Reversed-Phase Planar Chromatography of Enantiomeric Compounds on Microcrystalline Cellulose Triacetate (MCTA)

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Key Words:

Reversed-phase TLC
Microcrystalline cellulose triacetate
Pyrethroids
Flavanones
Unusual racemates
Unusual enantiomers

Summary

Several racemates have been resolved on home-made microcrystalline cellulose triacetate (MCTA) plates eluted with aqueous - organic mixtures containing methanol, ethanol, or 2-propanol. The roles of the chemical structures of the solutes and of the type and concentration of organic solvent on the resolving capability of this polysaccharide have been evaluated. Detection of enantiomeric mixtures in the ratios 50:1, 100:1, and 200:1 was performed by scanning densitometry of the MCTA chromatograms.

1 Introduction

In previous research home-made MCTA plates have been successfully used for the separation of several racemates and pure optical isomers [1,2]. Although the results were indicative of the role of chemical structure in the enantioseparation, prediction of whether it is possible to resolve a racemate into enantiomers on MCTA layers is at present based more on empirical experience than on a real knowledge of the mechanism involved in the chiral recognition process [3]. We have, therefore, investigated racemates structurally related to those studied previously [1,2] and new chiral compounds such as flavanone derivatives and pyrethroids. We have also investigated the use of scanning densitometry to determine optical purity. Because of the different biological activity of enantiomers, the preparation of compounds of high enantiomeric purity [4] and the study of new analytical procedures [5] are of notable importance.

2 Experimental

Analysis was performed with HPLC-quality solvents (Merck, Darmstadt, Germany).

MCTA for HPLC (particle size <10 μm) was purchased from Fluka (Buchs, Switzerland). A slurry of MCTA with silica gel 60 GF_{254} (particle size 15 μm; Merck, FRG) as binder was prepared by mixing the latter material (3 g) with distilled water (15 mL) for 5 min with a magnetic mixer then adding the MCTA (9 g) and ethanol (35 mL) and shaking the resulting product for 5 min before transferring it to the Chemetron (Camag, Muttenz, Switzerland) automatic plate spreader. The layers (10 × 20 cm, thickness 250 μm) were dried at room temperature (20°C, 65% relative humidity) and used within a few (2–5) hours.

Solutions (0.5–4 mg mL^{-1}) of racemates and pure optical isomers (Sigma, Aldrich, and ICN, USA; Fluka and Novabiochem, Switzerland) were prepared in methanol or aqueous ethanol (80%). These solutions (0.5–1 μL) were applied (Hamilton syringe; Alltech, Deerfield, IL, USA) 1 cm from the bottom and at least 1.5 cm from the sides of the plates.

The plates were chromatographed by ascending development in a Desaga (Heidelberg, FRG) thermostatted chamber (22 × 22 × 6 cm), saturated for 1 h at 25°C.

The spots were detected by UV illumination at $\lambda = 254$ and 364 nm. Densitometric measurements were performed in absorbance mode at $\lambda = 260$ nm with a Shimadzu CS-9001PC scanning densitometer coupled with a 486 IBM-compatible PC. Plates were scanned in the direction of development with a moving light spot in zigzag form over the sample zones. All functions of the scanner were controlled and the data were processed with TLC-specific soft-

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ware manufactured by Shimadzu. Real- time background correction was automatically performed in the zigzag system.

3 Results and Discussion

The structures of the test solutes are shown in **Figure 1**.

3.1 Mobile Phases

In addition to the eluents used previously (ethanol - water and 2-propanol - water [1,2]), aqueous solutions of methanol and ternary systems (*e.g.* methanol - ethanol - water) were also used. The retention of solutes increased notably with higher percentages of water, in agreement with the behavior predicted for reversed-phase chromatography. Significant changes in selectivity and in the resolution factors of analytes were obtained when ethanol was mixed with, or completely replaced by, either methanol or 2-propanol.

Table 1 shows the best chromatographic conditions for the resolution of 21 solutes determined by changing the type and composition of the eluent and, consequently, the separation time. (Three of the twenty- four solutes examined (catechin, fenvalerate, and styrene oxide), were not resolved under the experimental conditions used.)

The letters R and S and the symbols (+) and (-) are reported in Table 1 for those compounds whose enantiomers were commercially available. If the solutes have two chiral stereogenic centers, the sign of rotation is used; *e.g.* (4S,5R)-4-methyl-5-phenyl-2-oxazolidone is the laevorotatory (-) isomer and (4S,5R)-1,5-dimethyl-4-phenyl-2-imidazolidone is the dextrorotatory (+) isomer.

The data in Table 1 illustrate the high resolving capacity of MCTA for the majority of racemates. In fact, α values >1.2 (even $\geq$2 for some compounds) and R_M values >1 were generally found, evidence of the compactness of the spots.

Most separations were obtained by eluting with alcohol - water, 80 + 20 (*v/v*) but aqueous - organic solutions with higher percentages of water were useful for the resolution of less hydrophobic compounds, *e.g.* 2-phenylcycloheptanone, 2,3-*O*-isopropylidene-1,1,4,4-tetraphenylthreitol, and *N*-benzylproline ethyl ester. This behavior is in accord with that observed in previous studies [1,2]. Ethanol and 2-propanol are the most effective alcohols but methanol is essential for the resolution of the racemates of taxifolin, hesperetin, and naringenin. In general, the use of 2-propanol - water mixtures enabled the separation of enantiomers but notably increased the migration time. The separation times reported in Table 1 refer to alcohol - water, 80 + 20 (*v/v*); shorter development times ($\approx$ 5 h) were observed for levels of 2-propanol between 40 and 60%.

The enantioseparation of 4-methyl-5-phenyl-2-oxazolidone is of utmost importance because this compound is produced

by the reaction of nor-ephedrine with phosgene.
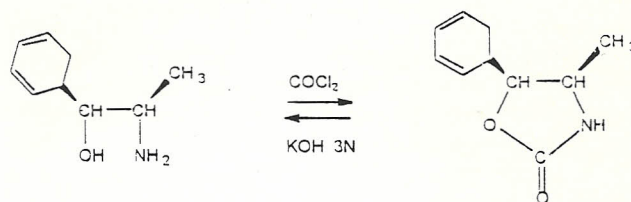
Conversion of the ephedrine enantiomers, which have different biological activity, into oxazolidones without racemization is the basis for the separation of the optical isomer of this sympathomimetic agent and a direct probe of the enantiomeric purity of nor-ephedrine [6].

MCTA columns were also used for the resolution of oxazolidones produced from the reaction of chiral β-blocking agents (metoprolol and propranolol) with specific reagents [7].

Table 1

Retention (hR_{F1}, hR_{F2})[a] and resolution (α, R_S)[b] data for enantiomeric compounds on microcrystalline cellulose triacetate plates with silica gel 60 GF$_{254}$ as binder (temperature 25°C).

Compound	Eluent	hR_{F1}	hR_{F2}	α	R_S
Alfamethrin	80:20[c]	23	29	1.37	1.7
Fenpropathrin	80:20[c]	30	34	1.20	1.2
Fenoxaprop-ethyl	80:20[d]	36 (R)	46 (S)	1.52	2.2
Taxifolin	80:20[e]	44	48	1.17	1.3
Hesperetin	80:20[e]	23	27	1.24	1.5
Naringenin	80:20[e]	23	28	1.30	1.6
Flavanone	80:20[c]	22	24	1.12	0.4
6-Methoxyflavanone	80:20[c]	24	27	1.17	0.8
6-Hydroxyflavanone	80:20[c]	36	39	1.14	0.8
(4S,5R)/(4R,5S)-4-Methyl-5-phenyl-2-oxazolidone	80:20[e]	62 (-)	72 (+)	1.58	2.6
(4R,5S)/(4S,5R)-1,5-Dimethyl-4-phenyl-2-imidazolidone	80:20[e]	75 (-)	86 (+)	2.06	2.5
trans-4-Chlorostilbene oxide	80:20[d]	25	40	2.00	4.3
2-Phenylcycloheptanone	60:40[d]	17	31	2.20	3.5
1-(9-Fluorenyl)ethanol	80:20[d]	26	44	2.24	3.0
N-Benzylproline ethyl ester	40:60[d]	19 (D)	22 (L)	1.20	1.0
γ-(Trityloximethyl)-γ-butyrolactone	70:30[d]	48 (-)	50 (+)	1.08	0.4
2,3-*O*-Isopropylidene-1,1,4,4-tetraphenylthreitol	50:50[d]	18 (-)	20 (+)	1.14	0.7
2-Methyl-1-indanone	80:20[e]	50	57	1.33	1.8
3-Methyl-1-indanone	80:20[e]	52	58	1.28	1.6
Troger's Base	80:20[c]	23 (+)	44 (-)	2.64	3.6
N-tBOC-3-(2-naphthyl)-Ala	80:20[c]	69 (D)	72 (L)	1.16	0.8

[a] $R_F \times 100$.
[b] $\alpha = (1/R_{F1}\text{-}1)/(1/R_{F2}\text{-}1)$; $R_S = 2 \times$ (distance between the centers of two adjacent spots) / (sum of the width of the two spots in the direction of development).
[c] Ethanol - water; migration distance 12 cm; separation time 3 h.
[d] 2-propanol - water; migration distance 14 cm; separation time 6 h.
[e] Methanol - water. migration distance 16 cm; separation time 2.5 h.

Styrene oxide

Taxifolin

trans-4-Chlorostilbene oxide

1-(9-Fluorenyl)-ethanol

Catechin

Naringenin

Hesperetin

R = H   Flavanone
R = OH   6-Hydroxy-flavanone
R = OCH₃   6-Methoxy-flavanone

1,5-Dimethyl-4-phenyl-2-imidazolidone

Troger's Base

R₁=CH₃, R₂=H   2-Methyl-1-indanone
R₁=H, R₂=CH₃   3-Methyl-1-indanone

4-Methyl-5-phenyl-2-oxazolidone

γ -(Trityloxymethyl)-γ -butyrolactone

2,3-O-Isopropylidene-1,1,4,4-tetraphenyl-threitol

N-tBOC-3-(2-naphtyl)-Ala

N-Benzylproline ethyl ester

Alphamethrin

Fenvalerate
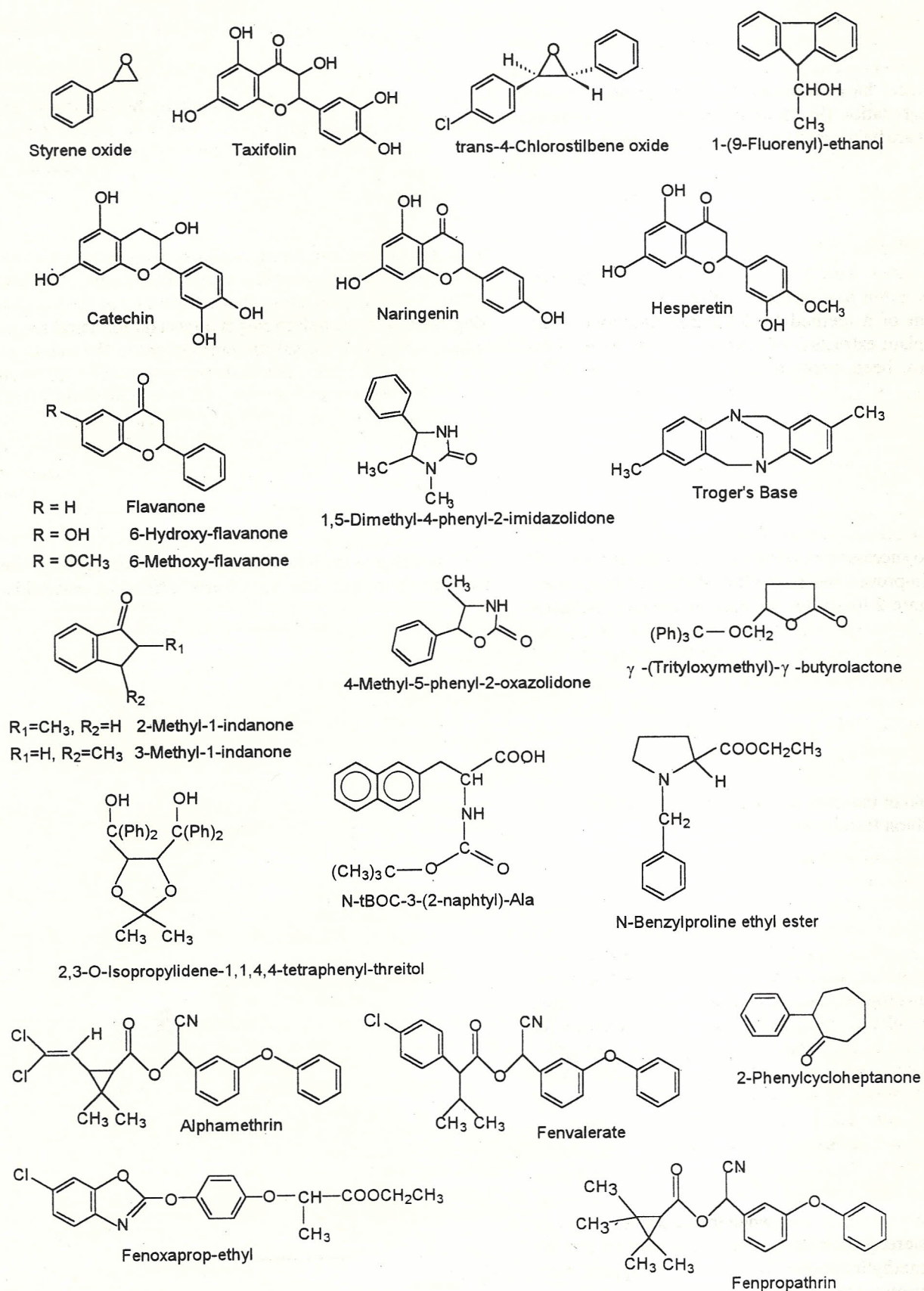
2-Phenylcycloheptanone

Fenoxaprop-ethyl

Fenpropathrin

Figure 1

The structures of the test solutes.

The enantiomeric separations of fenoxaprop-ethyl, alfamethrin (a racemate comprising the two *cis* isomers of cypermethrin) and fenpropathrin should be noted because their optical isomers have different biological activity [8] and rates of degradation [9]. In addition, synthetic pyrethroid insecticides are being used increasingly, because of their low mammalian toxicity [10].

The resolution data for Tröger's base are comparable with those obtained by other authors [11] on cellulose triacetate plates with ethanol - water, 80 : 20 (v/v).

Many flavanones have been isolated as secondary plant metabolites, often in a optically active form [12], and the development of a method to determine flavanone enantiomers in plant extracts is of primary interest. Several flavanones have been resolved into enantiomers on MCTA columns using methanol as eluent, but compounds with a low extent of substitution (hydroxyl and/or methoxy groups) were not resolved or only partially resolved [13]. The naturally occurring flavanones taxifolin, naringenin, and hesperetin were successfully resolved on MCTA layers eluted with methanol - water, 80 + 20 (v/v); partial resolution was observed for flavanone and 6-methoxy- and 6-hydroxyflavanone. Two successive developments with the same eluent effectively improved the resolution of the last three compounds. Figure 2 illustrates the chromatographic behavior of racemic flavanone, 6-hydroxy-flavanone, 6-methoxy-flavanone, and of two pyrethroids on MCTA layers after two successive developments with ethanol - water, 80:20 (v/v). Resolution values for the pyrethroids were higher than those reported in Table 1 and even the three flavanones are resolved.

3.2 The Role of the Chemical Characteristics of the Solutes in Chiral Recognition

Previous studies have pointed out the important role of the carbonyl group in the enantioseparation of benzoin and benzoin methyl ether, and the surprising resolution of racemic 1,1,2-triphenyl-1,2-ethandiol which contains two hydroxyl groups (as does hydrobenzoin) but no carbonyl group [1,2]. This separation was attributed to the substitution of an hydrogen atom of hydrobenzoin by a phenyl group; this results in highly hydrophobic characteristics [2] and changes the shape of the molecule. The favorable influence of the carbonyl group upon chiral recognition was, however, confirmed by the chromatographic behavior of racemic 2-methyl- and 3-methylindanone, the enantiomers of which are well resolved, and of (±)-2-phenylcycloheptanone for which the α and R_M values are 2.0 and 3.5, respectively.

The best results were obtained when the carbonyl group was close to the stereogenic center, as shown by the better resolution of 2-methylindanone (α position) compared with 3-methyl derivative (β position).

The separation of the optical isomers of *N*-tBOC-3-(2-naphthyl)-Ala confirms that the 2-position is an essential

factor in chiral recognition; this has previously been observed for γ-(2-naphthyl)-γ-butyrolactone and 1-(2-naphthyl)ethanol [1,2].

The enantiomers of γ-trityloxymethyl-γ-butyrolactone are partially resolved, behavior which might be a consequence of the high steric hindrance of the trityl group because the inclusion of a compound between the laminae of MCTA is very sensitive to the shape of the molecule.

The chromatographic behavior of flavanones enable us to study the influence of the substitution pattern of a molecule on chiral recognition. Table 1 shows that resolution is lowest for flavanone, indicating that substitution in the benzene ring fused with the hetero ring is important for chiral recognition, although these substituents are not in the vicinity of the stereogenic center. The best results were obtained when two hydroxyl groups were present in positions 5 and 7 (taxifolin, naringenin, and hesperetin); this is in agreement with results obtained on MCTA columns [13]. In this group of compounds naringenin and hesperetin have the highest α values, which indicates that an -OH or -OCH$_3$ group in the 3 and/or 4 position seems to have a favorable influence upon chiral recognition. In contrast, an hydroxyl group on the 3-position of the hetero ring, and, therefore, close to the stereogenic center, has an adverse effect on resolution.
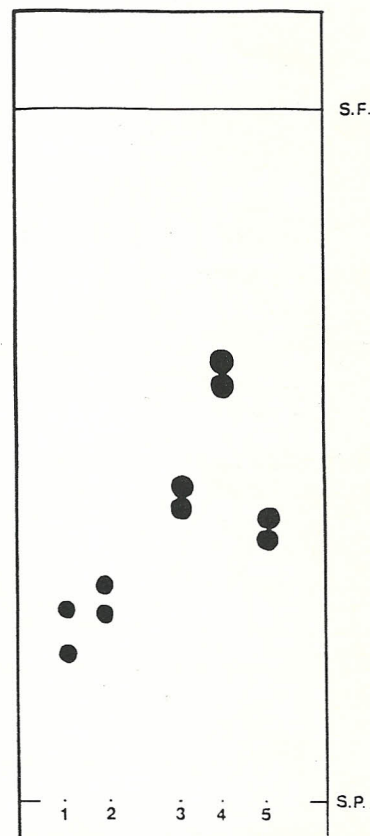

Figure 2

Chromatogram of racemic pyrethroids and flavanones on MCTA after two successive developments (distance 17 cm) with ethanol - water, 80 + 20 (v/v). The separation time for each run was 4.5 h. 1, (±)- alfamethrin; 2, (±)-fenpropathrin; 3, (±)-6-methoxyflavanone; 4, (±)-6-hydroxyflavanone; 5, (±)- flavanone.

Further, no resolution was observed for racemic catechin which is lacking the carbonyl group.

The enantiomers of 4-benzyl-2-oxazolidone were partially resolved on MCTA [2] because the benzyl group in the 4 position has a less favorable influence upon chiral recognition than the same group in the 5 position and, in fact, the optical isomers of 4-methyl-5-phenyl-2-oxazolidone and 5-phenyl-2-oxazolidone have been completely separated on MCTA plates and on cellulose triacetate columns [14], respectively.

The resolution of the racemic herbicide fenoxaprop-ethyl is not surprising because its chemical structure is similar to that of 2-aryl-substituted propionic acids, for which MCTA shows high enantioselectivity [1,2].

Comparison of the chromatographic behavior of the three pyrethroids examined (alfamethrin, fenproprathrin, and fenvalerate) indicates that the presence of the cyclopropane ring in the first two molecules is essential for resolution.
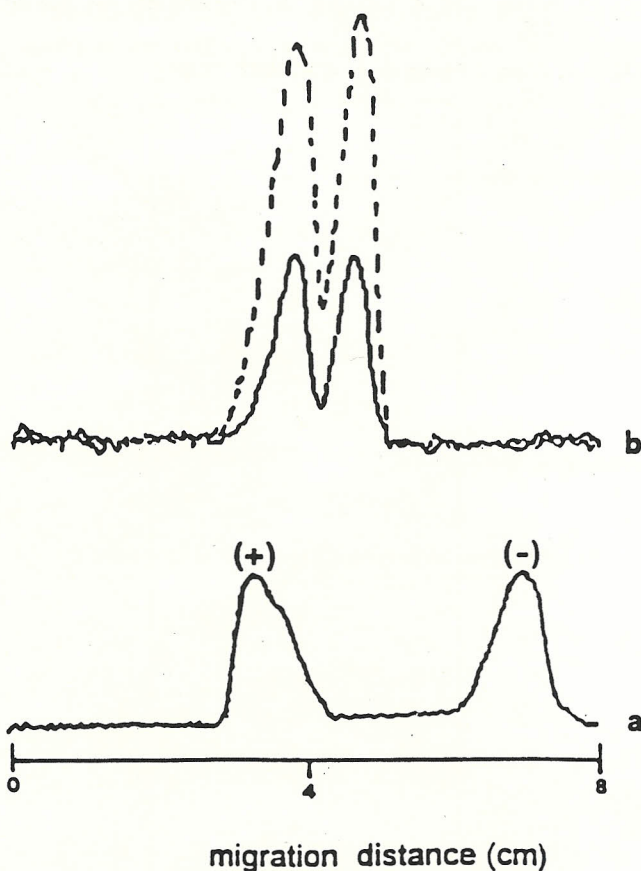

migration distance (cm)

Figure 3

Densitograms of racemic Troger's base (a) and naringenin (b) on MCTA layers eluted with ethanol - water, 80 + 20, and methanol - water, 80 + 20, respectively. The development distance was 15 cm; the separation times were 4 h with ethanol and 2 h with methanol. 0.5 μL (full line) and 1 μL (dashed line) of (±)-naringenin solution (4 mg mL^{-1}) and 1 μL of Troger's base solution (4 mg mL^{-1}) were applied to the plates.
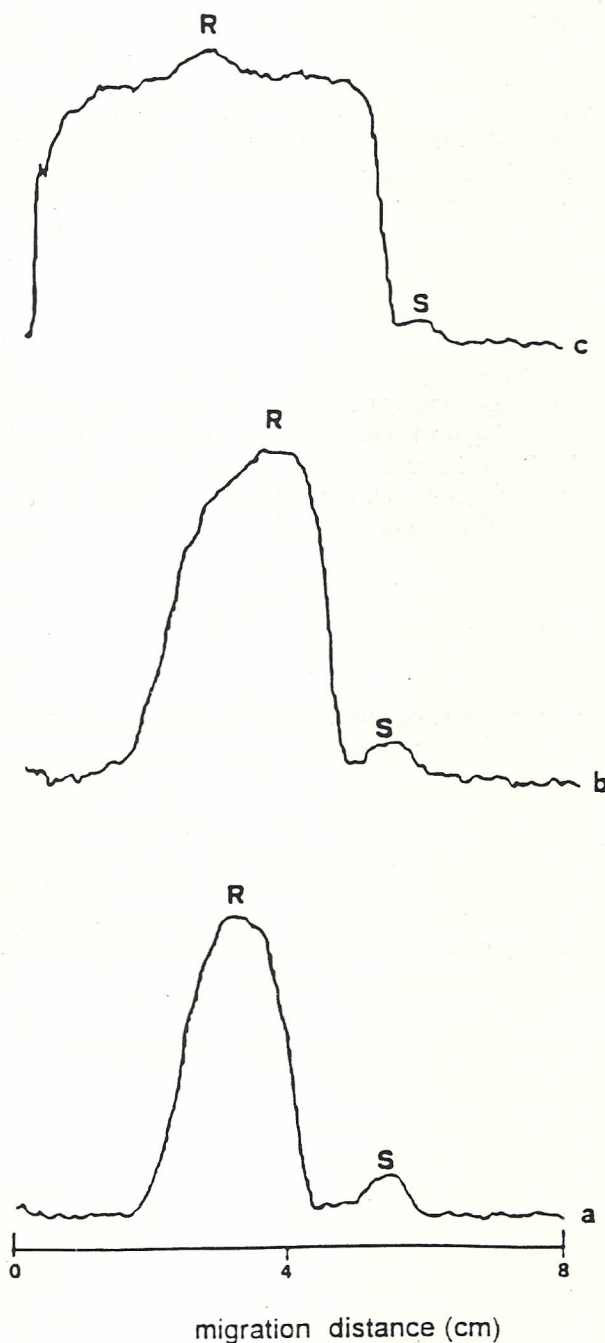

migration distance (cm)

Figure 4

Densitograms of racemic R- and S-1,1-binaphthyl-2,2-diamine mixtures in the ratios 50:1, 100:1 and 200:1 on MCTA layers eluted with ethanol - water, 80 + 20 (v/v). The development distance was 17 cm. (a) 10 μg R and 0.2 μg S; (b) 20 μg R and 0.2 μg S; (c) 40 μg R and 0.2 μg S.

The separation of enantiomeric 1-(9-fluorenyl)-ethanol is in accord with the previous resolution of (±)-2,2,2-trifluoro-1-(9-anthryl)-ethanol [11].

The observed correlations between structurally related racemates can be useful in predicting whether it is possible to resolve a racemate into enantiomers on MCTA layers.

4 Densitometric Measurements

Densitometric measurements were performed on the enantiomers of Troger's base and naringenin on MCTA plates developed with alcohol - water mixtures; the results are presented in **Figures 3a** and **3b**, respectively. The optical isomers of the first solute are resolved to baseline whereas naringenin was only partially resolved both at 2 μg and at 4 μg. Complete resolution of ($\pm$)-naringenin (0.8 μg) was obtained after application of 0.2 μL volumes of the solution to the plates.

Densitograms of synthetic mixtures of 1,1-binaphthyl-2,2-diamine atropisomers in the ratios 50:1, 100:1 and 200:1 are reported in Figure 4. The α and R_M values are 1.99 and 3.3 [1]. The R and S isomers were dissolved in ethanol - water, 80 + 20, at a concentration of 4 mg mL^{-1} and 0.2 mg mL^{-1}, respectively. 2.5, 5, and 10 μL volumes of the solution of the R isomer were applied in small portions (1 μL at a time). Baseline-resolved peaks were obtained for the two atropisomers even at a ratio of 100:1; partial resolution only was observed at a ratio of 200:1, but the S isomer is still visible. In conclusion, densitometric measurements on MCTA plates enable us to control the optical purity of 1,1-binaphthyl-2,2-diamine atropisomers.

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