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CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF ACIDS WITH QUININE AS CHIRAL COUNTER ION

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SUMMARY

A study of the separation of enantiomers of carboxylic and sulphonic acids as diastereomeric ion pairs with quinine as the chiral counter ion is presented. Different modified silica adsorbents have been used as stationary phases in order to regulate retention and stereoselectivity. The retention can also be controlled by the concentration of quinine and the acid in the mobile phase as well as by the relative amounts of the solvent components dichloromethane, hexane and pentanol. Stable and reproducible chromatographic systems are obtained with a low content of water (80 ppm) in the mobile phase.

INTRODUCTION

The chromatographic separation of enantiomers (optical isomers) is a subject of great interest in organic chemistry and biological sciences. Methods for the separation and determination of the individual enantiomers are of great importance, especially in the medical field, as it is known that the antipodes can give different effects in the human organism[1].

Different chromatographic techniques have been used for the separation of optical isomers (*cf.*, ref. 2). In liquid chromatography, separation of enantiomers can be effected by a chiral stationary phase as well as by a chiral complexing agent in the mobile phase giving diastereomeric complexes with the substrate. The complex formation can be based on hydrogen bonding[3], metal chelation[4–6] or ion-pair formation[7–10].

In this project, the ion-pair method has been used for the separation of amino alcohols with (+)-10-camphorsulphonic acid as chiral counter ion[7,8], whereas quinine and related cinchona alkaloids have been used for the chiral resolution of carboxylic and sulphonic acids[9]. The cinchona counter ions also have the advantage of improving the detection sensitivity. This paper presents further studies on chiral resolution with quinine and related amines as counter ions. The influence of the mobile phase composition on the retention and stereoselectivity has been studied in some detail. Different solid phases have been investigated with respect to retention and stereoselectivity. It is also shown that stable and reproducible chromatographic systems can be obtained by regulating the water content of the mobile phase.

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EXPERIMENTAL

Apparatus

The detector was an LDC Spectromonitor III, operating at 337 nm. The pump was an LDC ConstaMetric III and the injector a Rheodyne Model 7120 with a 20-μl loop.

The columns were of stainless steel with a polished inner surface, equipped with modified Swagelok connectors and Altex stainless-steel frits (2 μm). The column length was 100 or 150 mm and the I.D. was 3.0 mm. A water-bath, HETO Type 02 PT 923 TC (Birkerød, Denmark), was used to thermostat the column and the solvent reservoir.

The UV-absorbance spectra were recorded with an ACTA MIV spectrophotometer (Beckman) whereas the quantitative absorbance measurements were made with a Zeiss PMQ II Spectrophotometer.

Chemicals and reagents

LiChrosorb DIOL (5 μm), LiChrosorb Si 100 (5 μm) and LiChrospher Si 500 (10 μm) were obtained from E. Merck (Darmstadt, F.R.G.). Nucleosil CN (5 μm) was from Macherey-Nagel (Düren, F.R.G.).

Dichloromethane (LiChrosolv), 1-pentanol p.a. and glacial acetic acid GR were also obtained from Merck. Hexane (HPLC grade) was from Rathburn Chemicals (U.K.) and 1,2-butanediol was from Janssen Chimica (Belgium). Dichloromethane and hexane were freed from water by molecular-sieve treatment before use[7].

(−)-Di-O,O-*p*-toluoyl-L-tartaric acid, (+)-α-methoxy-α-trifluoromethylphenylacetic acid, (−)-α-methoxy-α-trifluoromethylphenylacetic acid, (+)-mandelic acid and racemic 10-camphorsulphonic acid were from Fluka (Buchs, Switzerland). (+)-10-Camphorsulphonic acid, (+)-N-(1-phenylethyl)phthalamic acid, (−)-N-(1-phenylethyl)phthalamic acid and quinine were obtained from Merck.

Racemic 2-phenoxypropionic acid, 2-(*o*-chlorophenoxy)propionic acid, 2-(*m*-chlorophenoxy)propionic acid, 2-(*p*-chlorophenoxy)propionic acid as well as quinine monohydrochloride were from Janssen Chimica. Racemic 2-(*p*-iodophenoxy)propionic acid, (+)-2-(*p*-bromophenoxy)propionic acid and (−)-2-(*p*-bromophenoxy)-propionic acid were supplied by the Department of Organic Pharmaceutical Chemistry, Uppsala University, Sweden. (+)-2-Phenoxypropionic acid was a gift from A. Collet at College de France (Paris, France).

N-*tert.*-Butoxycarbonyl-D-phenylalanine, N-*tert.*-butoxycarbonyl-L-phenylalanine, N-benzoxycarbonyl-D-phenylalanine, N-benzoxycarbonyl-L-phenylalanine, Dns-D-phenylalanine, Dns-L-phenylalanine, N-benzoxycarbonyl-D,L-valine, N-benzoxycarbonyl-L-valine, Dns-D,L-valine and Dns-L-valine were from Sigma. Quinine ethyl carbonate was from ICN-K & K Lab. (U.S.A.), (−)-10-Camphorsulphonate ammonium salt was from Aldrich. Naproxene [2-(6-methoxy-2-naphthyl)propionic acid] in (+) and (−) forms was kindly supplied by Astra (Södertälje, Sweden). 2,4-Dinitrophenol was from Vitrum (Sweden). It was recrystallized from ethanol. All other substances were of analytical or reagent grade and used without further purification.

Column preparation and chromatographic technique

The columns were packed by a slurry technique, using chloroform (LiChrosorb DIOL and Nucleosil CN) or dichloromethane–methanol (1:1) (LiChrosorb Si 100 and LiChrospher Si 500) as suspending liquid. They were tested before use with hexane–*n*-butanol (199:1) as eluent and 2,4-dinitrophenol and 2-phenylethanol as test solutes. Only columns giving a reduced plate height, $h = H/d_p$, where H = plate height and d_p = particle diameter), of less than 10 and asymmetry factors of less than 1.5 were used.

The columns were washed with water-free methanol, ethyl acetate and dichloromethane before introducing the mobile phase. After the breakthrough of the counter ion (measured by the UV detector), the system was arranged for recirculation with 300 ml of mobile phase in the reservoir.

Mobile phases containing quinine as hydrochloride were prepared from the monohydrochloride salt, whereas the other mobile phases were prepared by dissolving the acid and quinine as base in the mobile phase. In the studies with different water contents, the mobile phases were prepared by mixing dry and water-saturated dichloromethane. The water content in dry dichloromethane was determined by Karl Fischer titration to be less than 30 ppm. The water-saturated dichloromethane contains 1980 ppm at 25°C[11]. Before introducing a mobile phase with a higher water content, the column was washed with 150 ml of dichloromethane having the same water content as the mobile phase.

RESULTS AND DISCUSSION

The separation is based on the formation of diastereomeric ion pairs with a chiral counter ion, present in the mobile phase. The diastereomeric ion pairs are separated with conventional (non-chiral) solid phases by using an organic mobile phase of low polarity in order to promote a high degree of ion-pair formation. The ion-pair formation of the solute will also be affected by the concentration and strength of other protolytes present in the mobile phase.

Ion-pair formation in the mobile phase

When an acid and an amine react in an aprotic solvent of low polarity they can form an ion pair with electrostatic attraction and/or hydrogen bonds between the components. The acid–base reactions in such solvents have been studied with different techniques[12]. In this study, the ion-pair formation constants were determined by a spectrophotometric method, based on the use of an indicator acid, 2,4-dinitrophenol, with different absorbances as the acid and ion pair, which competes with the substrate acid for the amine. The acids are assumed to react with the amine as follows:

$$\text{HA (acid)} + \text{B (base)} \rightleftharpoons \text{HAB (ion pair)} \quad (1)$$

The reaction can be quantified by use of the equilibrium constant for the process, K_{HAB}.

The absorption spectra of 2,4-dinitrophenol as the acid and as the ion pair with triethylamine as well as of quinine as base are given in Fig. 1. The measurements

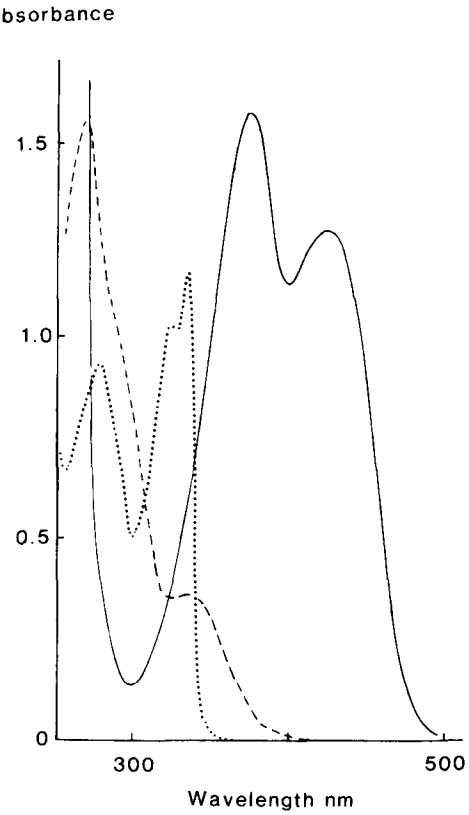


Fig. 1. Absorption spectra: ———, 0.104 m*M* 2,4-dinitrophenol and 0.037 *M* triethylamine; ---------, 0.104 m*M* 2,4-dinitrophenol; ....., 0.204 m*M* quinine. Solvent: dichloromethane (dry)–1-pentanol (199:1).

were performed at 418 nm, where only the ion pair of 2,4-dinitrophenol has significant absorbance within the concentration range studied. The ion-pair formation constant of 2,4-dinitrophenol (HA′) with quinine in dichloromethane–1-pentanol (199:1) was determined by plotting the reciprocal of the absorbance *versus* the reciprocal of the free amine concentration at a constant concentration of 2,4-dinitrophenol according to eqn. 2[13]

$$\frac{1}{Z} = \frac{1}{K_{HA'B}\varepsilon[B]C_{HA'}} + \frac{1}{\varepsilon C_{HA'}} \qquad (2)$$

where Z is the absorbance of the ion pair, $K_{HA'B}$ the ion-pair formation constant, [B] the concentration of quinine as free base, $C_{HA'}$ the initial concentration of 2,4-dinitrophenol and ε the molar absorptivity of the ion pair. The ion pair formation constant and the molar absorptivity were evaluated by linear regression analysis according to eqn. 2, which gave log ε = 3.92 and log $K_{HA'B}$ = 4.41. Previous studies of ion-pair formation between 2,4-dinitrophenol and tertiary amines in chloroform[13] have given log K_{HAB} values between 3.4 and 4.2, depending on the alkyl chain length.

The interaction of the indicator acid (HA') and the substrate acid (HA'') with quinine according to eqn. 1 can be expressed by the equilibrium constants $K_{HA'B}$ and $K_{HA''B}$:

$$K_{HA'B} = \frac{[HA'B]}{[HA'][B]} \tag{3}$$

$$K_{HA''B} = \frac{[HA''B]}{[HA''][B]} \tag{4}$$

From eqns. 3 and 4 and expressions for the total concentrations of the indicator acid, the substrate acid and the base, the following expression can be derived and used in the calculation of $K_{HA''B}$, *cf.*, ref. 14

$$K_{HA''B} = \frac{X - Y}{Y[C_{HA''} - (X - Y)]} \tag{5}$$

where $X = (C_{HA'}/L)(nL-Z)$ and $Y = (1/K_{HA'B})(Z/L-Z)$. Z is the absorbance at 418 nm, $C_{HA'}$ is the concentration of the indicator acid, 2,4-dinitrophenol, and $C_{HA''}$ that of the substrate acid, L is the limiting absorbance for the reaction of HA' with B and n is the molar concentration ratio between the base and the indicator acid.

Results from studies of the ion-pair formation of quinine with different acids are given in Table 1. At present, it is not possible to decide whether the variations in the results are due to the influence of side reactions or to low precision in the experimental and evaluation procedures. The precision can be illustrated by the fact that the maximum variation between four parallel determinations of the constants for acetic acid was 0.2 log units. The constants for the stronger acids in Table I are rather uncertain, but the found values give the approximate magnitude of the constants.

The ion-pair formation constant is considerably lower for acetic acid than for the other acids, which means that a higher fraction of the quinine is present as free amine when acetic acid is the acidic component in the mobile phase, conditions in other respects being the same. The use of a strong acid in the mobile phase might decrease the interaction between quinine and the enantiomers of a weaker substrate acid and give rise to a lower stereoselectivity.

Regulation of retention

The distribution of a solute as an ion pair or acid between the mobile and stationary phases is dependent on the concentrations of the counter ion and the acid present in the mobile phase, as they will affect the ion-pair formation of the solute. The retention of the solute will also be affected by the adsorption of the chiral counter ion as base and ion pair with the mobile phase acid.

Studies of the influence of the concentration of the chiral counter ion, quinine, were carried out in systems with constant acetic acid content in the mobile phase. The retention of the substrate acids decreases with increasing concentration of qui-

TABLE I

CONSTANTS FOR ION-PAIR FORMATION BETWEEN QUININE AND ACIDS

Solvent: dichloromethane (dry)–1-pentanol (199:1). Temperature: 25.0°C. Cell: 1.0 cm. Indicator acid: $1.001 \cdot 10^{-4}$ *M* 2,4-dinitrophenol. Wavelength: 418 nm.

	Quinine ($M \times 10^4$)	*Absorbance**	*log* $K_{HA''B}$
Acetic acid ($M \times 10^4$)			
0.541	0.501	0.259	3.6
1.037	0.500	0.247	3.6
1.037	1.001	0.426	3.5
2.075	1.001	0.359	3.8
2.075	2.002	0.558	3.8
N-tert.-Butoxycarbonyl-L-phenylalanine ($M \times 10^4$)			
0.500	0.500	0.114	5.5
0.500	1.000	0.329	5.1
1.000	0.500	0.050	5.5
1.000	1.000	0.164	5.5
1.000	2.000	0.516	4.9
2.000	1.000	0.057	5.5
2.000	2.000	0.233	5.5
(+)-10-Camphorsulphonic acid ($M \times 10^4$)			
0.250	1.004	0.388	5.6
0.501	1.505	0.494	4.9
1.001	2.002	0.473	5.6

* Absorbances below 0.2 are calculated from measurements with a 5-cm cell.

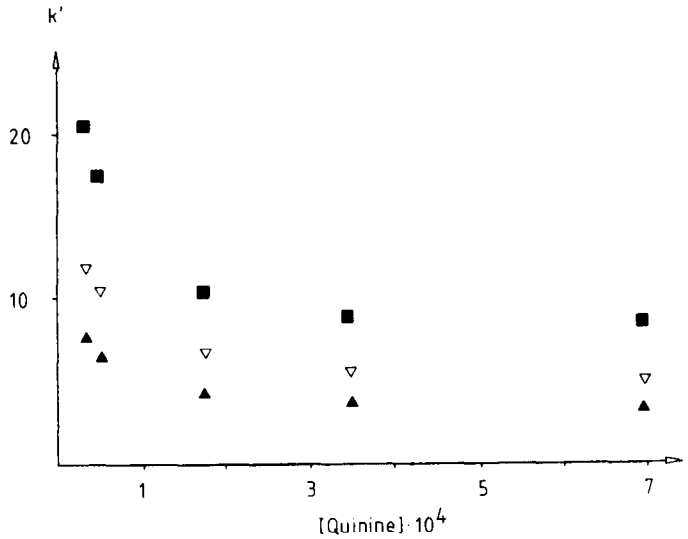


Fig. 2. Influence of counter ion concentration on retention. Solid phase: LiChrosorb DIOL. Mobile phase: quinine in dichloromethane (80 ppm water)–1-pentanol (199:1) containing 0.18 m*M* acetic acid. ■, (−)-10-Camphorsulphonic acid; ▽, L-N-*tert.*-butoxycarbonyl-phenylalanine; ▲, (−)-α-methoxy-α-trifluoromethylphenylacetic acid.

nine (Fig. 2), but it seems to reach a limiting value at higher concentrations of the counter ion. This indicates that the retention is not due to a simple Langmuir adsorption of ion pairs.

A further illustration of the influence of the solid phase is given by the adsorption isotherm of quinine, evaluated from the breakthrough volume in a series of chromatographic systems with different concentrations of quinine in the mobile phase. The reciprocal plot of the adsorption isotherm (Fig. 3) is non-linear, which shows a deviation from a simple Langmuir adsorption. This might be due to the fact that quinine can be adsorbed both as a base and as an ion pair with acetic acid, and the relative concentration of these species in the mobile phase changes with the quinine concentration since acetic acid is present in constant, fairly low concentration. The presence of different adsorption sites on the stationary phase can also be of importance.

Influence of the acid in the mobile phase

Addition of acid to the mobile phase presents further means for regulating the retention of the solutes in the chromatographic system. The influence of different acids in the mobile phase on the capacity factor and the stereoselectivity of 10-camphorsulphonic acid is shown in Table II. The retention of the solutes increases when a more hydrophobic acid is used in the mobile phase, *cf.*, acetic acid and di-*p*-tol-

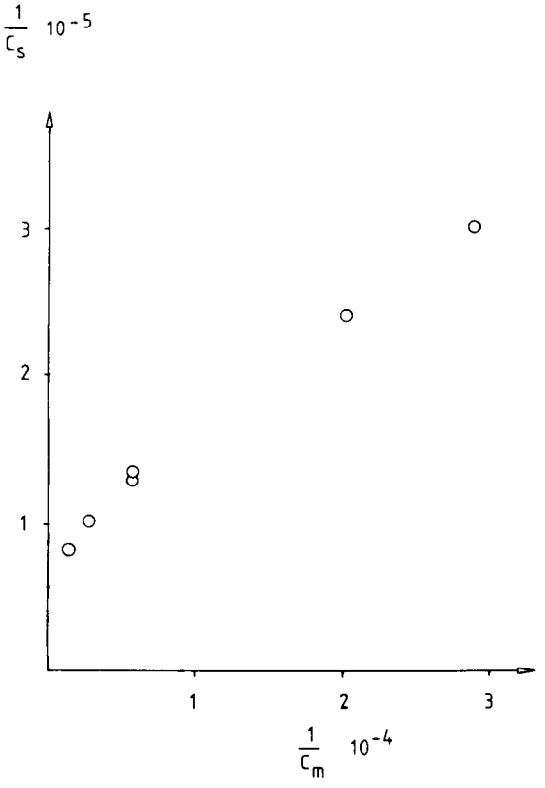


Fig. 3. Reciprocal plot of the adsorption isotherm of quinine with C_s (mol/g) and C_m (mol/l) as the concentrations in the solid and mobile phase, respectively. Solid phase and mobile phase as in Fig. 2.

TABLE II

INFLUENCE OF ACIDS IN THE MOBILE PHASE

Solid phase: LiChrosorb DIOL. Mobile phase: acid (HA) in dichloromethane (dry)–1-pentanol (99:1) containing 0.35 m*M* quinine. Sample: (+/−)-10-camphorsulphonic acid. $\alpha_{+/-} = k'_+/k'_-$.

Acid (HA)	C_{HA} *(mM)*	k'_+	$\alpha_{+/-}$
Hydrochloric	0.33	9.1	1.33
Mandelic	0.35	9.5	1.42
Di-*p*-toluoyltartaric	0.35	23.4	1.30
Acetic	0.35	8.5	1.41

TABLE III

INFLUENCE OF ACETIC ACID CONCENTRATION ON RETENTION AND STEREOSELECTIVITY

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid (HA) in dichloromethane (80 ppm water)–1-pentanol (199:1). $\alpha = k'_2$ (second eluted enantiomer)/k'_1 (first eluted enantiomer).

Compound	C_{HA} *(mM)*					
	0.035		*0.35*		*3.5*	
	k'_1	α	k'_1	α	k'_1	α
10-Camphorsulphonic acid	11.0	1.52	9.14	1.49	8.86	1.49
α-Methoxy-α-trifluoromethylphenylacetic acid	4.66	1.15	3.88	1.15	3.46	1.14
N-*tert.*-Butoxycarbonylphenylalanine	7.45	1.33	5.74	1.30	4.43	1.19

TABLE IV

INFLUENCE OF ACID ON RETENTION AND STEREOSELECTIVITY

Solid phase: LiChrosorb DIOL. Mobile phase: acid (HA) in dichloromethane (dry)–1-pentanol (199:1) containing 0.35 m*M* quinine.

Compound	*0.35 mM HCl*		*0.15 mM HCl*		*0.35 mM acetic acid*	
	k'_1	α	k'_1	α	k'_1	α
10-Camphorsulphonic acid	9.23	1.63	6.35	1.58	9.48	1.55
α-Methoxy-α-trifluoromethylphenylacetic acid	4.51	1.14	2.86	1.16	3.49	1.15
N-(1-Phenylethyl)phthalamic acid	7.93	1.07	9.97	1.16	10.2	1.14
N-*tert.*-Butoxycarbonylphenylalanine	5.68	1.11	4.40	1.29	4.82	1.31

uoyltartaric acid. The effect of the acid on the capacity factors of the enantiomers might be due to competing interaction with quinine in the mobile as well as in the stationary phase. The relatively small effect on the retention of the solutes when the acetic acid concentration is changed (Table III), and the fact that the retention slightly increases when the concentration of hydrochloric acid is increased (Table IV) indicate a rather complicated retention mechanism.

The stereoselectivity is also influenced by the nature and the concentration of the acid in the mobile phase, possibly due to a competing interaction with quinine, affecting the interaction with the chiral reagent. The results in Tables III and IV show that the stereoselectivity for two of the solutes increases when the strength and concentration of the mobile phase acid decreases, making a higher concentration of the chiral complexing agent available for interaction with the enantiomeric solutes. However, the fact that the solutes are influenced differently might indicate a more complex background for the improvement of the stereoselectivity.

Influence of solvent components

In systems of quinine ethyl carbonate as counter ion, the retention is low for hydrophobic solutes when dichloromethane is the main solvent in the mobile phase, but an increase can easily be obtained by addition of a non-polar modifier such as *n*-hexane (Table V). 1-Pentanol must be present in the mobile phase to obtain good efficiency and peak symmetry. The change of the stereoselectivity when *n*-hexane is introduced in the mobile phase is hardly significant.

TABLE V

INFLUENCE OF *n*-HEXANE IN THE MOBILE PHASE

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine ethyl carbonate and acetic acid in solvent. Sample: (+/−)-10-camphorsulphonic acid.

	Solvent: n-hexane–dichloromethane (dry)–1-pentanol			
	0:100:0	*0:99:1 (v/v)*	*10:89:1 (v/v)*	*50:49:1 (v/v)*
k'_1	2.28	1.68	2.03	10.5
$\alpha_{+/-}$	1.28	1.28	1.30	1.31

The eluting power of the mobile phase can be increased by a polar modifier like 1-pentanol[7–9]. Addition of more hydrophilic modifiers may be necessary when silica is used as adsorbent. A separation of the enantiomers of N-(1-phenylethyl) phthalamic acid with butanediol as modifier is shown in Fig. 4. The content of the hydrophilic modifier should be low, preferably less than 1%, since higher concentrations will decrease the stereoselectivity, especially for solutes with strongly hydrogen-bonding substituents[7,9].

The water content of the mobile phase will also have an effect on retention and chiral selectivity (Table VI). A water content of less than 30 ppm might be preferable for selectivity purposes, but the system requires a long equilibration time. Eighty ppm of water give a limited decrease in selectivity, and the system has the advantage of giving constant retention soon after breakthrough of the counter ion.

Influence of the solid phase

The retention of the solutes can also be affected by the nature of the stationary phase. Results with solid phases having different surface properties are given in Table VII. The unmodified silica LiChrospher Si 500 gives as expected the highest retention,

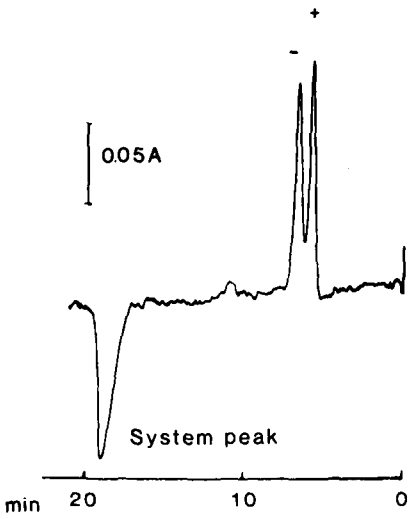


Fig. 4. Separation of enantiomers of N-(1-phenylethyl)phthalamic acid. Solid phase: LiChrosorb Si 100. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (dry)–1,2-butanediol (99:1).

TABLE VI

INFLUENCE OF WATER CONTENT IN THE MOBILE PHASE

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (with different water contents)–1-pentanol (199:1).

Compound	*Water content (ppm)*					
	30		*80*		*220*	
	k'_1	α	k'_1	α	k'_1	α
10-Camphorsulphonic acid	9.48	1.55	7.34	1.48	7.71	1.40
α-Methoxy-α-trifluoromethylphenylacetic acid	3.49	1.15	3.35	1.14	3.34	1.13
N-(1-Phenylethyl)phthalamic acid	10.2	1.14	9.39	1.15	9.77	1.12
N-*tert.*-Butoxycarbonylphenylalanine	4.82	1.31	4.61	1.34	4.89	1.27

TABLE VII

INFLUENCE OF THE SOLID PHASE

Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (dry)–1-pentanol (99:1).

Compound	*LiChrosorb DIOL*		*Nucleosil CN*		*LiChrospher Si 500*	
	k'_1	α	k'_1	α	k'_1	α
10-Camphorsulphonic acid	5.58	1.43	3.03	1.25	–	–
2-Phenoxypropionic acid	4.80	1.32	4.40	1.12	–	–
Naproxene	3.21	1.00	1.56	1.07*	14.1	1.09
α-Methoxy-α-trifluoromethylphenylacetic acid	2.18	1.15	0.77	1.14*	9.40	1.00

* 0.5% 1-pentanol.

whereas the DIOL phase gives somewhat higher retention than the nitrile phase. More interesting than the retention differences is the fact that the solid phases give different stereoselectivities when the same mobile phase composition is used. Naproxene, with a naphthalene ring but no other hydrogen-accepting groups in the vicinity of the chiral centre, cannot be separated into enantiomeric forms on LiChrosorb DIOL, while stereoselective retention of the enantiomers was obtained on silica. On the other hand, it is not possible to separate the enantiomers of α-methoxy-α-trifluoromethylphenylacetic acid on silica, whereas stereoselective retention was found on the DIOL and the nitrile phase. The difference in stereoselectivity on the solid phases indicates that the chiral resolution is due to different access of the diastereomeric ion pairs or the enantiomeric acids to the retaining functions on the surface.

Influence of the structure of the chiral counter ion

In the previous study with cinchona alkaloids as chiral counter ions[9] it was shown that small changes in the counter ion structure can have a drastic effect on the resolution of the enantiomers. Quinine ethyl carbonate, which has an ethyl ester function whereas quinine has an hydroxy group, is devoid of hydrogen-donating properties at the chiral centre C-9 and gives a significantly lower stereoselectivity than quinine in the examples shown in Table VIII. For one of the acids there is even a complete loss of stereoselectivity.

The loss of the hydroxy group in the chiral counter ion also gives rise to a significant decrease of the retention of the solutes. The k' value of 10-camphorsulphonic acid is four times lower with quinine ethyl carbonate than with quinine, and the separation of the enantiomers can be completed within less than 80 sec (Fig. 5).

Influence of sample structure

The separations of the diastereomeric ion pairs between the enantiomeric solutes and the chiral counter ion are dependent on the properties of the ion-pair components and the binding between them.

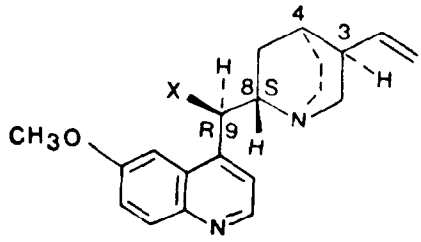
The separation of enantiomeric derivatives of 2-phenoxypropionic acid, Table IX, indicates that substituents in the aromatic ring can affect the stereoselectivity, although they are situated far from the chiral centre. The effect of the substituents is probably due to steric effects since the differences in acidic properties are very small. Introduction of a chloro atom in phenoxyacetic acid gives a change of less than 0.12 pK_a units[15] and the same is probably valid for the derivatives of 2-phenoxypropionic acid.

The effect of substituents in the vicinity of the chiral centre is highly dependent on the structure of the acid. Results obtained with different types of N-substituted derivatives of phenylalanine and valine are given in Table X. The differences in stereoselectivity for the N-benzoxycarbonyl, N-*tert.*-butoxycarbonyl and Dns derivatives of phenylalanine are small, whereas there is a significant increase in stereoselectivity when the benzoxycarbonyl group is exchanged for a dansyl group in valine. The resolution of the enantiomers of valine as their Dns derivatives is demonstrated in Fig. 6.

TABLE VIII

INFLUENCE OF COUNTER ION STRUCTURE ON STEREOSELECTIVITY

Solid phase: LiChrosorb DIOL. Mobile phases: A, 0.35 m*M* quinine and acetic acid in dichloromethane (dry)–1-pentanol (99:1). B, 0.35 m*M* quinine ethyl carbonate and acetic acid in dichloromethane (dry)–*n*-hexane–1-pentanol (49:50:1).



	X
quinine	OH
quinine ethyl carbonate	$OCOOC_2H_5$

Compound	*A*		*B*	
	k'_1	α	k'_1	α
10-Camphorsulphonic acid	5.6	1.43	10.5	1.31
α-Methoxy-α-trifluoromethylphenylacetic acid	2.2	1.14	1.7	1.09
N-(1-Phenylethyl)phthalamic acid	6.9	1.14	12.9	1.01
N-*tert.*-Butoxycarbonylphenylalanine	3.6	1.27	5.0	1.00

TABLE IX

STEREOSELECTIVE SEPARATION OF 2-PHENOXYPROPIONIC ACID DERIVATIVES

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (80 ppm water).

Solute	k'_1	α
2-Phenoxypropionic acid	7.49	1.55
2-(*o*-Chlorophenoxy)propionic acid	6.69	1.54
2-(*m*-Chlorophenoxy)propionic acid	6.65	1.44
2-(*p*-Chlorophenoxy)propionic acid	6.85	1.43
2-(*p*-Bromophenoxy)propionic acid	6.81	1.46
2-(*p*-Iodophenoxy)propionic acid	6.65	1.50

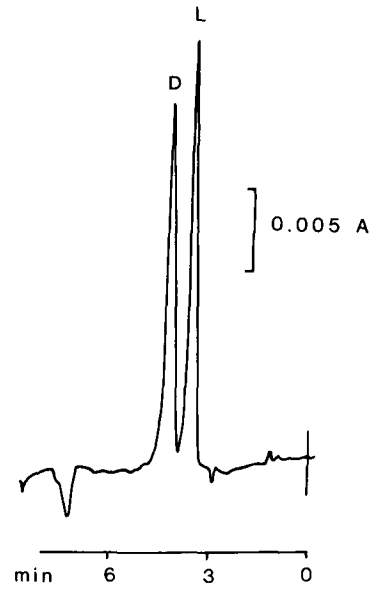


Fig. 5. Separation of enantiomers of 10-camphorsulphonic acid. Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine ethyl carbonate and acetic acid in dichloromethane (dry)–1-pentanol (99:1).

Fig. 6. Resolution of D,L-Dns-valine. Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (dry)–1-pentanol (99:1).

Stability and reproducibility of the chromatographic system

A chromatographic system with a mobile phase of low polarity is usually very sensitive to changes in the content of the ubiquitous water, and a change of the water content can affect both the retention of the solutes and the selectivity[16]. In the previous studies where the chiral counter ion was dissolved in dry dichloromethane (< 30 ppm water) with a low concentration of 1-pentanol, it was found that the capacity factors decreased during the 2–4 days needed for the stabilization of the system[8]. This was probably due to slow attainment of the distribution equilibrium of water between the mobile and the stationary phase. A chromatographic system with a mobile phase containing about 80 ppm of water is stable within less than 1 h after breakthrough of the mobile phase. The retention properties are also stable, as illustrated in Fig. 7: the change in capacity and separation factors was found to be less than 5% during the 7 days the system was studied.

The reproducibility of the chromatographic systems is good, as illustrated in Fig. 8, which shows chromatograms obtained in three different systems. The two upper chromatograms were obtained on the same column and with mobile phases of the same composition but on two different occasions. Between the two experiments, the column was used with mobile phases containing different kinds of counter ions. The retention times and stereoselectivity are almost identical. The reproduction of

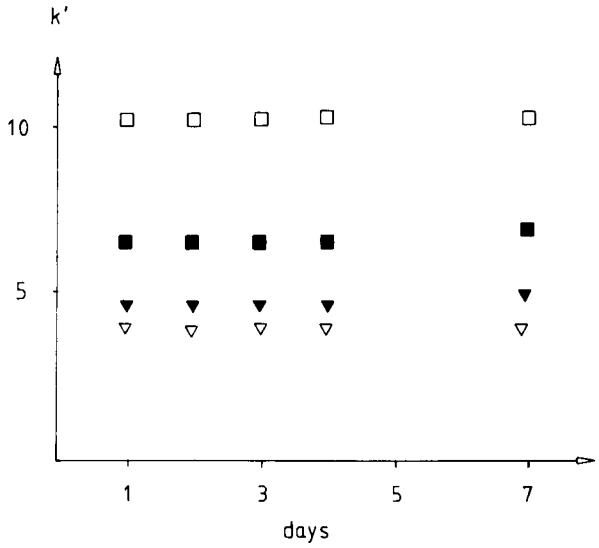


Fig. 7. Stability of chromatographic system. Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (80 ppm water). Samples: ■ □, (+, −)-2-phenoxypropionic acid; ▼ ▽, (+, −)-α-methoxy-α-trifluoromethylphenylacetic acid.

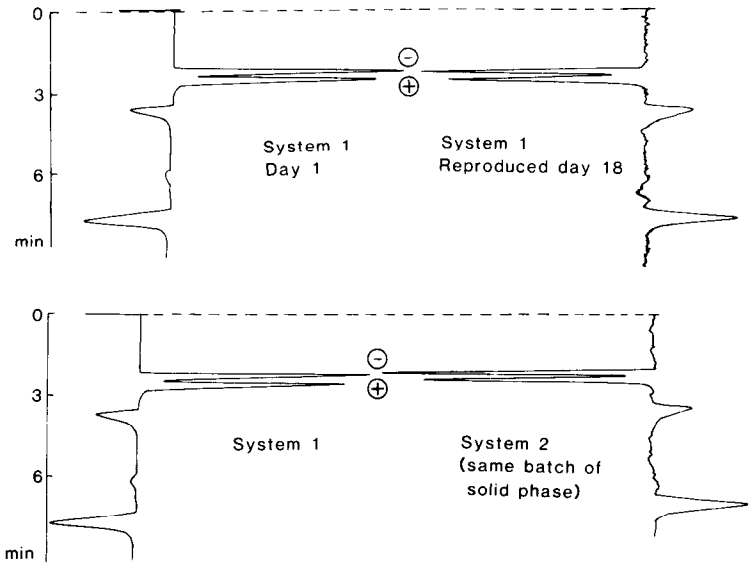


Fig. 8. Reproducibility of chromatographic system. Solid phase: LiChrosorb DIOL. Mobile phase: 0.18 m*M* quinine and acetic acid in dichloromethane (80 ppm water)–1-pentanol (199:1). Sample: (+,−)-α-methoxy-α-trifluoromethylphenylacetic acid.

TABLE X

STEREOSELECTIVE SEPARATION OF AMINO ACID DERIVATIVES

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 m*M* quinine and acetic acid in dichloromethane (dry)–1-pentanol (99:1). $\alpha_{D/L} = k'_D/k'_L$.

Compound	$\alpha_{D/L}$
N-*tert.*-Butoxycarbonylphenylalanine	1.27
N-Benzoxycarbonylphenylalanine	1.26
Dns-Phenylalanine	1.34
N-Benzoxycarbonylvaline	1.08
Dns-Valine	1.28

the chromatographic system on another column is illustrated in the lower two chromatograms in Fig. 8. The two columns were packed with the same batch of support, and the retention time as well as the stereoselectivity differed by less then 5%.

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