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CHROMSYMP. 107

CHIRAL RESOLUTION OF CARBOXYLIC AND SULPHONIC ACIDS BY ION-PAIR CHROMATOGRAPHY

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SUMMARY

Enantiomers (optical isomers) of carboxylic and sulphonic acids have been separated in a chromatographic system having a chiral counter ion in an organic solvent as mobile phase and a surface-modified, polar silica (LiChrosorb DIOL) as adsorbing stationary phase. Separation factors of 1.3–1.5 for the enantiomers of 10-camphorsulphonic acid and moderately hydrophobic carboxylic acids have been obtained with quinine as chiral counter ion. With UV-absorbing counter ions, such as quinine and quinidine, even non-UV-absorbing acids give a response in the UV detector. The detector response and the stereoselectivity can be regulated by polar additives, *e.g.*, 1-pentanol, and it will also depend on the anion in the mobile phase. The influence of solute and counter ion structure on the stereoselectivity is discussed. Applications of the chromatographic systems to practical problems are presented.

INTRODUCTION

The chromatographic separation of enantiomeric compounds has developed rapidly during the last years, stimulated by, *e.g.*, the need for such methods in biomedical science. Indirect resolution of enantiomers as diastereomeric derivatives and the use of chiral stationary phases that interact differently with the antipodes are common procedures in gas and liquid chromatography[1]. In liquid chromatography it is also possible to make use of chiral additives in the mobile phase to promote the separation of enantiomers. Resolution of optical isomers has been obtained with chiral metal chelates[2–5], albumin[6], optically active crown-ethers[7] and chiral counter ions[8–11].

In a previous study[11], it was briefly demonstrated that enantiomers of 10-camphorsulphonic acid could be separated by ion-pair chromatography on a hydrophilic adsorbent with a chiral cation, (+)-alprenolol, in the organic mobile phase. This paper presents further studies by the same principle with cinchona alkaloids (quinine, quinidine and cinchonidine) as chiral counter ions for the separation of enantiomers of, *e.g.*, different types of carboxylic acids. The ion-pair chromatographic technique has the further advantage of offering possibilities for the improvement of detection[12]. With highly UV-absorbing counter ions, even non-UV-absorbing

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acids and anions will give a response in the UV detector and can be followed in low concentrations.

Our studies have concentrated on the influence of mobile-phase composition on retention, stereoselectivity and UV response and on the relationship between stereoselectivity and molecular structure of the solute and the counter ion.

EXPERIMENTAL

Apparatus

The detector was an LDC Spectromonitor III, set at 337 nm unless otherwise stated. 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 I.D. 3.0 mm. A water-bath, HETO Type 02 PT 923 TC (Birkerød, Denmark), was used to thermostat the solvent reservoir, column and injector.

Chemicals and reagents

Dichloromethane (LiChrosolv) was obtained from E. Merck and was freed of water by molecular-sieve treatment before use[10]. 1-Pentanol and glacial acetic acid GR, were also obtained from Merck.

Racemic 10-camphorsulphonic acid, 2-oxo-3-bornanecarboxylic acid (3-camphorcarboxylic acid), tropic acid, 2-methoxymandelic acid, 3-methoxymandelic acid, 2-phenylbutyric acid and 2-methylbutyric acid, as well as (+)-mandelic acid, (−)-mandelic acid, *R*(−)-α-methoxyphenylacetic acid [(−)-O-methylmandelic acid], *R*(+)-α-methoxyphenylacetic acid [(+)-O-methylmandelic acid], *R*(+)-α-methoxy-α-trifluoromethylphenylacetic and *S*(−)-α-methoxy-α-trifluoromethylphenylacetic acid were obtained from Fluka. (−)-10-Camphorsulphonate ammonium salt was from Aldrich Chemical Company; (−)-cinchonidine, (+)-quinidine monohydrate, as well as racemic 2-phenoxypropionic acid, atrolactic acid hemihydrate and 2-phenylpropionic acid from Janssen Chimica; (−)-quinine, (+)-N-(1-phenylethyl)phthalamic acid, (−)-N-(1-phenylethyl)phthalamic acid and (+)-10-camphorsulphonic acid from E. Merck; (−)-quinine hydrochloride from Carl Roth; (−)-α-bromocamphor-8-sulphonate ammonium salt and (+)-α-bromocamphor-8-sulphonate ammonium salt from EGA. (+)-Naproxen and (−)-naproxen were kindly supplied by Astra (Södertälje, Sweden). N-*tert.*-Butoxycarbonyl-D-phenylalanine, N-*tert.*-butoxycarbonyl-L-phenylalanine, N-carbobenzoxy-D,L-leucine, N-carbobenzoxy-L-leucine, N-carbobenzoxy-D-phenylalanine, N-carbobenzoxy-L-phenylalanine, N-carbobenzoxy-D,L-valine and N-carbobenzoxy-L-valine were obtained from Sigma. (+)-Alprenolol chloride was kindly supplied by Hässle (Mölndal, Sweden). All other substances and solvents were of analytical or reagent grade and used without further purification.

Chromatographic systems

LiChrosorb DIOL, 5 μm (Merck) was used as the solid stationary phase. The mobile phase was a solution of the counter ion as salt in a mixture of dichloromethane

and 1-pentanol. Systems with the chiral amine as acetate were prepared by adding amine base and acetic acid in equal concentrations to the solvent.

Column preparation and chromatographic technique. The LiChrosorb DIOL columns were packed by a slurry technique with chloroform as the suspending medium. The columns were tested by using *n*-hexane–*n*-butanol (199:1) as eluent and 2,4-dinitrotoluene and 2-phenylethanol as test solutes, giving k' of about 1 and 8 respectively. Only columns giving a reduced plate height, $h = H/d_p$, of less than 10 were used in the studies.

The columns were washed with water-free methanol, dichloromethane and *n*-hexane before the mobile phase was introduced. 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. The mobile-phase reservoir, injector and column were thermostatted in a water-bath at 25.0°C. The samples were introduced as acids or salts, dissolved in the mobile phase.

Columns prepared from different batches of LiChrosorb DIOL gave slightly different separation factors for the enantiomeric acids, in spite of very uniform preparation conditions. However, all results given in a certain table have been obtained with the same batch of the solid stationary phase.

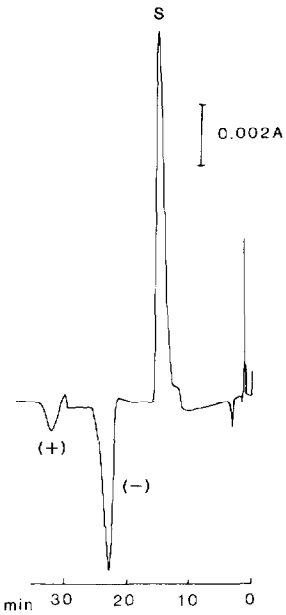
RESULTS AND DISCUSSION

Previous studies of the separation of enantiomeric aminoalcohols by the ion-pair chromatographic technique have led to the assumption that the basic process is an interaction of the chiral counter ion with the enantiomers to give two diastereomeric ion pairs. The resolution is due to the different distribution of the diastereomeric ion-pairs between the organic mobile phase and the adsorbing stationary phase[11].

Detection principle

The ion-pair chromatographic technique can be used for UV detection of non-UV-absorbing samples in reversed-phase chromatography with aqueous mobile phases[12]. The principle has also been applied in a system with an organic mobile phase and a hydrophilic adsorbent as stationary phase for the detection of enantiomers of 10-camphorsulphonic acid by using the UV-absorbing (+)-alprenolol as counter ion[11].

The response of the UV detector seems to be due to the following process. Injection of a sample affects the distribution of the counter ion between the adsorbent and mobile phase in the starting zone. Mobile phase with a constant content of the counter ion is fed into the system and a new equilibrium is reached where each sample component gives a migrating zone having a content of the UV-absorbing counter ion which deviates from that of the mobile phase. The UV-absorbing counter ion also gives migrating zones with such a content that it compensates for the changes in the solute zones. These zones give rise to extra peaks with constant retention, the so-called system peaks. They can be identified by injection of mobile phase containing an excess or a deficiency of the counter ion. Sample peaks with shorter retention than the most retained system peak are positive while sample peaks with higher retention are negative. When system peaks are discussed below, it refers to the most retarded.



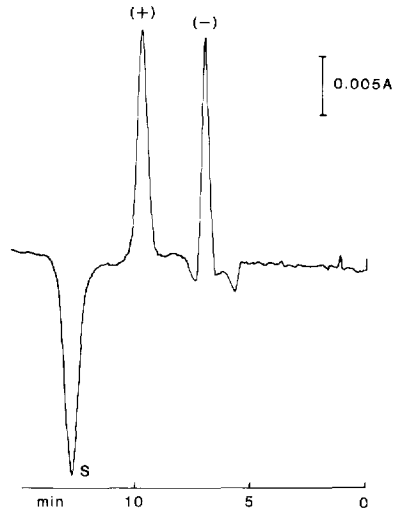


Fig. 1. Resolution of (±)-10-camphorsulphonic acid. Solid phase: LiChrosorb DIOL. Mobile phase: quinine chloride $3.0 \cdot 10^{-4}$ *M* in dichloromethane–1-pentanol (199:1) S = system peak.

Fig. 2. Resolution of (±)-10-camphorsulphonic acid. Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane–1-pentanol (99:1) S = system peak.

An illustration is given in Figs. 1 and 2, which show chromatograms given by the enantiomers of 10-camphorsulphonic acid in two different systems where the solutes are more retained (1) and less retained (2) than the system peak. The enantiomers are without inherent absorbance at the measuring wavelength (337 nm) and are detected solely by the change in the counter ion concentration in the solute peaks.

The presence of the injected compound in the solute peak can be confirmed by detection at a wavelength where the solute has a measurable absorbance. A study on (+)-N-(1-phenylethyl)phthalamic acid can be used as an illustration. When the chromatogram was recorded at 337 nm and 254 nm, a positive solute peak and a negative system peak were obtained. At 337 nm, where the carboxylic acid is without absorbance, the area ratio between the solute and system peak was 1.0, *i.e.*, complete compensation in the system peak. At 254 nm, where both counter ion and solute have some absorbance, a peak area ratio of 1.51 was obtained, which shows an increase of the area of the positive solute peak due to the absorbance of the acid.

The UV response to an injected compound can be expressed quantitatively by a conditional molar absorptivity, ε^*, defined by

$$\varepsilon^* = Y s u / m d b \qquad (1)$$

where Y is the peak area, s is the sensitivity setting of the detector, u is the flow-rate, m is the amount of compound, d is the chart speed and b is the path length in the detector cell[12].

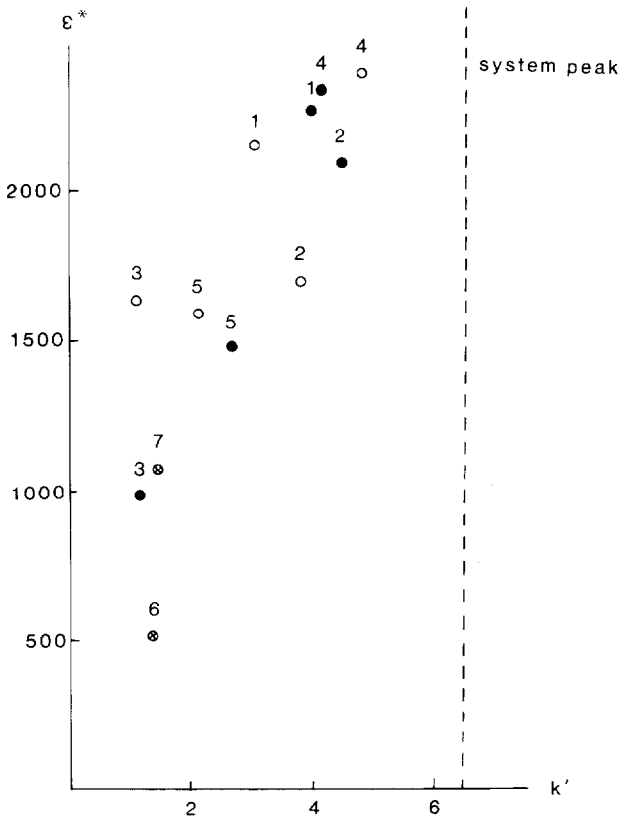


Fig. 3. Response for different solutes. ○, (+)-Enantiomer; ●, (−)-enantiomer; ⊗, racemate. Solutes: 1 = 10-camphorsulphonic acid; 2 = O-methylmandelic acid; 3 = α-methoxy-α-trifluoromethylphenylacetic acid; 4 = N-(1-phenylethyl)phthalamic acid; 5 = 2-phenoxypropionic aid (retention order for enantiomers not determined); 6 = 2-phenylbutyric acid; 7 = 2-methylbutyric acid.

Fig. 3 gives ε^* for compounds without absorbance at the detection wavelength with different retention in a system with quinidine acetate ($3.5 \cdot 10^{-4}$ *M*) as UV-absorbing component in dichloromethane–1-pentanol (99:1). It is obvious that the response increases with the retention of the compound relative to the system peak, but there is also a dependence on the nature of the compound, since quite different conditional molar absorptivities have been obtained at about the same capacity ratios. It was suggested previously[11] that the response might be due to a non-stoichiometric displacement process between the ion pairs present in the system and ion pairs formed between the solute and the UV-absorbing counter ion. Further studies are needed to elucidate whether this view is consistent with the results in Fig. 3.

Influence of 1-pentanol

The retention of injected solutes decreases with increasing concentration of 1-pentanol in the mobile phase (Table I), but the possibilities of regulating the retention by the alcohol concentration are limited due to its negative effect on the stereoselectivity, especially for compounds with strong hydrogen-bonding functions.

TABLE I

INFLUENCE OF 1-PENTANOL ON RETENTION AND STEREOSELECTIVITY

Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinine and $3.5 \cdot 10^{-4}$ *M* in dichloromethane–1-pentanol. $\alpha = k'_2$ (second eluted enantiomer)/k'_1 (first eluted enantiomer).

Compound	*1-Pentanol (%) v/v*					
	0.5		*1*		*2*	
	k'_1	α	k'_1	α	k'_1	α
10-Camphorsulphonic acid	9.4	1.52	5.6	1.43	4.7	1.19
α-Methoxy-α-trifluoromethylphenyl-acetic acid	3.7	1.14	2.2	1.14	1.8	1.10
N-(1-Phenylethyl)phthalamic acid	10.8	1.16	6.9	1.14	5.2	1.09

However, it is usually possible to maintain a separation factor of 1.1–1.2 at a pentanol content of 2%, which will enable a resolution of even fairly hydrophilic enantiomers whithin a reasonable time.

The same negative effect of 1-pentanol on the stereoselectivity was observed by separation of enantiomers of aminoalcohols with (+)-10-camphorsulphonic acid as counter ion[10,11]. The influence of pentanol might be due to competitive interaction with functional groups in the ions, thereby disturbing the selective interaction between the ions in the diastereomeric ion pairs.

The pentanol concentration can also be used to improve the detection for a certain compound in the chromatographic system. The response is dependent on the relative retention between the solute and the system peak which to some extent can be controlled by the content of pentanol in the mobile phase. Some examples are shown in Table II. The conditional molar absorptivity was increased by more than a factor of 2 for (–)-N-(1-phenylethyl)phthalamic acid when the pentanol concentration was decreased from 1 to 0.5 %. For less strongly retained compounds the change is more limited, as illustrated by α-methoxy-α-trifluoromethylphenylacetic acid.

TABLE II

INFLUENCE OF 1-PENTANOL ON RELATIVE RETENTION AND DETECTION SENSITIVITY

Chromatographic system: see Table I. $\alpha_s = k'$ (sample) / k' (system peak); ε^* defined by eqn. 1; $\varepsilon^{337}_{Quinine} = 2020$ (as measured by the detector).

Compound	*1-Pentanol (%), v/v)*							
	0		*0.5*		*1*		*2*	
	α_s	ε^*	α_s	ε^*	α_s	ε^*	α_s	ε^*
(–)-N-(1-Phenylethyl)phthalamic acid	1.26	1780	0.89	5889	0.79	2450	0.84	3680
(–)-α-Methoxy-α-trifluoromethyl-phenylacetic acid	0.35	2230	0.33	2060	0.32	2000	0.28	1830

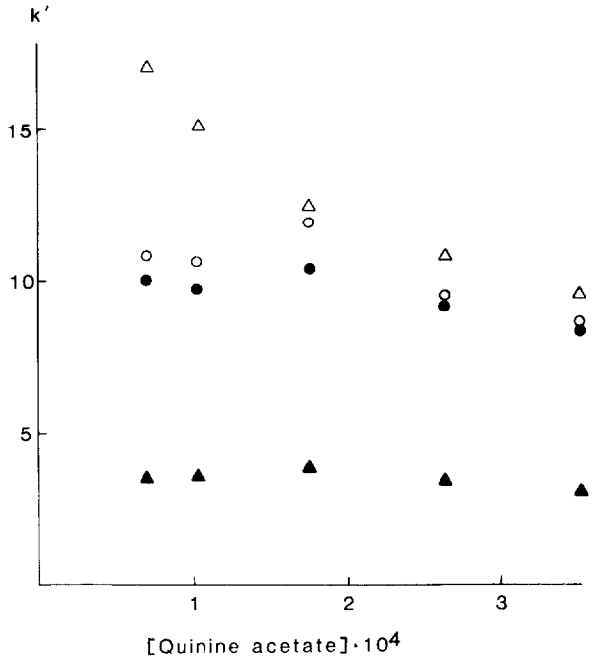


Fig. 4. Influence of quinine acetate concentration on retention. Solid phase: LiChrosorb DIOL. Mobile phase: quinine and acetic acid in dichloromethane 1-pentanol (99:1). △, System peak; ○, (+)-N-(1-phenylethyl)phthalamic acid; ●, (+)-10-camphorsulphonic acid; ▲, (+)-α-methoxy-α-trifluoromethylphenylacetic acid.

Influence of quinine acetate concentration

The response pattern upon injection of a solute indicates that ion pairs of quinine with the solute and with the anion in the mobile phase (acetate) compete for a limited adsorption capacity at the stationary phase. The effect of the concentration of quinine acetate on the capacity ratio of three acids is shown in Fig. 4. The k' values of the solutes increase with decreasing concentration of the counter ion down

TABLE III

INFLUENCE OF THE ANION IN MOBILE PHASE ON STEREO SELECTIVITY AND DETECTION SENSITIVITY

Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* salt of quinine in dichloromethane–1-pentanol (99:1). $\alpha = k'_2 / k'_1$; α_S = k' (sample) / k' (system peak).

Compound	*Enantiomer*	*Quinine chloride*			*Quinine acetate*		
		α	α_S	ε^*	α	α_S	ε^*
α-Methoxy-α-trifluoromethylphenylacetic acid	+	1.10	0.35	1670	1.12	0.34	2100
	−		0.32	1550		0.30	2080
N-*tert.*-Butoxycarbonylphenylalanine	+	1.06	0.44	590	1.26	0.54	1480
	−		0.42	550		0.43	1590

TABLE IV

INFLUENCE OF COUNTER ION STRUCTURE ON THE STEREOSELECTIVE RETENTION OF 10-CAMPHORSULPHONIC ACID

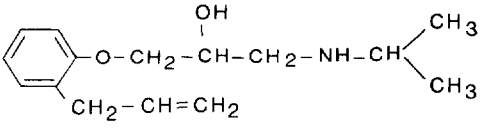
Solid phase: LiChrosorb DIOL. Mobile phase: salt of chiral cation in dichloromethane–1-pentanol. Structures as in Fig. 5. α_{+-} = k' of (+)-form / k' of (−)-form.

Chiral salt	*Content (mol/l × 10^4)*	*1-Pentanol (%, v/v)*	α_{+-}
(+)-Alprenolol chloride	10.0	0	0.95
(−)-Quinine chloride	3.0	1	1.33
(−)-Quinine acetate	3.5	1	1.47
(+)-Quinidine acetate	3.5	1	0.77
(−)-Cinchonidine acetate	3.5	1	1.24

to $2 \cdot 10^{-4}$ M. At lower concentrations, k' is constant or decreases slightly. The deviation at low concentrations of quinine acetate might be due to dissociation in the mobile phase, which increases with decreasing concentration of the ion pairs[13].

The nature of the anion in the mobile phase has an influence on the retention and stereoselectivity, especially for carboxylic acids. Two examples are given in Table III, which shows results obtained with quinine chloride and quinine acetate as ion pairs in the mobile phase. Quinine acetate seems to be preferable: it gives a significantly higher conditional molar absorptivity, *i.e.*, detection sensitivity, and a much better stereoselectivity for the N-blocked amino acid. It is not yet possible to give an

1.



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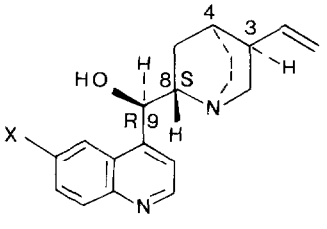

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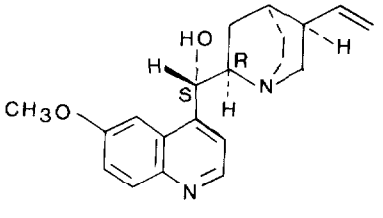


Fig. 5. Counter ion structures: 1 = alprenolol; 2 = quinine, X = OCH_3 [3(*R*), 4(*S*), 8(*S*), 9(*R*)], or cinchonidine, X = H [3(*R*), 4(*S*), 8(*S*), 9(*R*)]; 3 = quinidine [3(*R*), 4(*S*), 8(*R*), 9(*S*)].

TABLE V

INFLUENCE OF COUNTER ION ON STEREOSELECTIVITY AND RETENTION ORDER

Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* chiral amine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane 1-pentanol (99:1). $\alpha = k'_2 / k'_1$.

Compound	*Quinine*		*Quinidine*		*Cinchonidine*	
	α	k'_1	α	k'_1	α	k'_1
10-Camphorsulphonic acid	1.47	5.15 (–)	1.30	3.04 (+)	1.24	6.69 (–)
N-(1-Phenylethyl)phthalamic acid	1.14	6.86 (+)	1.15	4.16 (–)	1.18	9.65 (+)
O-Methylmandelic acid	1.12	7.80 ( )	1.18	3.80 (+)	1.02	8.74*
α-Methoxy-α-trifluoromethylphenyl-acetic acid	1.16	2.35 ( )	1.11	1.07 (+)	1.00	2.64

* Retention order uncertain.

explanation for the difference in the influence of chloride and acetate. The relative retention compared to the system peak is about the same in the two systems.

Influence of counter ion structure on stereoselectivity

The separation of enantiomers is probably due to their different distributions as diastereomeric ion pairs with the chiral counter ion between the organic mobile phase and the adsorbing stationary phase.

Previous studies on the separation of enantiomers of aminoalcohols with (+)-10-camphorsulphonic acid as counter ion have indicated that a simultaneous electrostatic interaction and hydrogen bonding between the ions is vital for the separation[11]. Only aminoalcohols having an alkyl chain of two carbons between the hydroxy group and the amine function could be resolved.

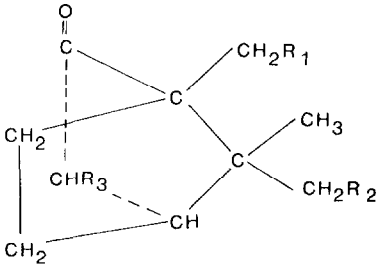
Further structural relationships of importance for stereoselectivity have been observed by the separation of enantiomeric 10-camphorsulphonic acids with aminoalcohols as counter ions. Table IV gives results obtained with different counter ions, all having a distance of two carbons between the hydroxy and amino functions (Fig. 5). (+)-Alprenolol with both functional groups in a straight chain gives a fairly low stereoselectivity. Considerably higher separation factors are obtained with quinine and other cinchona alkaloids, which contain a tertiary amino group in a ring system. It is possible that the improved stereoselectivity is due to the introduction of bulky and rigid groups in the vicinity of the chiral centre in the counter ion, which might increase the difference in interaction with the enantiomers. For a long time the cinchona alkaloids have been used in the separation of enantiomers, quinine is, next to brucine, the leading reagent for the resolution of optically active carboxylic acids by selective crystallization[14].

Quinine and cinchonidine have the same absolute configurations at the chiral centres and give the same retention order for the enantiomers of 10-camphorsulphonic acid. Quinidine has *R* and *S* configuration at the C-8 and C-9 atoms[15,16] and gives the opposite retention order for the enantiomers of the sulphonic acid. The same change in retention order was found for enantiomers of carboxylic acids (Table V).

TABLE VI

SEPARATION OF ENANTIOMERIC CAMPHOR DERIVATIVES

Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinidine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane–1-pentanol (99:1).



Compound	R_1	R_2	R_3	α_+
10-Camphorsulphonic acid	SO_3H	H	H	0.77
3-Bromo-8-camphorsulphonic acid	H	SO_3H	Br	1.00
3-Camphorcarboxylic acid	H	H	COOH	1.09*

* k'_2/k'_1, determined from injection of racemate.

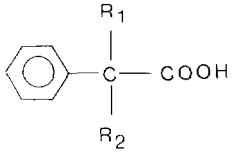
The different separation factors obtained with quinine and quinidine may be due to the fact that they are diastereoisomers, not enantiomers. There is, furthermore, a difference in solubility between quinine and quinidine which might be due to differences in internal hydrogen bonding[15]. In quinidine there are possibilities for internal hydrogen bonding between the hydroxyl group at C-9 and the tertiary amine function. The decrease in stereoselectivity for 10-camphorsulphonic acid observed when quinidine is used as the counter ion might be due to a competition between the internal hydrogen bonding and the hydrogen bonding between the anion and cation. Quinine, which has no possibility of internal hydrogen bonding, gives, as expected, a higher stereoselectivity.

The change in stereoselectivity for compounds without strongly hydrogen-

TABLE VII

SEPARATION OF ENANTIOMERIC MANDELIC ACID DERIVATIVES

Chromatographic system: see Table VI. $\alpha = k'_2 / k'_1$.

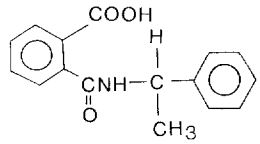


Compound	R_1	R_2	α
O-Methylmandelic acid	H	OCH_3	1.18
α-Methoxy-α-trifluoromethyl-phenylacetic acid	CF_3	OCH_3	1.11
Atrolactic acid	CH_3	OH	1.00
Tropic acid	H	CH_2OH	1.15

TABLE VIII

SEPARATION OF ENANTIOMERIC CARBOXYLIC ACIDS

Chromatographic system: see Table VI.

Compound	α
2-Phenoxypropionic acid	1.30
2-Phenylpropionic acid	1.00
Naproxen [2-(6-methoxy-2-naphthyl)propionic acid]	1.00
	
N-(1-Phenylethyl)phthalamic acid	1.15

bonding groups indicates that other interactions between the ion-pair components can also be important (Table V). The decrease in the stereoselectivity for O-methylmandelic acid and α-methoxy-α-trifluoromethylphenylacetic acid observed when quinine is exchanged for cinchonidine stresses the importance of the aromatic methoxy moiety in the counter ion for the resolution process.

Stereoselectivity and solute structure

The stereoselectivity for different classes of compounds with quinidine or quinine as chiral counter ions is demonstrated in Tables VI–IX.

The acidic camphor derivatives (Table VI) have a strongly hydrogen-accepting oxo group that can give a hydrogen bond with the hydroxyl group in the counter ion. The enantiomers of 10-camphorsulphonic acid are easily separated in systems with quinine and quinidine as counter ions, giving separation factors of 1.5 and 1.3, respectively. The enantiomers of 3-bromo-8-camphorsulphonic acid are not separated in these systems, and previous studies with (+)-3-bromo-8-camphorsulphonic acid as counter ion have indicated a very low stereoselective interaction with aminoalcohols[10]. A separation factor of 1.09 was observed for the enantiomers of 3-camphorcarboxylic acid, but the retention order has not been established due to lack of the individual enantiomers.

Chiral resolution of mandelic acid derivatives with quinidine as counter ion is shown in Table VII. The results indicate that a strong hydrogen bonding between

TABLE IX

SEPARATION OF ENANTIOMERIC AMINO ACID DERIVATIVES

Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane 1-pentanol (99:1).

Compound	α
N-Carbobenzoxyleucine	1.08
N-Carbobenzoxyvaline	1.08
N-Carbobenzoxyphenylalanine	1.26
N-*tert.*-Butoxycarbonylphenylalanine	1.26

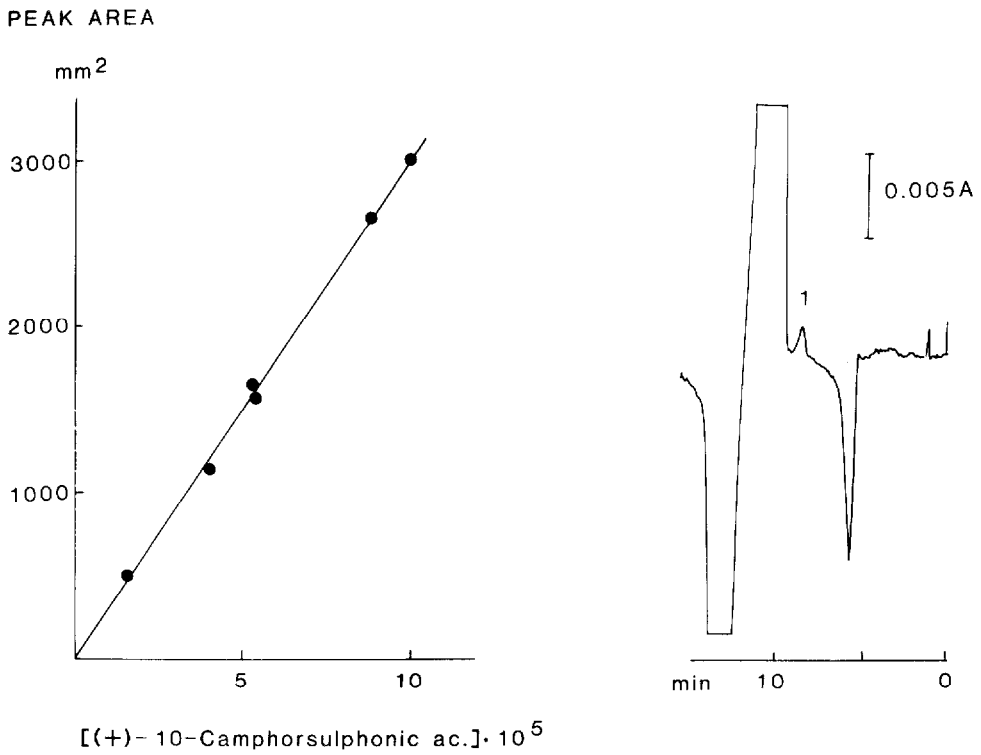


Fig. 6. Quantitation of (+)-10-camphorsulphonic acid. Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane–1-pentanol (99:1).

Fig. 7. Determination of optical impurity in (+)-10-camphorsulphonic acid. Solid phase: LiChrosorb DIOL. Mobile phase: $1.7 \cdot 10^{-4}$ *M* quinine and $1.7 \cdot 10^{-4}$ *M* acetic acid in dichloromethane–1-pentanol (99:1). Peak 1 = (−)-camphorsulphonic acid.

the anion and cation is not an absolute prerequisite for selective retention of diastereomeric ion pairs, as the enantiomers of O-methylmandelic acid are separated although they only contain a relatively weakly hydrogen-accepting methoxy group. Exchange of atoms and groups at the chiral centre has a drastic effect on the stereoselectivity: enantiomers of tropic acid are separated, but no resolution is obtained for the structurally closely related atrolactic acid. Chiral selectivity has also been observed for mandelic acid and for mandelic acid with a methoxy substituent in the aromatic ring, but the separating efficiency is low for these hydrophilic compounds.

Carboxylic acids like 2-phenylpropionic acid and naproxen with an aromatic ring structure and a methyl group attached to the chiral centre cannot be separated with cinchona alkaloids as counter ions (Table VIII). Polar functions, such as a phenoxy group, in the vicinity of the chiral centre seem to be necessary for stereoselective retention. The separation of the enantiomers of N-(1-phenylethyl)phthalamic acid indicates that the carboxy group need not be bonded directly to the asymmetric carbon atom.

The N-blocked amino acids in Table IX also have carboxylic and other polar functions in the vicinity of the chiral centre, and stereoselective retention is obtained with quinine as counter ion.

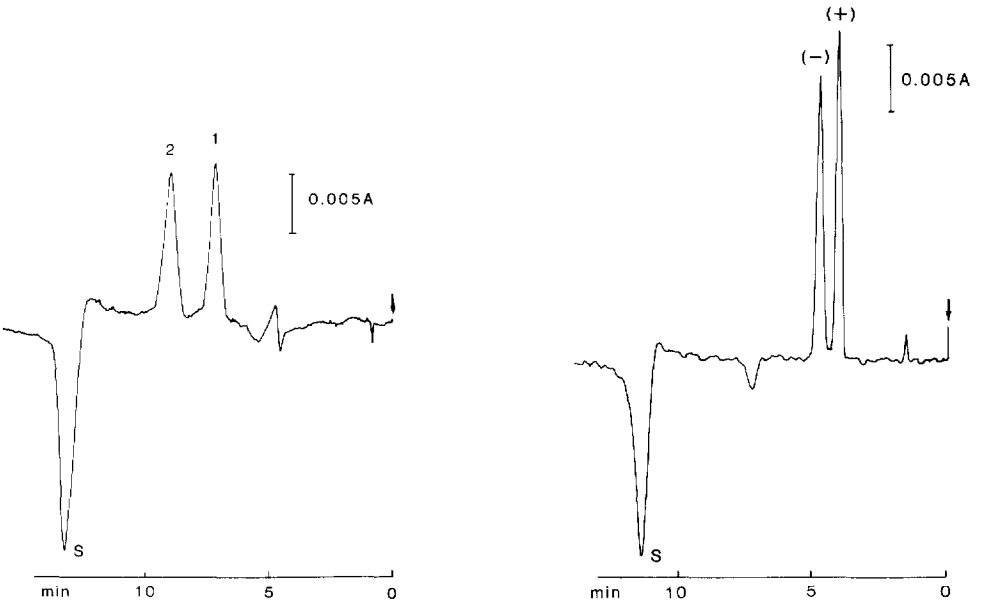


Fig. 8. Resolution of (±)-2-phenoxypropionic acid (order between enantiomers 1 and 2 not determined). Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane–1-pentanol (199:1).

Fig. 9. Resolution of (±)-N-*tert.*-butoxycarbonyl-phenylalanine. Solid phase: LiChrosorb DIOL. Mobile phase: $3.5 \cdot 10^{-4}$ *M* quinidine and $3.5 \cdot 10^{-4}$ *M* acetic acid in dichloromethane 1-pentanol (99:1).

Applications

The enantiomers can be quantitated by measurement of peak height or area. The linearity of the response is demonstrated in Fig. 6, which is obtained with quinine as the UV-absorbing counter ion.

These systems can be used for detection of optical impurities in low concentrations, as shown in Fig. 7. The small peak given by 0.1 nmol of the laevorotary enantiomer represents an impurity of 0.7% in (+)-10-camphorsulphonic acid, which gives the main positive peak. Impurities of the dextrorotary enantiomer are preferably determined in a system with quinidine as counter ion, where the retention order between the optical isomerers is reversed.

The complete resolution of enantiomers is facilitated by good peak symmetry, as demonstrated in Figs. 8 and 9 with quinine and quinidine as chiral counter ions. The enantiomers of 2-phenoxypropionic acid and N-*tert.*-butoxycarbonylphenylalanine are resolved with R_S of 2.1 and 1.3, respectively.

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