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Enantiomeric separation of amines using N-benzoxycarbonylglycyl-L-proline as chiral additive and porous graphitic carbon as solid phase[a]

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ABSTRACT

A study of the separation of enantiomeric amines as diastereometric complexes (ion pairs) with N-benzoxycarbonylglycyl-L-proline (L-ZGP) as chiral additive is presented. The chromatographic performances of two solid stationary phases, porous graphitic carbon (PGC) and LiChrosorb DIOL, were compared and the carbon-based phase was found to have advantages with regard to column efficiency and short equilibration times. Less than 15 column volumes of the mobile phase (L-ZGP in dichloromethane) were sufficient to obtain constant chromatographic conditions with porous graphitic carbon. The water content of the organic mobile phase had no significant effect on the equilibration time of the PGC phase. The chiral counter ion (N-benzoxycarbonylglycyl-L-proline) could be used with aqueous mobile phase on PGC for chiral separations of enantiomeric polyaromatic amines.

INTRODUCTION

Several enantiomeric amino alcohols, *e.g.*, propranolol, metoprolol and alprenolol, have been resolved in the direct separation mode using ion-pair chromatography with N-benzoxycarbonylglycyl-L-proline (L-ZGP) as the chiral counter ion [1,2]. The L-ZGP was dissolved in dichloromethane (containing 30–500 ppm of water) in order to promote a high degree of ion-pair formation. A non-chiral solid phase, *e.g.*, LiChrosorb DIOL, was used to separate the diastereomeric complexes (ion pairs) of enantiomeric amines with L-ZGP. The chromatographic system showed good stability and has recently been applied to the determination of propranolol enantiomer in plasma [3]. However, a relatively large volume of mobile phase (300–400 ml) was needed before constant retention times were obtained. The gradual decrease in retention during the equilibration was probably due to the deactivation of strong adsorption sites on the solid phase. Water dissolved in organic solvents of low polarity

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adsorbs and changes the properties of polar solid phases, *e.g.*, silica or surface-modified silica [4].

Recently, a new high-performance liquid chromatographic (HPLC) support, porous graphitic carbon (PGC), developed by Gilbert *et al.* [5], was made commercially available (Hypercarb; Shandon, Runcorn, U.K.). The PGC phase is a strongly hydrophobic adsorbent with a flat surface consisting of layers of hexagonally arranged carbon atoms [6]. PGC has mainly been used as an alternative to reversed-phase bonded silica gels, but the planar and rigid surface structure also gives new and interesting separation capabilities for normal-phase chromatography. One advantage of the PGC phase is the homogeneous surface of the carbon molecule layers as they only possess a minimum of polar groups at their edges [6]. Further, as PGC has low contents of polar functionalities, the retention and selectivity of solutes might be less susceptible to small changes in the concentration of water and polar modifiers when applying organic mobile phases of low polarity.

The aim of this investigation was to compare a silica-based support, LiChrosorb DIOL, and PGC as adsorbing phases in chiral ion-pair chromatography. The possible effects of the stationary phase on the stereoselectivity in separations with a chiral counter ion are discussed.

In the comparison of the phases, particular emphasis was placed on the volume of mobile phase necessary to obtain constant retention times and stereoselectivity and also the chromatographic performance (efficiency and peak symmetry). The mobile phases were L-ZGP in dichloromethane with different contents of water. The effect of adding triethylamine (TEA) to the mobile phase as a "tailing reducer" was also studied.

Enantioselective separations for various classes of amines are given in order to illustrate the potential of porous graphitic carbon as adsorbing phase in chiral ion-pair chromatography. The separation of racemic 1-phenyl-2-aminopropane was of special interest as it has no polar functions apart from the amino group.

The successful application of PGC for the separation of enantiomeric amines using L-ZGP in aqueous mobile phases is also demonstrated.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Constametric III (LDC, Riviera Beach, FL, U.S.A.) pump, a 7125 injecor (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl loop and a Spectromonitor III UV detector (LDC) with a 12-μl cell.

The LiChrosorb DIOL column (150 × 3.0 mm I.D.) was packed by a slurry technique using chloroform as suspending medium [1]. The PGC columns (100 × 4.7 mm I.D.) were packed with 7-μm particles. The PGC 134 column (Batch PGC 93) was a gift from Professor J. H. Knox (Department of Chemistry, University of Edinburgh). The Hypercarb column was supplied by Shandon. Columns and solvent reservoir were thermostated at 25.0 ± 0.1°C with a HETO type 2 pt 293 TC water-bath (Birkerød, Denmark).

Chromatographic technique

The columns were pre-equilibrated with 250–300 ml of dry dichloromethane (containing <30 ppm of water) before the mobile phase was introduced.

The chromatographic performance was characterized by reduced plate height (h), enantioselectiviy (α) and the peak asymmetry factor (Asf) obtained at a reduced velocity of 1.9 mm/s. Measurements were made using standard equations [7], but as the peak frequently showed severe tailing the data were used only as a qualitative measure of the peak performance. The void volume was determined by injection of chloroform, which was assumed to be unretained.

Chemicals

LiChrosorb DIOL (5 μm), dichloromethane (LiChrosolv), chloroform (analytical-reagent grade), methanol (analytical-reagent grade) and molecular sieves 4Å were obtained from E. Merck (Darmstadt, Germany).

Dichloromethane dried with molecular sieves had a water content of less than 30 ppm. The water contents of the eluents were adjusted by mixing dry and water-saturated (2100 ppm of water [8]) dichloromethane.

N-Benzoxycarbonylglycyl-L-proline was obtained from Nova Biochem (Switzerland). (*R*)- and (*S*)-propranolol · HCl was supplied by ICI (Macclesfield, U.K.), (*R,S*)-atenolol · HCl, (*R,S*)-2′-methylpropanolol · HCl and (*R,S*)-tocainide · HCl were gifts from Hässle (Mölndal, Sweden), (*R*)- and (*S*)-prilocaine · HCl was obtained from Astra (Södertälje, Sweden), (*R,S*)-mexiletine · HCl from Boehringer (Ingelheim, Germany), racemic and (*S*)-1-phenyl-2-aminopropane sulphate were gifts from the Department of Organic Pharmaceutical Chemistry (Uppsala, Sweden) and (*R.S*)-promethazine · HCl was provided by Kabi-Pharma (Stockholm, Sweden).

RESULTS AND DISCUSSION

Solid phase

LiChrosorb DIOL had a very strong affinity for amines and thus high counter-ion concentrations (L-ZGP) were required in order to elute the amines from the column, (Table I). The chromatographic performance was unsatisfactory with poorly shaped peaks and low efficiency that impaired the chiral resolution. A previous study [2] had shown that the addition of an amine, *e.g.*, triethylamine, to the mobile phase could enhance the resolution by increasing the efficiency and improving the peak shape. In this study acceptable peak symmetries (Asf = 1.2) and complete resolution (R_S = 2.0) of propranolol and metoprolol enantiomers could be obtained by using 10 m*M* L-ZGP and 0.20 m*M* TEA in dichloromethane as the eluent (Table I). The modified silica surface is energetically heterogeneous [9] and the observed improvement in the chromatographic performance was probably due to deactivation of strong adsorption sites by the TEA. The presence of the amine in the mobile phase also reduced the retention times, but had only a negligible effect on the enantioselectivity.

At low counter ion concentration, asymmetric peaks were obtained with porous graphitic carbon as the adsorbing phase. The bad peak shape might be due to retention of the free amine on strong adsorption sites with limited capacities as addition of competing amine, triehylamine, improved the peak performance (Table II). Further, high L-ZGP concentrations, *i.e.*, a high degree of ion-pair formation and retention as ion pairs, improved the peak symmetry and efficiency.

TABLE I

INFLUENCE OF TRIETHYLAMINE AND L-ZGP CONCENTRATIONS ON CHROMATOGRAPHIC PERFORMANCE WITH LICHROSORB DIOL AS STATIONARY PHASE

Mobile phase: L-ZGP and TEA in dichloromethane (containing 80 ppm of water).

Mobile phase	Parameter[a]	Solute	
		Metoprolol	Propranolol
[L-ZGP] = 10 m*M*, [TEA] = 0 m*M*	k'_R	5.1	5.8
	h	56	61
	Asf	2.5	2.3
	α	1.40	1.36
[L-ZGP] = 2.5 m*M*, [TEA] = 0.25 m*M*	k'_R	5.2	7.4
	h	32	32
	Asf	1.4	1.4
	α	1.40	1.37
[L-ZGP] = 10 m*M*, [TEA] = 0.20 m*M*	k'_R	3.0	3.5
	h	26	22
	Asf	1.2	1.2
	α	1.43	1.36

[a] k'_R = capacity factor for *R* enantiomer; h = observed reduced plate height; *Asf* = asymmetry factor; $\alpha = k'_S/k'_R$.

TABLE II

INFLUENCE OF TRIETHYLAMINE AND L-ZGP CONCENTRATIONS ON CHROMATOGRAPHIC PERFORMANCE WITH HYPERCARB AS STATIONARY PHASE

Mobile phase: L-ZGP and TEA in dichloromethane (containing 80 ppm of water).

Mobile phase	Parameter[a]	Solute	
		Metoprolol	Propranolol
[L-ZGP] = 2.5 m*M*, [TEA] = 0 m*M*	k'_R	2.7	20
	h	20	—[b]
	Asf	2.6	—[b]
	α	1.24	1.58
[L-ZGP] = 10 m*M*, [TEA] = 0 m*M*	k'_R	2.4	13
	h	8	11
	Asf	1.4	2.0
	α	1.25	1.48
[L-ZGP] = 2.5 m*M*, [TEA] = 0.25 m*M*	k'_R	2.2	13
	h	8	—[b]
	Asf	1.7	—[b]
	α	1.24	1.47
[L-ZGP] = 10 m*M*, [TEA] = 0.25 m*M*	k'_R	2.3	11
	h	7	8
	Asf	1.0	1.7
	α	1.26	1.48

[a] See Table I.
[b] Severe tailing.

Addition of triethylamine had no significant effect on the enantioselectivity except for a minor decrease in the separation factor of propranolol.

Regarding column efficiency, the PGC phase was found to be superior to the LiChrosorb DIOL phase. However, it was not possible to eliminate completely the bad peak shape for the propranolol enantiomers obtained on PGC with 0.25 m*M* TEA added to the mobile phase, whereas the metoprolol enantiomers eluted as symmetrical peaks (Table II).

The reason for the change in stereoselectivity on replacing LiChrosorb DIOL with PGC (*cf.*, Tables I and II) has not yet been elucidated, but it may be due to differences in the adsorption of the two diastereomeric ion pairs [(*R*)-A–(*S*)-HX and (*S*)-A–(*S*)-HX] to the solid phases. The apolar PGC phase will interact with the ion pair and its components by dispersion forces and π–π interactions, whereas LiChrosorb DIOL gives mainly polar interactions (*e.g.*, hydrogen bonding). Previously it has been demonstrated, by using L-ZGP [2] and 10-camphorsulphonic acid [10] as chiral counter ions, that the hydrogen-bonding properties of different modified silica phases affected the enantioselectivity and resolution of enantiomeric amines.

As discussed by, *e.g.*, Davankov [11], in systems having a chiral additive several different chiral mechanisms responsible for the stereoselectivity are possible. A chiral acid might promote stereoselective ion-pair formation in the mobile phase and a selective distribution of the two diastereomeric ion pairs. The enantioselective separation may also be due to stereoselective interaction of the enantiomeric amines with the chiral acid adsorbed on the solid phase.

The retention of the enantiomeric amine, (*R*)-A, as an ion pair [(*R*)-A–(*S*)-HX] with the chiral acid, (*S*)-HX, can be expressed by the equilibria

$$(R)\text{-A}_m + (S)\text{-HX}_m \overset{K_{RS}}{\rightleftharpoons} (R)\text{-A–(}S\text{)-HX}_m \overset{K^*_{RS}}{\rightleftharpoons} (R)\text{-A–(}S\text{)-HX}_s \qquad (1)$$

where m and s refers to the solid and mobile phase, respectively, and K_{RS} and K^*_{RS} are constants for the ion-pair formation and distribution of the ion pair between the mobile and stationary phase, respectively. The free amine might also be retained by adsorption on the solid phase:

$$(R)\text{-A}_m \overset{K_1}{\rightleftharpoons} (R)\text{-A}_s \qquad (2)$$

The adsorption constant K_1 is the same for the (*R*)- and (*S*)-amines as the binding to the solid phase of the free amine is non-stereoselective.

So far it has not been possible to predict the change in the separation factor, $\alpha_{S/R}$, on replacing, *e.g.*, LiChrosorb DIOL with PGC. This would require a knowledge of the adsorption constants for the free amine (K_1) and the ion-pairs (K^*_{RS}, K^*_{SS}) and also the ion-pair formation constants (K_{RS}, K_{SS}) in the mobile phase. The stationary phase with the highest selectivity for diastereomeric ion-pair adsorption ($\alpha = K^*_{SS}/K^*_{RS}$) also gives the highest enantioselectivity provided that the enantioselective ion-pair formation is not dominating and counteracting (*i.e.*, $K_{RS} > K_{SS}$) the selective ion-pair adsorption.

Influence of water content on the equilibration of the chromatographic system

Fig. 1 shows the number of column volumes (V_m) that had to pass the PGC column in order to obtain stable chromatographic conditions. The breakthrough volumes of L-ZGP were only slightly higher than the void volume, demonstrating a low

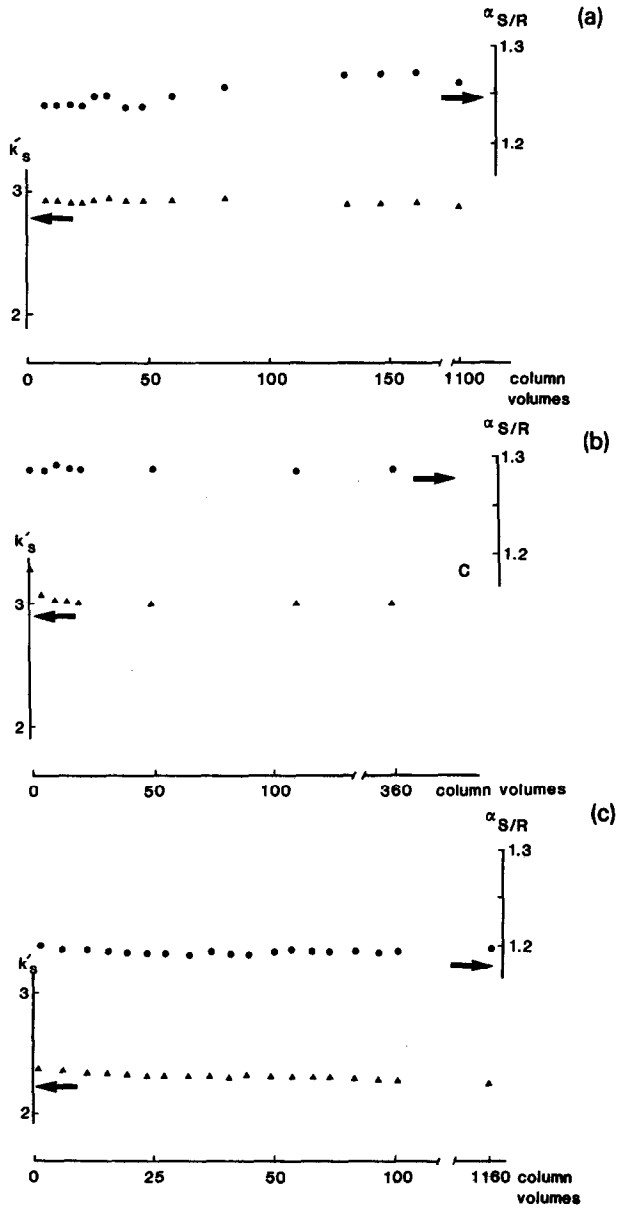

Fig. 1. Equilibration of chromatographic system. Stationary phase, Hypercarb; mobile phase, 10 mM L-ZGP in dichloromethane (containing X ppm water); solute, (R,S)-metoprolol. (a) X = 80; (b) X = 500; (c) X = 2100. (▲) Capacity factors; (●) separation factors.

adsorption constant of L-ZGP with respect to the PGC phase. Furthermore, thermodynamic equilibrium in the column, *i.e.*, constant retention times and separation factors, were obtained within less than fifteen column volumes, (Fig. 1). The water content of the mobile phase had no significant effect on the equilibration time. This indicates a low adsorption of water on PGC.

A decrease in the capacity and separation factors was observed when the mobile phase was saturated with water (2100 ppm of water) (Fig. 1). High concentrations of water gave more effective solvation of the diastereomeric ion pairs and their components which probably affected their stabilities and their distribution properties. Thus, a water content of about 500 ppm was preferable as it gave negligible differences in retention and stereoselectivity compared with mobile phases containing 80 ppm of water (Fig. 1b and a). However, it is more convenient to prepare and easier to control a mobile phase with a higher water content (*e.g.*, 500 ppm).

In comparison, 300 column volumes of mobile phase were required in order to obtain constant chromatographic conditions with LiChrosorb DIOL as stationary phase (Fig. 2). Interestingly, the separation factor becomes constant after 50 column volumes, whereas more than 300 column volumes had to pass the column before constant retention times were obtained. This indicated that the retentions of the enantiomers were affected to the same extent during the equilibration of the column. As discussed above, with silica-based phases it was necessary to include an amine in the mobile phase in order to obtain a good chromatographic performance. The deactivation of the LiChrosorb DIOL was dependent on the concentrations of amine, counter ion and water. It was extremely time consuming to condition LiChrosorb DIOL when the triethyamine ws omitted from the mobile phase. More than 500 ml of

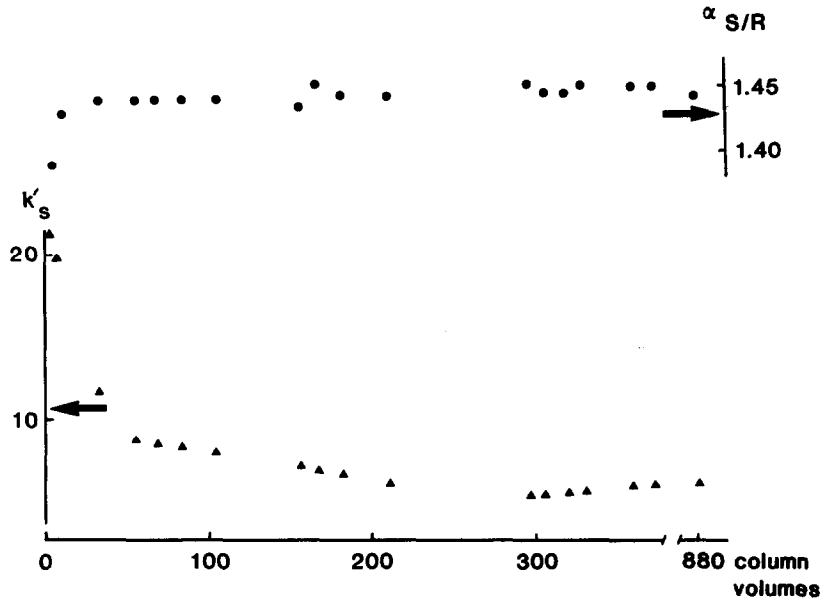

Fig. 2. Equilibration of chromatographic system. Stationary phase, LiChrosorb DIOL; mobile phase, 10 m*M* L-ZGP and 0.20 m*M* TEA in dichloromethane (containing 500 ppm of water). Symbols as in Fig. 1.

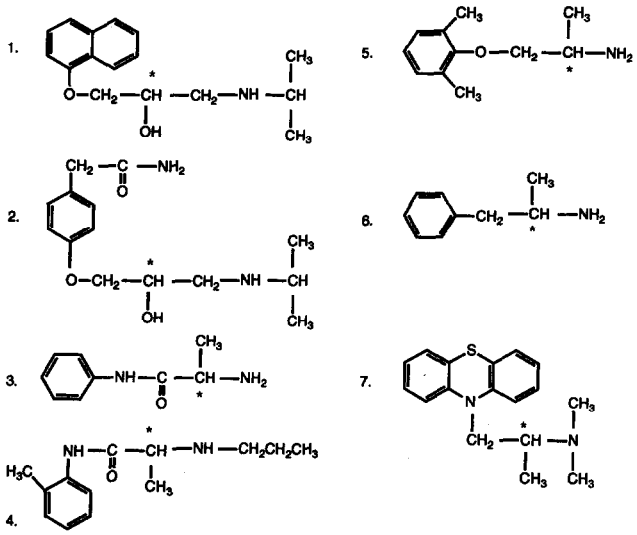

Fig. 3. Structures of solutes (see Table III). ★, Asymmetric centre.

dichloromethane containing 500 ppm of water and 10 m*M* L-ZGP were required to obtain a constant retention and selectivity. Close control of the water content and the presence of an amine in the mobile phase were essential in order to obtain rapid equilibration and reproducible systems when applying silica-based phases.

Solute structure and enantioselectivity

Chiral separations of some pharmaceutical compounds (Fig. 3) with L-ZGP as the counter ion and porous graphitic carbon as the adsorbing phase are shown in Table III. The highest separation factors were observed for the amino alcohols (propranolol and atenolol) which can give multi-point interactions (electrostatic attraction and hydrogen bonding) with the counter ion. This is in accordance with previous findings using modified silica supports (LiChrosorb DIOL and Nucleosil CN) [2]. The surface-modified silica phases gave more or less the same stereoselectivity (α = 1.2–

TABLE III

INFLUENCE OF SOLUTE STRUCTURE ON RETENTION AND STEREOSELECTIVITY

Stationary phase, PGC 134; mobile phase, 10 m*M* L-ZGP in dichloromethane (containing 80 ppm of water).

No.[a]	Solute	k'_1	α^b
1	Propranolol	11	1.47
2	Atenolol	4.1	1.35
3	Tocainide	3.4	1.20
4	Prilocaine	0.91	1.12
5	Mexiletine	3.9	1.21
6	1-Phenyl-2-aminopropane	3.8	1.15
7	Promethazine	1.0	1.15

[a] For structures see Fig. 3.

[b] k' (second-eluted enantiomer)/k' (first-eluted enantiomer).

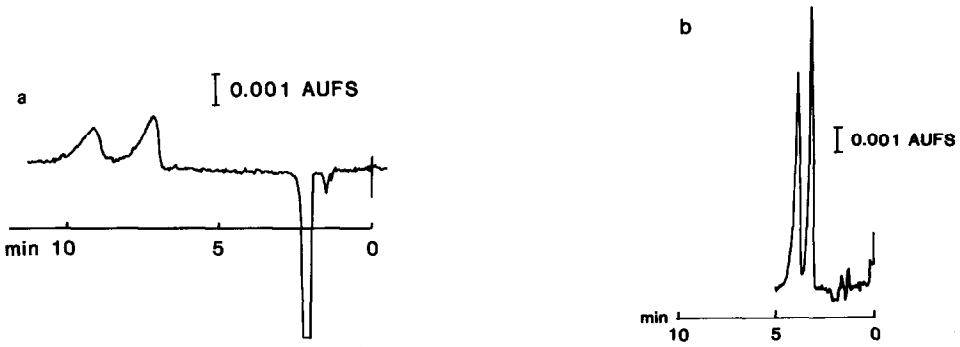

Fig. 4. Resolution of (*R,S*)-atenolol. Stationary phase, PGC 134; mobile phase, 10 m*M* L-ZGP and *X* m*M* TEA in dichloromethane (containing 80 ppm of water). (a) $X = 0$ m*M*; (b) $X = 1.0$ m*M*.

1.8) as PGC but they retained the hydrophilic amino alcohols (*e.g.*, atenolol) more strongly than propranolol. As discussed above, high concentrations of L-ZGP and addition of TEA to the mobile phase were necessary in order to obtain a reasonable retention time with the modified silica phases. Thus, hydrophilic chiral amines and amino alcohols are preferably separated using PGC, as demonstrated by the separation of (*R,S*)-atenolol in Fig. 4a. A complete resolution of the enatiomers was obtained within less than 10 min using 10 m*M* L-ZGP in dichloromethane as the eluent. As indicated in Table II, the bad peak shape could be improved by an addition of triethylamine (Fig. 4b).

The combination of L-ZGP as the chiral additive and a modified silica as the adsorbing phase generally separated enantiomeric β-amino alcohols but not amines lacking a strong hydrogen bonding group in the vicinity of the asymmetric carbon atom [1,2]. With PGC, on the other hand, separations of several different classes of enantiomeric amines (Table III) were possible. Interestingly, chiral recognition was observed for 1-phenyl-2-aminoproprane, an amine with no polar function attached to the chiral centre apart from the amino group. The separation (Fig. 5) indicated that

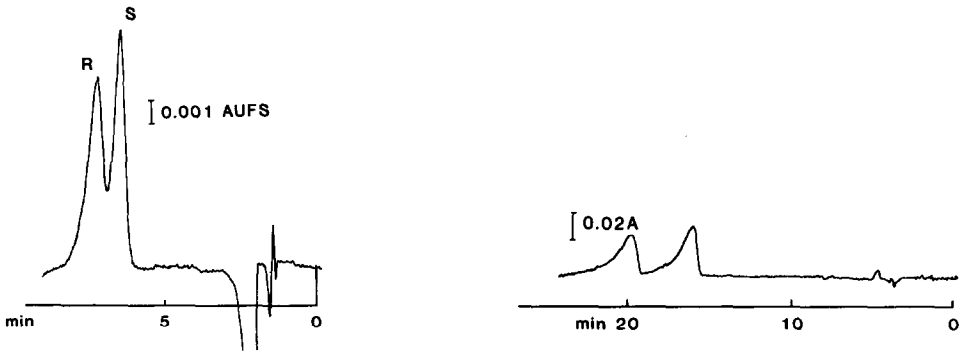

Fig. 5. Separation of racemic 1-phenyl-2-aminopropane. Conditions as in Fig. 4a.

Fig. 6. Reversed-phase separation of (*R,S*)-promethazine. Stationary phase, PGC 134; mobile phase: 5.2 m*M* L-ZGP in methanol–water (95:5).

ion-pair formation and the possibility of additional weaker hydrophobic or steric interaction between the L-ZGP and enantiomeric amines were sufficient for chiral resolution. However, introducing a polar group, *e.g.*, –O– (mexiletine, Table III) or –CONH– (tocainide and prilocaine, Table III) in the vicinity of the chiral centre improved the stereoselectivity. It is possible that two or more strong interactions (electrostatic attraction, hydrogen bonding, dipole–dipole) between the ions enhanced the differences in stability or distribution properties of the diastereomeric ion pairs.

Chiral separations were also carried out for tertiary amines, *e.g.*, promethazine (Table III).

Chiral separations in reversed-phase systems

The enantioselectivity obtained using L-ZGP in water-saturated dichloromethane suggested the possibility of chiral recognition in mobile phases of high polarity. Indeed, L-ZGP dissolved in methanol–water (95:5) promoted the separation of enantiomeric amines with a polyaromatic structure structure (Fig. 6). The column efficiency was moderate but a complete resolution of racemic promethazine was possible owing to the high stereoselectivity ($\alpha = 1.25$).

Based on the limited data available, it was not possible to elucidate if the decrease in capacity factors at the higher L-ZGP concentration (Table IV) was due to an increased formation of less retained ion pairs or to a more effective competition by the counter ion for the limited adsorption capacity of the stationary phase. Further, the mixed effects on the separation factors by the 3-fold increase in the counter ion concentration indicated complex retention mechanism(s).

CONCLUSION

The study of PGC has revealed some advantageous properties as an HPLC stationary phase. Chiral separations of amines with different hydrogen-bonding capabilities were possible using N-benzoxycarbonylglycyl-L-proline dissolved in organic mobile phases with high water contents. Fast equilibration (less than fifteen column volumes) were obtained without the addition of an amine to the mobile phases, whereas LiChrosorb DIOL as adsorbing phase gave long equilibration times despite the use of triethylamine in the mobile phase. The bad peak shape obtained on the

TABLE IV

CHIRAL SEPARATION USING AQUEOUS MOBILE PHASES

Stationary phase, PGC 134; mobile phase, L-ZGP in methanol–water (95:5).

Solute	L-ZGP concentration (m*M*)			
	5.22		19.0	
	k'_1	α	k'_1	α
Propranolol	32	1.08	5.4	1.12
Promethazine	17	1.25	3.9	1.20
2'-Methylpropranolol	57	1.06	5.5	1.0

DIOL and PGC phases was improved by the addition of triethylamine to the mobile phase.

Enantiomeric amines having a polyaromatic ring were resolved with L-ZGP dissolved in an aqueous mobile phase.

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