Journal of Chromatography, 585 (1991) 35–44
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 507

(2*R*,3*R*)-Dicyclohexyl tartrate as a chiral mobile phase additive

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(First received April 19th, 1991; revised manuscript received May 27th, 1991)

ABSTRACT

The enantiomers of acids, esters and amino alcohols of moderate hydrophobicity containing two hydrogen bonding functions were separated in underivatized form using (2*R*,3*R*)-dicyclohexyl tartrate (DCHT) in phosphate buffer (pH 3) as mobile phase and porous graphitic carbon (Hypercarb) as stationary phase. The retention of the solutes can be controlled by the concentration of DCHT in the mobile phase without affecting the enantioselectivity. A low pH in the mobile phase gave optimum enantioselectivity for both acidic and basic solutes. DCHT, which is water soluble up to 0.5 m*M*, has a high affinity for the graphitic carbon phase when using phosphate buffer as the mobile phase. It seems to be adsorbed on the support as a monolayer as changes in the concentration of the mobile phase have only a minor influence on the amount adsorbed (0.14 mmol/g support). Addition of acetonitrile to the mobile phase in higher amounts decreased the amount of DCHT adsorbed on the carbon support and also decreased the retention and the stereoselectivity.

INTRODUCTION

The separation of enantiomers is of particular interest in the biomedical field as it has been shown that the enantiomeric forms of a drug may have different biological activities [1]. A number of chiral solid phases using different principles for enantiomeric recognition [2] are now commercially available.

A chiral mobile phase additive that is dynamically coated on an achiral solid phase may be used as an alternative to covalently bound stationary phases. The technique is flexible and permits screening for new chiral selectors and reversals of the retention order of enantiomers [3]. Lindner *et al.* [4] and Davankov *et al.* [5,6] have demonstrated the optical resolution of amino acids using ligand-exchange chromatography after coating of the support with N-alkyl-L-amino acids. The use of chiral crown ethers dynamically coated on C_{18} material for the resolution of the enantiomers of amino acids and amines has been presented by Shinbo *et al.* [7]. A liquid ester of tartaric acid [(2*R*,3*R*)-di-*n*-butyl tartrate (DBT)] coated on silica-based reversed-phase materials has been used as the chiral stationary phase in the liquid–liquid chromatography (LLC) of amino alcohols [8,9]. Esters of tartaric acid dissolved in organic solvents have also been applied as stationary phases [10].

The studies with DBT dissolved in organic solvents indicated that the solutes were adsorbed on the support [10]. High separation factors were observed for some ephedrine analogues when using a support with a minimum [11] number of polar groups, namely porous graphitic carbon, Hypercarb.

The aim of this study was to evaluate the stereoselective properties of a hydrophobic chiral selector, (2*R*,3*R*)-dicyclohexyl tartrate (DCHT) dynamically coated on Hypercarb.

EXPERIMENTAL

Apparatus

The pump used was a Beckman (Fullerton, CA, USA) Model 114 M. Detection was performed at 254 nm using a SpectroMonitor 3100 (Milton Roy, Riviera Beach, FL, USA); the injector was a Rheodyne (Berkeley, CA, USA) Model 7120 with a 20-μl

0021-9673/91/$03.50

loop. The injector, column and solvent reservoir were kept at 25°C using a thermostat HETO type 02 PT 923 TC water-bath (Birkerød, Denmark).

Column equilibration

The columns were equilibrated by pumping the mobile phase containing the selector. The equilibration was followed by recording the absorbance of the eluent at 195 nm. The breakthrough volume was used to calculate the amount of DCHT adsorbed on the support. The studies carried out under various conditions were performed during the first day after the breakthrough had been observed.

Capacity factors (k') were calculated on the assumption that sodium nitrate is unretained. The separation factor is given as $\alpha = k'_2/k'_1$.

Columns

The Hypercarb columns were either obtained prepacked in 100 × 4.7 mm I.D. stainless-steel columns from Shandon (Astmoor, UK) or packed in the laboratory by a slurry technique in stainless-steel columns (100 × 3.0 mm I.D.). Chloroform was used as the slurry liquid and *n*-hexane as packing liquid. After thorough washing with chloroform and methanol, the columns were tested in a system with a mobile phase consisting of 3.8 *M* acetonitrile–phosphate buffer (pH 2.8) (20:80, v/v). (*R*)- and (*S*)-mandelic acid, phenol, racemic atropine, racemic 2-amino-1-(4-nitrophenyl)propanol and racemic 2-amino-1-[spiro(cyclopropane-1,1′-inden)-3′-yl]ethanol were used as test solutes. The columns were rinsed with 100 ml of ethyl acetate followed by 150 ml of dioxane or dioxane–methanol (25:75) after each chromatographic system. The column performance was checked regularly using the 3.8 *M* acetonitrile–phosphate buffer (pH 2.8) (20:80) mobile phase described above.

μBondapak phenyl material was packed according to procedures described earlier [9].

Capcell PAK C_{18} SG 120 (5-μm particles) was a kind gift from Professor Nabuo Tanaka (Kyoto Institute of Technology) and was obtained packed in a stainless-steel column (250 × 4.6 mm I.D.).

Chemicals

Acetonitrile (LiChrosolv), methanol (Li-Chrosolv), ethyl acetate (analytical-reagent grade) and phenol (analytical-reagent grade) were from E. Merck (Darmstadt, Germany) and isopropanol (HPLC grade) and dioxane (HPLC grade) from Lab Scan (Dublin, Ireland). (2*R*,3*R*)-Dicyclohexyl tartrate was synthesized according to procedures described earlier [10]. The solutes used are presented in Table I. All other chemicals were of analytical-reagent grade and were used without further purification.

RESULTS AND DISCUSSION

Support

Previous liquid–liquid chromatographic studies [10] with DBT as chiral selector showed that the separation factors were highest when using graphitic carbon as support. The lower separation factors obtained with chemically modified silica as support (*e.g.*, μBondapak phenyl) might be due to an achiral retention, *e.g.*, by residual silanols. End-capping of the material has become the usual way of treating residual silanols but these materials still show polar properties [12]. A polymer-coated silica (Capcell PAK C_{18}), suitable for protonated amines in regular chromatographic systems [13], was therefore included in this study (Table II).

Addition of DCHT to the mobile phase gave reduced retention and improved peak symmetry for the enantiomeric solutes with all supports. In agreement with previous observations [10], the enantioselectivity was much higher using graphitic carbon as the support (Table II). Calculations of the coverage of DCHT on the supports based on the stated area of underivatized silica particles and of the carbon phase showed that the two hydrophobic supports, Capcell PAK and Hypercarb, had about a tenfold higher coverage than μBondapak. Despite this fact, the peak symmetry and separation factors were similar on the two silica-based supports. It might be that the conformation adopted by DCHT depends on the three-dimensional structure of the packing material. In addition, the polar groups in DCHT, probably needed for the chiral interaction with the solutes, may interact with residual silanols on the silica support. In contrast, the interaction between DCHT and graphitic carbon is of apolar character and does not involve the polar groups of DCHT.

TABLE I

SOLUTES

No.	Name	Source[a]
1	(1*R*,2*S*)-N-Methylephedrine	Fluka
2	(1*S*,2*R*)-N-Methylephedrine	Fluka
3	(1*R*,2*R*)-Pseudoephedrine	Sigma
4	(1*S*,2*S*)-Pseudoephedrine	Sigma
5	(1*R*,2*S*)-Ephedrine · HCl	Fluka
6	(1*S*,2*R*)-Ephedrine · HCl	Merck
7	(1*R*,2*S*)-Norephedrine · HCl	Serva
8	(1*S*,2*R*)-Norephedrine · HCl	Janssen
9	Racemic 2-amino-1-(4-methanesulphonamidophenyl)ethanol	Leo
10	Racemic 2-amino-1-(4-nitrophenyl)-ethanol · HCl	Leo
11	Racemic 2-amino-1-(3-nitrophenyl)-ethanol · HCl	Leo
12	Racemic 2-amino-1-(3-nitrophenyl)-propanol	Leo
13	Racemic 2-amino-1-(2,4-dichlorophenyl)ethanol	Leo
14	Racemic 2-amino-1-[1,1-dimethyl-inden-3-yl]ethanol	Kabi
15	Racemic 2-amino-1-[spiro(cyclopropane-1,1′-inden)-3′-yl]ethanol	Kabi
16	Racemic 3-phenyllactic acid	Fluka
17	(−)-Phenyllactic acid	Fluka
18	(*R*)-Mandelic acid	Fluka
19	(*S*)-Mandelic acid	Fluka
20	Racemic 3-hydroxy-4-methoxy-mandelic acid	Fluka
21	Racemic tropic acid	Sigma
22	(*R*)-2-Phenylpropionic acid	Sigma
23	(*S*)-2-Phenylpropionic acid	Sigma
24	(2*S*,3*S*)-2-Benzyloxy-1,3,4-butanetriol	Janssen
25	(2*R*,3*S*)-2-Benzyloxy-1,3,4-butanetriol	Janssen
26	(*R*)-Phenyl-1,2-ethanediol	Janssen
27	(*S*)-Phenyl-1,2-ethanediol	Janssen
28	(*R*)-2-Phenylpropanol	Janssen
29	(*S*)-2-Phenylpropanol	Janssen
30	(*R*)-Ethyl mandelate	Aldrich
31	(*S*)-Ethyl mandelate	Aldrich
32	Racemic homatropine	Merck
33	Racemic N-methylhomatropine	Merck
34	Racemic atropine	Merck
35	(*S*)-Atropine	Sigma
36	Racemic N-methylatropine	Merck
37	Racemic ipratropine	Draco

[a] Leo (Helsingborg, Sweden); Draco (Lund, Sweden); Kabi (Stockholm, Sweden); Serva (Heidelberg, Germany); Janssen Chimica (Beerse, Belgium); Fluka (Buchs, Switzerland); E. Merck (Darmstadt, Germany); Sigma (St. Louis, MO, USA); Aldrich (Milwaukee, WI, USA).

Solute structure and stereoselectivity

Graphitic carbon dynamically modified with DCHT can separate the enantiomers of amines, acids and non-protolytic solutes (Tables III–V). An example is given in Fig. 1, which shows the separation of the enantiomers of homatropine (**32**). Complete resolution was obtained within 10 min using a flow-rate of 1.0 ml/min.

Amines. A higher enantioselectivity was observed (Table III) for the primary amine norephedrine (**7** and **8**) than for the corresponding secondary amine [ephedrine (**5** and **6**)] and the tertiary amine [N-methylephedrine (**1** and **2**)]. Similar observations have been made in liquid–liquid chromatographic studies with DBT as selector [9]. As has been concluded earlier [9], the amine function in these ephedrine analogues is most likely needed for the interaction with the selector. In contrast to what was seen in the liquid–liquid studies, a methyl substituent on the α-carbon slightly decreased the enantioselectivity (*e.g.*, **11** and **12** in Table III). In the adsorption systems the substituents in the aromatic ring also seem to be of importance for chiral discrimination (see Table III, **7–13**), possibly owing to steric effects and/or the changed electron configuration [14].

Included in Table III are two solutes with similar indene structures (**14** and **15**). Exchange of two methyl substituents on carbon one for a cyclic dimethylene group, giving the molecule a more rigid structure, drastically increased the stereoselectivity.

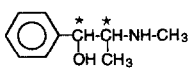
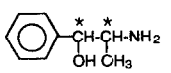
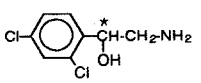
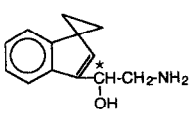
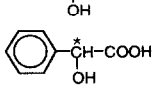
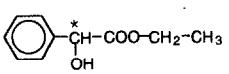
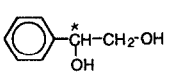
Acidic and non-protolytic solutes. The separation of enantiomers of hydroxy acids is presented in Table IV. With a mobile phase of pH 3 the acids are present mainly in uncharged form. The pK_a of mandelic acid is 3.4 [15]. The enantioselectivity was favoured by a hydroxyl group directly attached to the chiral carbon [mandelic acid (**18** and **19**) and tropic acid (**21**)]. Substitution in the aromatic ring seems to improve the stereoselectivity [*e.g.*, 3-hydroxy-4-methoxymandelic acid (**20**)].

Table V shows the retention and the stereoselectivity of some esters of mandelic acid and tropic acid at pH 3. Homatropine (**32**), which is a tropyl ester of mandelic acid (**18** and **19**), is separated with higher enantioselectivity than mandelic acid and ethyl mandelate (**30** and **31**). Atropine (**34** and **35**), which is a tropyl ester of tropic acid (**21**), shows a lower separation factor than homatropine (**32**). Substitu-

TABLE II

SOLID SUPPORTS

Mobile phase: 0.31 m*M* DCHT in phosphate buffer (pH 2.8) (I = 0.1). $asf_{10\%}$ = Asymmetry factor measured at 10% of the height.

Solute		Support								
		Hypercarb ($9.9 \cdot 10^{-7}$ mol/m²)[b]			μBondapak phenyl ($7.0 \cdot 10^{-8}$ mol/m²)[b]			Capcell PAK C_{18} ($6.6 \cdot 10^{-7}$mol/m²)[b]		
		k'_1	α	$asf_{10\%}$	k'_1	α	$asf_{10\%}$	k'_1	α	$asf_{10\%}$
5,6		0.09	>1.00	1.9	1.20	1.03	1.2	0.90	1.04	1.2
7,8		0.07	>1.00	2.2	0.96	1.04	1.3	0.79	1.04	1.3
13[a]		5.90	1.41	2.8	12.0	1.0	–	15.2	1.00	1.4
15[a]		11.8	1.76	3.5	14.9	1.0	–	13.8	1.00	1.4
18,19		2.08	1.08	1.8	2.04	1.01	1.9	1.75	1.02	1.8
30,31		4.43	1.13	1.3	12.0	1.00	–	11.2	1.01	3.1
26,27		1.02	1.07	1.4	2.18	1.03	–	2.06	1.02	1.4

[a] The solute is injected as the racemic only.
[b] Amount of DCHT adsorbed.

tion on the nitrogen affects the selectivity, probably owing to steric effects as a bulky substituent [ipratropine (**37**)] was less favourable than a methyl group [N-methylatropine (**36**)]. The higher stereoselectivity obtained for the tropyl esters may be due to spatial fitting. It seems unlikely that the nitrogen in the tropane function takes part in any hydrogen bonding as stereoselectivity was obtained for the quaternary ammonium compounds (**33**, **36** and **37**).

Among the aprotic solutes in Table V, the diol 1-phenyl-1,2-ethanediol (**26** and **27**) gave about the same enantioselectivity as mandelic acid (**18** and **19**). The enantiomers of 2-phenyl-1-propanol (**28** and **29**) containing one hydrogen-accepting/donating group only were not separated.

Control of retention and stereoselectivity

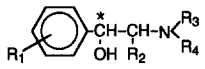
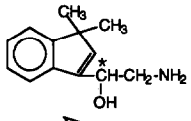
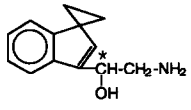
Influence of pH. The stereoselectivity of both acids and bases (amino alcohols) was favoured by a low pH in the mobile phase (Fig. 2). As could be expected, neither the retention nor the stereoselectivity of the aprotic solutes was affected by pH.

An increase in pH was followed by an enhanced retention of the amino alcohols (Fig. 2). If the

TABLE III

SOLUTE STRUCTURE AND STEREOSELECTIVITY FOR AMINO ALCOHOLS

Solid support: Hypercarb. Mobile phase: 0.25 m*M* DCHT in phosphate buffer (pH 2.8) (I = 0.1).

Solute					k'_1	α
	R_1	R_2	R_3	R_4		
1,2	H	CH_3	CH_3	CH_3	0.16	1.04
3,4	H	CH_3	CH_3	H	0.13	1.10
5,6	H	CH_3	CH_3	H	0.15	1.16
7,8	H	CH_3	H	H	0.13	1.22
9	*p*-$NHSO_2CH_3$	H	H	H	0.51	1.41
10	*p*-NO_2	H	H	H	1.13	1.20
11	*m*-NO_2	H	H	H	0.92	1.19
12	*m*-NO_2	CH_3	H	H	1.20	1.17
13	*o*,*p*-di-Cl	H	H	H	7.61	1.42
14					7.69	1.00
15					15.8	1.76

retention (k') is regarded as a retention composed of two different mechanisms, one chiral (k'_{chir}) and one without stereoselectivity (k'_{achir}), the separation factor is given by

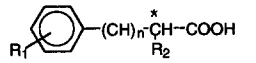
$$\alpha_{+/-} = \frac{k'_{chir(+)} + k'_{achir}}{k'_{chir(-)} + k'_{achir}} \quad (1)$$

The increased retention with increase in pH is the

TABLE IV

SOLUTE STRUCTURE AND STEREOSELECTIVITY FOR ACIDS

Solid support: Hypercarb. Mobile phase as in Table III.

Solute				k'_1	α
	R_1	R_2	n		
16,17	H	OH	1	7.68	1.10
18,19	H	OH	0	2.69	1.08
20	*m*-OH, *p*-OCH_3	OH	0	2.85	1.12
21	H	CH_2OH	0	3.22	>1.0[a]
22,23	H	CH_3	0	25.0	1.00

[a] Two columns.

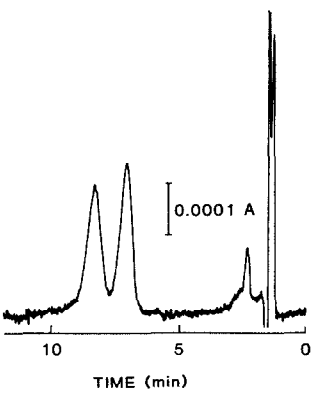


Fig. 1. Resolution of homatropine. Support: Hypercarb. Mobile phase: 0.063 m*M* DCHT and 0.13 *M* isopropanol in phosphate buffer (pH 2.8) (I = 0.1).

result of an increased retention as base. This binding seems to have an achiral character as it gives rise to a lower α and, according to the equation above, this is the effect of an increase in k'_{achir}.

Atropine (**34** and **35**) and homatropine (**32**) were affected differently to the bases discussed above. An increased retention was observed at higher pH, but the stereoselectivity was not affected. This is in accordance with the hypothesis that the nitrogen is not involved in the hydrogen bonding between selector and solute.

The acidic solutes were less retained at higher pH and at the same time a decreased stereoselectivity was observed (Fig. 2). It is likely that is the uncharged acid that takes part in the chiral interaction.

Influence of DCHT concentration. In mobile phases containing DCHT and 0.13 *M* isopropanol in phosphate buffer (pH 3), the retention could be regulated by the concentration of DCHT without a significant effect on the stereoselectivity (Figs. 3 and 4). Only small changes in adsorption of DCHT were obtained within the concentration range studied (see Fig. 5) and it is therefore likely that the amount of DCHT adsorbed was close to a monolayer. Assuming a monolayer, the area occupied by each

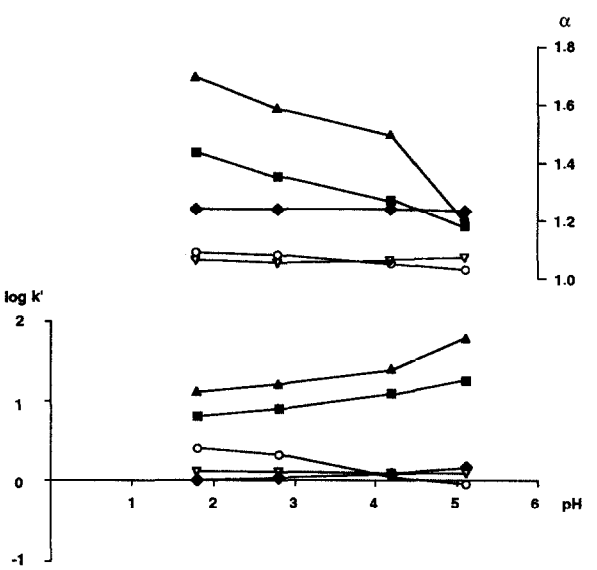


Fig. 2. Influence of pH on the capacity factor for the first-eluted peak (k'_1) and the separation factor (α). Mobile phase: 0.25 m*M* DCHT and 0.13 *M* isopropanol in phosphate buffer (I = 0.1). Solutes: ▲ = racemic 2-amino-1[spiro(cyclopropane-1,1'-inden)-3'-yl]ethanol; ■ = racemic 2-amino-1-(2,4-dichlorophenyl)ethanol; ◆ = homatropine; ▽ = 1-phenyl-1,2-ethanediol; ○ = mandelic acid.

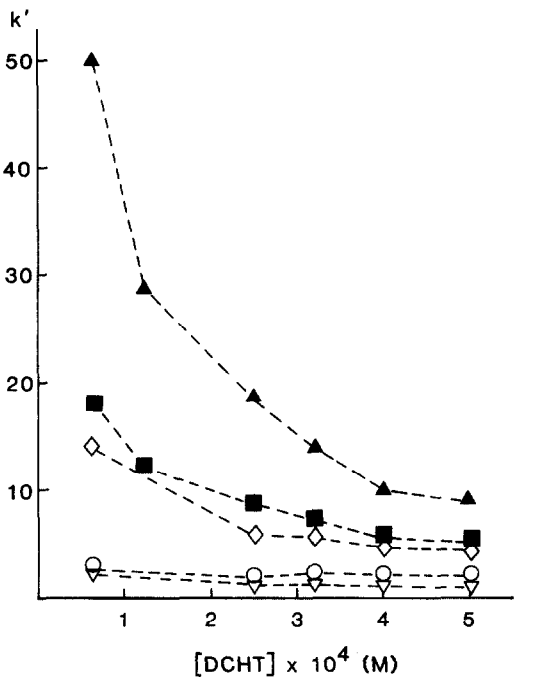


Fig. 3. Influence of DCHT concentration on the capacity factor for the first-eluted enantiomer (k'_1). Mobile phase: DCHT and 0.13 *M* isopropanol in phosphate buffer (pH 2.8) (I = 0.1). Solutes: ▲ = racemic 2-amino-1[spiro(cyclopropane-1,1'-inden)-3'-yl]ethanol; ■ = racemic 2-amino-1-(2,4-dichlorophenyl)ethanol; ◇ = (*R*)-ethanol mandelate; ▽ = (*R*)-1-phenyl-1,2-ethanediol; ○ = (*R*)-mandelic acid.

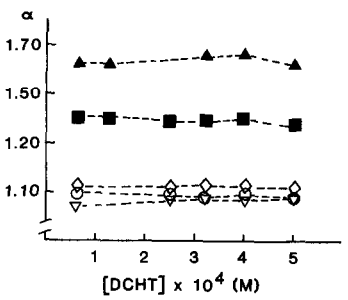


Fig. 4. Influence of DCHT concentration on the separation factor (α). Conditions as in Fig. 3.

molecule of DCHT was around 170 Å^2. Further, the high loading of DCHT even at low concentrations implies a high affinity of DCHT for the graphitic carbon.

However, increased concentrations of DCHT gave a decreased retention (Fig. 3). The process responsible for this decrease has so far not been elucidated, but it seems to be without influence on the ratio between chiral and achiral retention (see eqn. 1) as the separation factor is not altered.

Influence of organic modifier. A low content of a hydrogen donor such as isopropanol or a hydrogen acceptor such as acetonitrile had no significant effect on the amount of DCHT adsorbed on Hypercarb. However, the retention and the stereoselectivity for most of the solutes were affected (see Table VI).

When the concentration of acetonitrile was increased from 0.95 to 3.8 *M*, the amount of DCHT adsorbed was decreased by 60% (Table VI). Simultaneously, a decrease in both retention and stereoselectivity was observed. The lower stereoselectivity is probably due to the smaller amount of DCHT adsorbed, giving a higher proportion of achiral retention (see eqn. 1). On increasing the concentration of DCHT in the mobile phase containing 3.8 *M* acetonitrile, a higher loading of DCHT was obtained (Fig. 6), the retention was lower (Fig. 7) and the stereoselectivity was improved (Fig. 8). These results strongly support the assumption of a negative influence of achiral retention. The highest stereoselectivities observed were still lower than the separation factors achieved with mobile phases without acetonitrile.

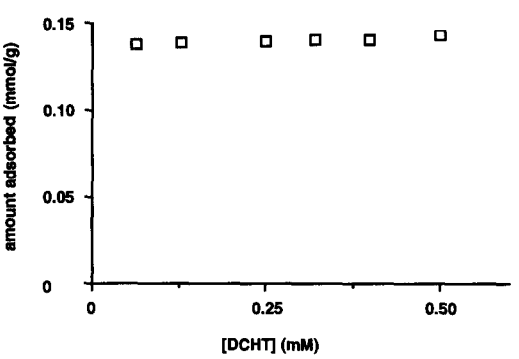
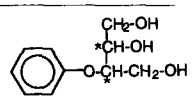
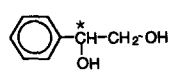
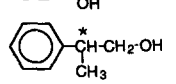
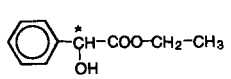


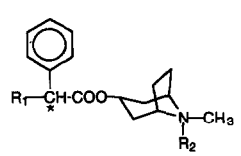
Fig. 5. Influence of DCHT concentration on the adsorption of DCHT. Conditions as in Fig. 3.

TABLE V

SOLUTE STRUCTURE AND STEREOSELECTIVITY FOR APROTIC SOLUTES AND HYDROXY ESTERS

Solid support: Hypercarb. Mobile phase as in Table III.

Solute	Structure	k'_1	α
24,25		3.42	1.01
26,27		1.43	1.08
28,29		14.2	1.0
30,31		6.13	1.13



	R_1	R_2		
32	OH	H	1.21	1.26
33	OH	CH_3	0.87	1.25
34,35	CH_2OH	H	1.42	1.21
36	CH_2OH	CH_3	1.07	1.19
37	CH_2OH	$CH(CH_3)_2$	1.35	1.15

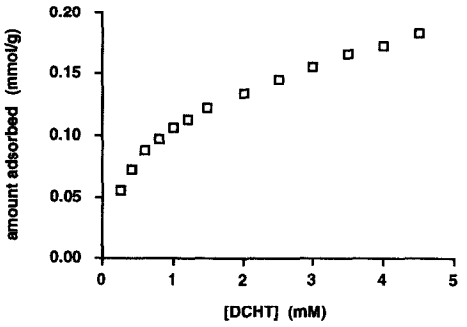


Fig. 6. Influence of DCHT concentration on the adsorption of DCHT with acetonitrile in the mobile phase. Mobile phase: DCHT and 3.6 *M* acetonitrile in phosphate buffer (pH 2.8) (I = 0.1).

Stability and reproducibility of chromatographic systems

The stability of a system with Hypercarb as support was studied for 6 days (1500 column volumes). The capacity factors (Fig. 9) and separation factors (Fig. 10) of the enantiomers of uncharged solutes were stable, whereas a 10–14% increase in retention was observed for the amino alcohols. The prolonged retention is probably due to hydrolysis giving monocyclohexyl tartrate, which may act as a counter ion.

Good agreement for retention and stereoselectivity (Table VII) was observed on applying the chromatographic system to three different batches of graphitic carbon.

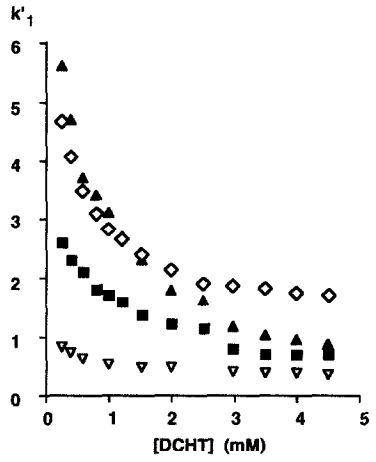


Fig. 7. Influence of DCHT concentration on the capacity factor for the first-eluted enantiomer with acetonitrile in the mobile phase. Conditions as in Fig. 6. Solutes: ▲ = racemic 2-amino-1[spiro(cyclopropane-1,1′-inden)-3′-yl]ethanol; ■ = racemic 2-amino-1-(2,4-dichlorophenyl)ethanol; ◇ = (*R*)-ethyl mandelate; ▽ = (*R*)-1-phenyl-1,2-ethanediol.

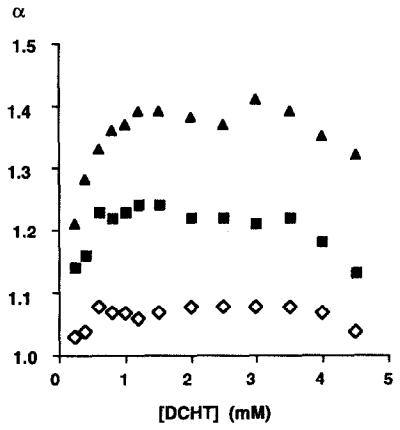


Fig. 8. Influence of DCHT concentration on the stereoselectivity with acetonitrile in the mobile phase. Conditions as in Fig. 6. The α values for 1-phenyl-1,2-ethanol could not be determined at enhanced concentrations of acetonitrile and are therefore not given.

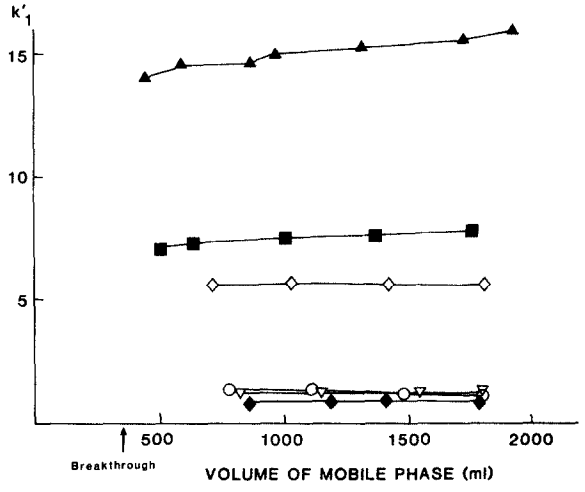
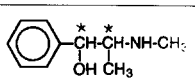
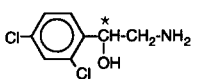
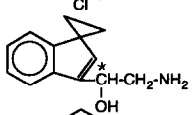
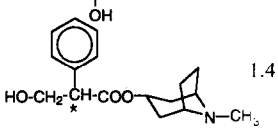
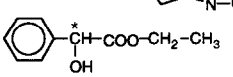
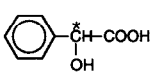
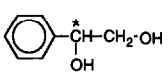


Fig. 9. Stability of chromatographic system: capacity factor (k'_1). Mobile phase: 0.32 m*M* DCHT and 0.13 *M* isopropanol in phosphate buffer (pH 2.8) (I = 0.1). Solutes: ▲ = racemic 2-amino-1[spiro(cyclopropane-1,1′-inden)-3′-yl]ethanol; ■ = racemic-2-amino-1-(2,4-dichlorophenyl)ethanol; ◆ = homatropine; ◇ = (*R*)-ethylmandelate; ▽ = (*R*)-1-phenyl-1,2-ethanediol; ○ = (*R*)-mandelic acid.

TABLE VI

ORGANIC MODIFIERS

Mobile phase: organic modifier and 0.25 m*M* DCHT in phosphate buffer (pH 2.8).

Solute		— (0.13 *M*) 0.137[a]		Isopropanol (0.95 *M*) 0.142[a]		Acetonitrile (1.9 *M*) 0.144[a]		Acetonitrile (3.8 *M*) 0.124[a]		Acetonitrile 0.057[a]	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
5,6		0.15	1.16	0.28	1.13	0.36	1.06	0.27	1.04	0.17	1.00
13		7.6	1.42	8.8	1.39	9.4	1.39	—	—	—	—
15		15.8	1.76	18.7	1.62	14.2	1.57	13.9	1.55	5.5	1.23
34,35		1.4	1.21	1.2	1.21	2.4	1.15	1.9	1.13	1.2	1.0
30,31		6.1	1.13	6.0	1.12	7.0	1.11	6.8	1.10	5.4	1.04
18,19		**2.6**	**1.08**	**1.9**	**1.09**	**1.6**	**1.09**	**1.6**	**1.08**	**1.2**	**1.03**
26,27		1.4	1.08	1.2	1.07	1.3	1.05	1.2	1.02	0.99	1.01

[a] Amount of DCHT adsorbed (mmol/g).

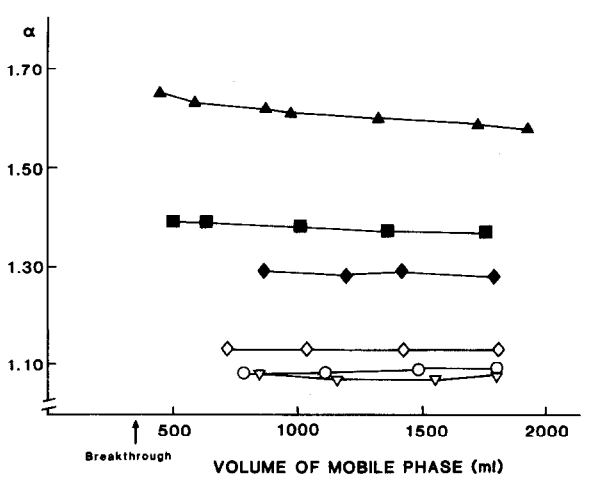


Fig. 10. Stability of chromatographic system: separation factor (α). Conditions as in Fig. 9.

CONCLUSIONS

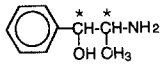
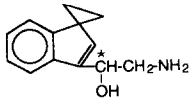
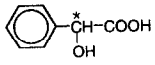
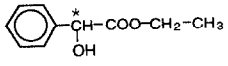
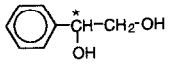
The enantiomers of amino alcohols, acids, hydroxy esters and diols were separated by using DCHT dynamically adsorbed on graphitic carbon. All the separated solutes contained at least two hydrogen-bonding functions. The character of the hydrogen-bonding groups and the presence of bulky groups are important for chiral recognition. For example, the stereoselectivity increased in the order mandelic acid < ethyl mandelate < homatropine.

Among the amino alcohols, where at least one point of interaction is provided by the nitrogen, the separation factor increased with decreasing degree of substitution on the nitrogen, *i.e.*, methylephedrine < ephedrine < norephedrine.

TABLE VII

REPRODUCIBILITY OF CHROMATOGRAPHIC SYSTEM

Mobile phase: 0.25 m*M* DCHT and 1.9 *M* acetonitrile in phosphate buffer (pH 2.8) (I = 0.1).

Solute		Hypercarb column (batch No.)					
		99		100		102	
		k'_1	α	k'_1	α	k'_1	α
7,8		0.22	1.14	0.21	1.14	0.20	1.16
15		14.2	1.56	15.5	1.49	14.5	1.56
18,19		1.8	1.07	1.7	1.08	1.6	1.07
30,31		7.0	1.10	6.6	1.10	6.8	1.10
26,27		1.3	1.04	1.2	1.02	1.2	1.02

ACKNOWLEDGEMENTS

We are grateful to Professor Douglas Westerlund for his interest in this project. This work was supported by the Swedish Natural Science Research Council. Research grants from the Swedish Academy of Pharmaceutical Sciences and the I.F. Foundation for Pharmaceutical Research are gratefully acknowledged.

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