetonitrile–0.151 *M* β-CD (30;70)
Dansyl-DL-phenylalanine	0.35	0.39	1.11	1.4	Acetonitrile–0.151 *M* β-CD (30:70)
Dansyl-DL-serine	0.41	0.47	1.15	1.5	Acetonitrile–0.133 *M* β-CD (20:80)
Dansyl-DL-aspartic acid	0.64	0.70	1.09	1.8	Acetonitrile–0.133 *M* β-CD (25:75)
Dansyl-DL-tryptophan	0.43	0.45	1.05	0.8	Acetonitrile–0.231 *M* β-CD (35:65)
Dansyl-DL-threonine	0.42	0.51	1.24	2.0	Methanol–0.151 *M* β-CD (30:70)

* D-Isomer was eluted first.

** Solutions also contained urea and sodium chloride (see Experimental section).

TABLE II

SEPARATION DATA FOR ENANTIOMERIC COMPOUNDS

Compounds	R_{F1}	R_{F_2}	α	R_s	*Mobile phase**	*Detection method*
Mephenytoin	0.32	0.38	1.19	1.5	Methanol–0.308 *M* β-CD (35:65)	UV (254 nm)
(±)*S*-(1-Ferrocenyl-2-methylpropyl)-thioethanol	0.42	0.51	1.21	2.0	Acetonitrile–0.125 *M* β-CD (15:85)	TLC scanner**
(±)*S*-(1-Ferrocenylethyl)thiophenol	0.38	0.42	1.07	0.5	Acetonitrile–0.151 *M* β-CD (30:70)	TLC scanner**
N′-Benzylnornicotine	0.29	0.34	1.17	1.7	Methanol–0.200 *M* β-CD (60:40)***	UV (254 nm)
N′-(2-Naphthylmethyl)nornicotine	0.19	0.24	1.26	1.7	Methanol–0.200 *M* β-CD (60:40)***	UV (254 nm)
(±)2-Chloro-2-phenylacetyl chloride	0.02	0.07	3.5	0.55	Acetonitrile–0.151 *M* β-CD (30:70)	TLC scanner**
DL-Alanine-2-naphthylamide hydrochloride	0.59	0.66	1.12	1.2	Methanol–0.163 *M* β-CD (35:65)	TLC scanner**
(1*R*,2*S*,5*R*)-(−)-Menthyl-(*S*)- and (1*S*,2*R*,5*S*)-(+)-menthyl-(*R*)-*p*-toluenesulfinate	0.06	0.08	1.33	0.6	Acetonitrile–0.151 *M* β-CD (30:70)	TLC scanner**
(±)2,2′-Binaphthyldiyl-N-benzylmonoaza-16-crown-5	0.05	0.08	1.60	0.6	Methanol–0.265 *M* β-CD (60:40)	TLC scanner**

* Solutions also contained urea and sodium chloride (see Experimental section).

** Wavelengths for detection were 254, 280 and 230 nm.

*** 1% Aqueous triethyl ammonium acetate (pH 7.1).

Another interesting aspect of this technique is that sometimes there seems to be no relation between the ease of separation with the β-CD mobile phase modifier *versus* the β-CD bonded phase. Many compounds are separated equally well by both techniques with the expected reversal in retention order as the main difference. However, some racemates can be resolved by one method but not the other. For example, and menthyl-*p*-toluenesulfinate (Table II) and dansyl glutamic acid and aspartic acid (Table I) are difficult to separate on β-CD bonded phase LC columns. Conversely, some chiral crown ethers[34] and racemic metallocenes[35] could not be resolved with β-CD mobile phase additives and RP-TLC even though they were easily resolved by HPLC with a β-CD bonded phase. This is interesting because the chiral resolving agent (β-CD) is the same in both cases. Clearly, the mechanism of chiral recognition and resolution is not always analogous in the two related methods. While the reasons for these differences in chiral selectivity are not yet clear, there are a number of possible factors. In the case of the bonded phase, the cyclodextrin is linked to the silica gel via one to three, eight atom spacer arms. The spacer arms can restrict the motion of the cyclodextrin and provide an additional possible interaction site for a complexed molecule. Also, the surface density and configuration of the cyclodextrin on the bonded phase media may be more fixed than when using the cyclodextrins as mobile phase modifiers. As a mobile phase additive, the cyclodextrin is readily

TABLE III

SEPARATION DATA FOR DIASTEREOMERIC COMPOUNDS

UV lamp (254 nm) was used for detection of spots.

Compounds	R_F	α	R_s	*Mobile phase**
Labetalol	0.49 0.53	1.08	0.7	Methanol–0.262 *M* β-CD (35:65)
Cinchonine Cinchonidine	0.21 0.18	1.16	0.9	Acetonitrile–0.133 *M* β-CD (20:80)
Quinine Quinidine	0.19 0.25	1.32	1.5	Methanol–0.250 *M* β-CD (40:60)
N′-(Menthoxycarbonyl)-anabasine	0.04 0.07	1.75	2.0	Methanol–0.200 *M* β-CD (60:40)**
N′-(menthoxycarbonyl)-3-pyridyl-1-aminoethane	0.24 0.30	1.25	3.1	Methanol–0.200 *M* β-CD (60:40)**
17α,20α-Dihydroxy-4-pregnen-3-one 17α,20β-Dihydroxy-4-pregnen-3-one	0.56 0.48	1.17	2.7	Acetonitrile–0.151 *M* β-CD (30:70)
17α,20α,21-Trihydroxy-4-pregnene-3,11-dione 17α,20β,21-Trihydroxy-4-pregnene-3,11-dione	0.82 0.68	1.20	1.5	Methanol–0.151 *M* β-CD (30:70)
20α-Hydroxy-4-pregnen-3-one 20β-Hydroxy-4-pregnen-3-one	0.59 0.33	1.79	5.6	Methanol–0.151 *M* β-CD (30:70)

* Solutions also contained urea and sodium chloride (see Experimental section).

** 1% aqueous triethylammonium-acetate (pH 7.1).

available for multiple complexation[36]. Currently the effect of multiple complexation and equilibria on chiral recognition is unknown. When using cyclodextrins as mobile phase modifiers they are both adsorbed on the achiral stationary phase and present as carriers in solution. If solution demixing occurs during development and a racemate travels ahead of the cyclodextrin solvent front, it would not be expected to resolve. Indeed, this may be occuring at the higher organic modifier concentrations. However, this cannot explain why some racemates resolve via the mobile phase additive method but not on the chiral stationary phase.

It is not surprising that a number of diastereomeric compounds are more easily separated by "chiral LC" technique than by conventional normal and reversed-phase methods. Likewise, RP-TLC with chiral cyclodextrin mobile phase additives effectively separates a variety of these isomers. Table III gives a few typical examples including steroid epimers and alkaloids. Coupling the differential affinity of cyclodextrins for many different isomers with planar chromatography allows one to evaluate many different solvent systems and isomeric mixtures simultaneously and inexpensively.

CONCLUSIONS

Given the paucity of readily available planar chromatographic techniques for resolving enantiomers, the "cyclodextrin mobile phase approach" is particularly facile and attractive. It also seems that this method can be more than a simple alternative for LC on cyclodextrin bonded phases. The fact that racemates can be resolved via the mobile phase additive approach which cannot be resolved on the analogous CSP and *vice versa* raises a number of mechanistic questions involving chiral recognition. The TLC resolution of enantiomers occurs only under a fairly narrow range of mobile phase conditions. As such, some knowledge as to the effect of organic modifier and cyclodextrin concentration is essential for the successful utilization of this method.

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