Journal of Chromatography, 218 (1981) 547–557
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,994

LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

XV*. RESOLUTION OF α-AMINO ACIDS ON REVERSED-PHASE SILICA GELS COATED WITH N-DECYL-L-HISTIDINE

V. A. DAVANKOV*, A. S. BOCHKOV and Yu. P. BELOV
Institute of Organo-Element Compounds, Academy of Sciences of the U.S.S.R., Moscow 117813 (U.S.S.R.)

SUMMARY

Zorbax ODS, Zorbax C_8 and LiChrosorb RP-18 columns were coated with N-decyl-L-histidine (C_{10}-L-His) and charged with copper(II) ions. The factors controlling the retention and resolution of racemic amino acids such as column temperature, pH and ionic strength of the eluent, concentration of copper ions and content and type of organic component in the eluent were examined. The best results were obtained with a 10^{-4} *M* copper(II) acetate solution in pure water. With the exception of aspartic acid, L-isomers of amino acids always eluted with smaller k' values than do the D-antipodes. The enantioselectivity (α) did not exceed 2, which is most convenient for analytical-scale separations. The possible nature of the interactions between the hydrophobic sorbent surface and the components of ternary sorption complexes is discussed.

INTRODUCTION

Chiral modification of commercially available high-performance liquid chromatographic (HPLC) columns by adsorption of appropriate chiral ligands[2] combines important advantages of the two approaches in resolving racemates by means of ligand-exchange chromatography, those of the "chiral eluent" method[3–9] and the use of chiral stationary phases[10,11]. Among these advantages are the possibility of using available chromatographic sorbents and applying desired chiral coating agents; the high stability of the chiral phase system; the unique possibility of eluating the modifier, thus regenerating both the column and the resolving agent; and, the possibility of preparative resolutions because of the absence of disturbing organic contaminants in the eluted fractions.

N-Alkyl-L-hydroxyproline[2] and -L-proline grafted to linear polyacrylamide[12] have been used to modify reversed-phase and normal-phase silica gels, respectively. L-Prolyl-N-dodecylamide was also found to adhere strongly enough to C_{18} Hypersil to

* For Part XIV, see ref. 1.

0021-9673/81/0000–0000/$02.50

render completely superfluous the addition of this chiral ligand to a water-rich eluent[6].

In this work, N-decyl-L-histidine (C_{10}-L-His) has been used as a chiral coating on reversed-phase silica gels, and the resolution of unmodified amino acids in the presence of copper(II) ions has been examined.

EXPERIMENTAL

Commercially available columns (250 × 4.6 mm I.D.) were used, packed with Zorbax C_8 and Zorbax ODS (10 μm) reversed-phase sorbents. The packings were coated with C_{10}-L-His by passing a 1 % ethanolic solution of this compound through the columns followed by an aqueous solution of copper(II) acetate. The reproducibility of the parameters (retention and enantioselectivity) of columns that were coated in this manner is not very high, so that no systematic or significant difference between the behaviour of C_8 and C_{18} packings could be established.

The 250-mm columns were used in combination with a DuPont Model 850 liquid chromatograph, operated with an 8 mm^3 detection cell at 254 nm and a Rheodyne injection loop of 16 mm^3 volume. The elution rate was 2 ml/min.

With more strongly retained solutes, a shorter column was used (80 mm), which was packed with LiChrosorb RP-18 (5 μm) (E. Merck, Darmstadt, G.F.R.) by the usual slurry technique. This column was combined with a Tswett 304 liquid chromatograph (U.S.S.R.) with detection at 254 nm.

The retentions of L- and D-amino acid enantiomers (k'_L and k'_D) were calculated, based on a slightly modified eluent as a t_0 marker, the enantioselectivity was expressed as the ratio $\alpha = k'_D/k'_L$.

RESULTS AND DISCUSSION

General

Coating the reversed-phase columns by using a nearly saturated ethanolic solution of C_{10}-L-His results in adsorption of sufficient amounts of the chiral ligand on the hydrophobic sorbent surface.

As indicated in a previous paper[2], *n*-decyl groups of the chiral coating must be integrated in some way between the alkyl chains of the interface layer. This kind of arrangement seems to be attainable when ethanol is used as the medium during the coating procedure. However, water-rich eluents used in chromatography do not solvate the alkyl chains of the graft and coating, so that the hydrocarbonaceous interface layer must become more compact, thus entrapping the alkyl tail of the chiral ligand. The hydrophilic amino acid part of the latter should be oriented towards the polar eluent and should be accessible for the formation of ternary complexes with metal ions and mobile ligands.

It is interesting that the C_8 packing is generally as good as the C_{18} packing, although the C_{10}-alkyl chains of the coating can be fully embedded in the ODS layer alone (if a parallel orientation of alkyl chains is assumed). The higher efficiency of the Zorbax C_8 column and the higher capacity values (k') of the Zorbax ODS column are worth mentioning.

The main difference between the C_{10}-L-His coating studied here and the C_{10}-L-

hydroxyproline modification investigated previously[2] is the markedly lower enantioselectivity of the C_{10}-L-His system. However, this is not a disadvantage when the analysis of the enantiomeric composition of a certain solute is the purpose of the chromatography: an enantioselectivity exceeding $\alpha = 1.5$ leads to a decrease in the precision of evaluating the ratio of two chromatographic peaks. In contrast, for preparative resolutions one would prefer coatings with a higher enantioselectivity. Generally, the enantioselectivity of L-histidine-incorporated sorbents is lower than that of L-proline- or L-hydroxyproline-incorporated sorbents[10,11].

Considering analytical applications of enantioselective phase systems involving strong fixation (via chemical bonds or adsorption) of resolving chiral ligands on a sorbent matrix, we should emphasize their principal advantage over the "chiral eluent" method which was developed specially for analytical purposes. When a chiral agent is present in the eluent, the two enantiomers resolved in the column enter the detector cell in the form of ternary complexes with the resolving agent and metal ions. These two complexes are diastereomeric and therefore possess different molar absorptivities[13]. Direct spectrophotometric detection of the resolved enantiomers must be based on a special calibration for each racemate–chiral eluent pair. The correction coefficients may change with varying elution conditions because of the varying extent of formation of diastereomeric complexes in solution. This important property was overlooked in some publications[5,7–9]. On the other hand, if the eluent is free of any chiral components, no diastereomeric species can form in solution and any type of detection would immediately produce the desired ratio of two enantiomers.

For the above reasons, the C_{10}-L-His-incorporated reversed-phase system is convenient for the enantiomeric analysis of most amino acids. Of 28 racemates examined, only basic amino acids (Arg, Lys, His), asparagine, glutamic acid and citrulline could not be resolved at the plate number of *ca.* 3000 achieved so far. It is also advantageous that pure water [containing trace amounts of Cu(II) ions] was found to be the best eluent in amino acid analysis.

TABLE I

RETENTION AND SEPARATION ENANTIOSELECTIVITY OF AMINO ACIDS ON ZORBAX C_8 AS A FUNCTION OF pH AND IONIC STRENGTH OF AQUEOUS ELUENTS CONTAINING 10^{-4} *M* COPPER(II) ACETATE AT 35°C

Substance	*pH 6.18 (without NH_4Cl)*			*pH 4.85 (0.1 M NH_4Cl)*		
	k'_L	k'_D	α	k'_L	k'_D	α
Asp	0.86	0.63	1.37	0.50	0.50	1.00
His	0.50	0.50	1.00	0.20	0.20	1.00
Arg	4.77	4.77	1.00	0.63	0.63	1.00
Thr	1.03	1.33	1.29	0.35	0.35	1.00
Pro	4.66	6.70	1.44	2.00	3.00	1.50
Abu	7.20	11.60	1.61	2.30	5.61	2.44
Val	19.33	35.33	1.83	7.41	9.26	1.25
Met	34.00	43.00	1.26	11.45	15.11	1.32
Tyr	31.78	44.21	1.39	9.17	13.29	1.45

Factors controlling retention and enantioselectivity

Several factors significantly influence the retention and resolution selectivity of amino acids on reversed-phase packings coated with C_{10}-L-His.

Addition of 0.1 *M* ammonium chloride to 10^{-4} *M* copper acetate solution leads to a decrease in pH from 6.18 to 4.85. As can be seen from the Table I, the capacity factors, k'_L and k'_D, decrease rapidly owing to the combined action of (i) increasing ionic strength, (ii) partial protonation of both fixed and mobile amino acid ligands and (iii) increasing concentration of ligands (NH_3) competing for coordination with Cu(II) ions. Hence hydrophilic amino acids leave the column with very small k' values with no resolution into isomers. For more hydrophobic solutes, the enantioselectivity remains nearly the same.

TABLE II

RETENTION AND SEPARATION ENANTIOSELECTIVITY OF RACEMIC AMINO ACIDS AS A FUNCTION OF COPPER ACETATE CONCENTRATION IN THE ELUENT (ETHANOL–WATER, 15:85; ZORBAX C_8; 35°C)

Substance	$0.5 \cdot 10^{-4}$ *M*			$1 \cdot 10^{-4}$ *M*			$2 \cdot 10^{-4}$ *M*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Orn	0.83	0.83	1.00	0.71	0.71	1.00	0.54	0.54	1.00
allo-Hyp	1.17	1.17	1.00	0.90	0.90	1.00	0.67	0.67	1.00
Ser	2.00	2.00	1.00	1.40	1.40	1.00	1.31	1.31	1.00
Asn	0.98	0.98	1.00	0.82	0.82	1.00	0.66	0.66	1.00
Glu	1.67	1.67	1.00	1.36	1.36	1.00	1.17	1.17	1.00
Thr	2.00	2.24	1.12	1.60	1.80	1.13	1.33	1.52	1.14
allo-Tyr	1.96	1.96	1.00	1.90	1.90	1.00	1.50	1.50	1.00
Cit	1.40	1.40	1.00	1.20	1.20	1.00	1.18	1.18	1.00
Pro	2.40	3.05	1.27	1.82	2.37	1.30	1.38	1.84	1.33
Asp	1.69	1.24	1.36	1.50	1.13	1.33	1.36	0.96	1.42
His	5.50	5.50	1.00	1.88	1.88	1.00	1.72	1.72	1.00
Aaa*	1.95	1.95	1.00	1.60	1.60	1.00	1.47	1.47	1.00
Ala	2.17	2.17	1.00	1.80	1.80	1.00	1.50	1.50	1.00
Abu**	3.40	3.40	1.00	2.76	2.76	1.00	2.57	2.57	1.00
Tyr	9.33	11.00	1.18	6.50	7.75	1.19	6.33	7.66	1.21
Lys	6.67	6.67	1.00	3.80	3.80	1.00	3.50	3.50	1.00
Ival	6.50	6.95	1.07	5.60	6.00	1.07	3.83	4.25	1.11
Val	7.60	9.35	1.23	5.30	6.70	1.26	4.67	6.02	1.29
Arg	8.20	8.20	1.00	5.70	5.70	1.00	4.17	4.17	1.00
Met	11.40	12.06	1.06	8.00	8.50	1.06	7.25	7.76	1.07

* α-Aminoadipic acid.

** Aminobutyric acid.

An increasing concentration of Cu(II) ions in the eluent produces a similar decrease in k' values (Table II). All the above factors control mainly the extent of formation of ternary sorption complexes, but do not affect the stereochemical interactions within these complexes which are responsible for enantioselectivity.

The type and amount of organic components in hydro-organic eluents strongly influence hydrophobic interactions in the system, including those within the sorption complex. The less organic solvent is present, the higher are the k' and α values (Table

III). Best results are obtained in pure water. This is especially important for small amino acids, which are weakly retained and are resolved only in the absence of organic solvents (Ala, *allo*-Thr). It is interesting that an increasing proportion of water in the eluent does not enhance the retention of highly hydrophilic solutes (Asp, Ser). Nevertheless, distinct improvements in column efficiency and enantioselectivity are observed even in this instance. The last column in Table III is the most important, as it gives the resolutions attainable in pure water for small and polar amino acids.

TABLE III

RETENTION AND RESOLUTION SELECTIVITY OF AMINO ACIDS WITH DIFFERENT RATIOS OF WATER TO ETHANOL IN THE ELUENT [10^{-4} *M* COPPER(II) ACETATE; ZORBAX C_8; 35°C]

Substance	*Ratio (water–ethanol)*											
	70:30			*85:15*			*92.5:7.5*			*100*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Ser	1.57	1.57	1.00	1.40	1.40	1.00	1.15	1.33	1.16	1.23	1.54	1.25
Asn	0.86	0.86	1.00	0.82	0.82	1.00	0.82	0.82	1.00	0.63	0.63	1.00
Glu	1.50	1.50	1.00	1.36	1.36	1.00	0.93	0.93	1.00	2.39	2.39	1.00
Thr	1.69	1.92	1.14	1.60	1.80	1.13	1.50	1.75	1.17	1.03	1.33	1.29
allo-Thr	2.29	2.29	1.00	1.90	1.90	1.00	1.88	1.88	1.00	3.31	4.00	1.21
Cit	1.29	1.29	1.00	1.20	1.20	1.00	1.83	1.83	1.00	2.00	2.00	1.00
Pro	1.71	2.21	1.29	1.82	2.37	1.30	2.00	2.66	1.33	4.66	6.70	1.44
Asp	1.71	1.29	1.33	1.50	1.13	1.33	1.47	1.09	1.35	0.86	0.63	1.37
His	1.67	1.67	1.00	1.88	1.88	1.00	1.88	1.88	1.00	0.50	0.50	1.00
Aaa*	1.47	1.47	1.00	1.60	1.60	1.00	2.00	2.00	1.00	5.46	6.43	1.18
Ala	1.86	1.86	1.00	1.80	1.80	1.00	1.71	1.71	1.00	2.48	3.35	1.35
Abu	2.79	2.79	1.00	2.76	2.76	1.00	3.25	3.88	1.19	7.20	11.60	1.61
Lys	3.07	3.07	1.00	3.80	3.80	1.00	7.33	7.33	1.00	3.29	3.29	1.00
Ival	1.71	1.71	1.00	5.60	6.00	1.07	6.88	7.63	1.11	19.57	24.33	1.24
Val	3.43	3.43	1.00	5.30	6.70	1.26	8.00	10.33	1.29	19.33	35.33	1.83
Arg	5.73	5.73	1.00	5.70	5.70	1.00	7.50	7.50	1.00	4.77	4.77	1.00
Met	4.00	4.24	1.06	8.00	8.50	1.06	10.25	11.38	1.11	34.00	43.00	1.26
Tyr	4.86	4.86	1.00	6.50	7.75	1.19	7.33	9.67	1.32	31.78	44.22	1.39

* α-Aminoadipic acid.

Different organic solvents were examined, namely acetonitrile (polarity $P' = 6.0$, viscosity $\eta = 0.34$), ethanol ($P' = 4.3$, $\eta = 1.08$) and methanol ($P' = 5.1$, $\eta = 0.54$); the retention of hydrophobic solutes gradually increased on passing from acetonitrile to methanol when mixed in equal proportions with water (Table IV).

An increase in the temperature of the column from 18 to 45°C reduces the elution time and slightly enhances the overall resolution (Table V). A drift of the detection baseline at temperatures above 45°C may result from partial desorption of fixed chiral C_{10}-L-His ligands.

Strongly retained hydrophobic amino acids were analysed on a short column. All of them can be resolved in the reversed-phase system (Table VI).

TABLE IV

RETENTION AND RESOLUTION SELECTIVITY OF AMINO ACIDS AS A FUNCTION OF THE ELUENT COMPOSITION [10^{-4} *M* COPPER(II) ACETATE; 35°C]

Substance	*Water–acetonitrile (85:15)*						*Water–methanol (85:15)*					
	Zorbax C_8			*Zorbax ODS*			*Zorbax* C_8			*Zorbax ODS*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
allo-Hyp	0.71	0.71	1.00	1.00	1.00	1.00	0.82	0.82	1.00	0.93	0.93	1.00
Ser	0.74	0.74	1.00	1.75	1.75	1.00	1.12	1.12	1.00	1.23	1.23	1.00
Asn	0.43	0.43	1.00	1.40	1.40	1.00	0.61	0.61	1.00	0.75	0.75	1.00
Glu	0.57	0.57	1.00	0.88	0.88	1.00	0.81	0.81	1.00	0.96	0.96	1.00
Thr	0.59	0.59	1.00	1.75	1.75	1.00	1.66	1.91	1.15	2.31	2.59	1.12
allo-Thr	0.44	0.44	1.00	1.67	1.67	1.00	2.11	2.11	1.00	3.21	3.21	1.00
Cit	0.79	0.79	1.00	2.40	2.40	1.00	1.75	1.75	1.00	3.02	3.02	1.00
Pro	1.00	1.51	1.51	3.20	4.80	1.50	3.51	4.86	1.38	4.62	6.05	1.31
Asp	0.78	0.44	1.77	1.06	0.66	1.61	0.83	0.51	1.63	1.21	0.81	1.50
Aaa*	0.52	0.52	1.00	2.25	2.25	1.00	1.13	1.13	1.00	1.98	1.98	1.00
Ala	0.96	0.96	1.00	3.01	3.01	1.00	1.83	1.83	1.00	2.37	2.37	1.00
Abu	3.24	3.24	1.00	3.67	3.67	1.00	4.52	4.52	1.00	6.31	6.31	1.00
Lys	2.00	2.00	1.00	5.57	5.57	1.00	5.72	5.72	1.00	7.36	7.36	1.00
Ival	4.75	5.75	1.21	11.37	13.67	1.20	9.00	10.71	1.19	14.17	17.15	1.21
Val	3.58	5.84	1.63	10.60	16.85	1.59	8.27	10.67	1.29	13.92	16.98	1.22
Met	5.25	6.63	1.26	13.51	16.48	1.22	12.33	14.54	1.18	17.81	20.48	1.15
Tyr	4.67	5.88	1.26	6.68	8.41	1.26	12.33	15.33	1.24	15.02	17.72	1.18

* α-Aminoadipic acid.

Column efficiency

The plate number of a 250-mm column calculated for the peak of L-proline in water ($k' \approx 5$) was 3000 and the reduced plate height was 8.3. This relatively low column efficiency cannot be ascribed to the ligand-exchange mechanism or to the influence of the chiral coating. On chromatography of a test mixture of benzene, nitrobenzene and naphthalene in methanol–water (85:15), the plate number was only 20–30% higher, which may well result from the difference in the eluent viscosity.

Also for L-proline, the peak asymmetry factor was calculated to be 1.6, skew 1.15. The column porosity (ε) was 68 and the Knox–Parcher ratio was 8.46. The sample loading capacity was 2 mg of L-proline.

As far as column stability is concerned, it can be stated that 500 analyses on each of the columns tested did not cause any change in their retention parameters, enantioselectivity or efficiency.

The reproducibility of results was within 1–2% and the detection limit was 10^{-8}–10^{-9} g of amino acid (proline).

Mechanism of retention and chiral recognition of amino acids

Of all solutes examined, amino acids carrying large aliphatic or aromatic substituents at the α-carbon atom display the highest k' values. This indicates that hydrophobic interactions with the sorbent surface are mainly responsible for the retention of these solutes. Naturally, retention decreases with increasing content of organic

TABLE V

RETENTION AND RESOLUTION SELECTIVITY OF AMINO ACIDS AS A FUNCTION OF COLUMN TEMPERATURE [WATER–ETHANOL, 85:15; 10^{-4} *M* COPPER(II) ACETATE; ZORBAX C_8]

Substance	*18°C*			*35°C*			*45°C*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Orn · HCl	0.87	0.87	1.00	0.71	0.71	1.00	0.50	0.50	1.00
allo-Hyp	0.95	0.95	1.00	0.90	0.90	1.00	0.63	0.63	1.00
Ser	1.45	1.45	1.00	1.40	1.40	1.00	1.33	1.33	1.00
Asn	0.88	0.88	1.00	0.82	0.82	1.00	0.50	0.50	1.00
Glu	1.63	1.63	1.00	1.36	1.36	1.00	1.00	1.00	1.00
Thr	1.71	1.94	1.14	1.60	1.80	1.13	1.29	1.48	1.15
allo-Thr	2.27	2.27	1.00	1.90	1.90	1.00	1.36	1.36	1.00
Cit	1.33	1.33	1.00	1.20	1.20	1.00	0.88	0.88	1.00
Pro	2.01	2.54	1.27	1.82	2.37	1.30	1.72	2.27	1.32
Asp	2.07	1.50	1.38	1.50	1.13	1.33	1.39	0.98	1.42
His · HCl	2.40	2.40	1.00	1.88	1.88	1.00	1.77	1.77	1.00
Aaa*	1.80	1.80	1.00	1.60	1.60	1.00	1.36	1.36	1.00
Ala	1.85	1.85	1.00	1.80	1.80	1.00	1.38	1.38	1.00
Abu	3.13	3.13	1.00	2.76	2.76	1.00	2.70	2.70	1.00
Lys	4.47	4.47	1.00	3.80	3.80	1.00	2.88	2.88	1.00
Ival	6.33	6.64	1.05	5.60	6.00	1.07	4.00	4.36	1.09
Val	6.21	7.50	1.20	5.30	6.70	1.26	3.60	4.64	1.29
Arg	6.23	6.23	1.00	5.70	5.70	1.00	5.21	5.21	1.00
Met	10.80	11.34	1.05	8.00	8.50	1.06	6.43	6.94	1.08
Tyr	11.93	14.55	1.22	6.50	7.75	1.19	5.28	6.50	1.23

* α-Aminoadipic acid.

component in the eluent and with the replacement of methanol with acetonitrile. These regularities do not hold for hydrophilic amino acids, where the contribution from hydrophobic interactions to the total retention is small compared with the contribution from coordination interactions. The latter are especially evident on changing the concentration of copper(II) ions in the eluent or its pH and content of mineral salt. Thus, the retention of amino acids in the chromatographic column is governed by the formation of ternary sorption complexes [with Cu(II) ions and C_{10}-L-His] at the interface layer, hydrophobic interactions contributing considerably to the stability of these complexes.

The formation of ternary complexes is the only process responsible for the chiral recognition of mobile ligands. As the eluents used in the present work are free of any chiral agents, localization of the ternary complexes on the hydrophobic surface is a matter of course. In this respect, the system developed here is similar to the previously described enantioselective LiChrosorb RP-18 coating with N-alkyl-L-hydroxyproline[2]. The main difference is the much lower overall enantioselectivity of the C_{10}-L-His-incorporated system. The highest α values observed for the C_7-L-Hyp and C_{10}-L-His coatings were 16.4 (ref. 2) and 1.83, respectively, so that the latter is more convenient for analytical separations and the former for preparative separations.

We explain the lower enantioselectivity of the histidine-type fixed ligand in terms of the structural ambiguity of its sorption complexes. Whereas in copper(II)

TABLE VI

RETENTION AND RESOLUTION SELECTIVITY OF STRONGLY RETAINED AMINO ACIDS ON A 80 × 4.6 mm I.D. COLUMN PACKED WITH LICHROSORB RP-18 (5 μm)

Amino acid	*10^{-4} M Cu^{2+}; water–ethanol (85:15)*						*10^{-4} M Cu^{2+}; 18°C*						*Water–ethanol (85:15); 18°C*					
	18°C			*35°C*			*Water–ethanol (70:30)*			*Water–ethanol (92.5:7.5)*			*$5 \cdot 10^{-5}$ M Cu^{2+}*			*$2 \cdot 10^{-4}$ M Cu^{2+}*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Ph-Ser	4.75	10.74	2.26	3.67	8.00	2.18	2.91	5.00	1.72	5.64	13.48	2.39	5.24	11.16	2.13	4.01	9.38	2.34
Leu	5.00	9.00	1.40	4.33	7.67	1.77	3.17	3.83	1.21	6.91	10.50	1.52	6.71	9.13	1.36	4.12	6.22	1.51
Norleu	8.25	13.78	1.67	6.33	10.30	1.62	5.29	6.29	1.19	10.39	18.80	1.81	11.31	17.98	1.59	6.11	10.88	1.78
Phe	25.00	40.00	1.60	13.70	21.65	1.58	14.30	17.73	1.24	43.60	75.43	1.73	29.06	45.04	1.55	21.10	36.29	1.72
Trp	40.50	51.00	1.26	33.00	42.60	1.29	17.10	18.63	1.09	85.90	113.38	1.32	48.20	59.77	1.24	35.30	46.24	1.31

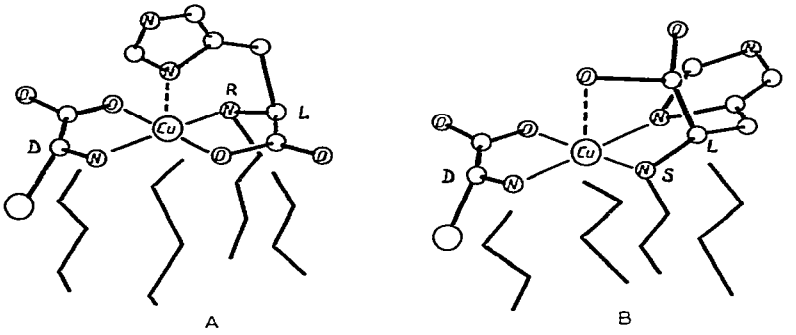

Fig. 1. "Glycine-type" (A) and "diamine-type" (B) ternary sorption complexes.

complexes of N-alkyl-L-proline, the configuration of the asymmetric nitrogen is always unique; in the primary amino group of L-His any of two hydrogen atoms can be replaced with an alkyl group, thus leading the way to two different configurations of the nitrogen, which can interchange even after coordination of a copper ion.

One of these configurations of nitrogen, (*R*), corresponds to a "glycine-type" coordination of copper where a carboxy group and an α-amino group are situated in the main coordination square of copper and the imidazole nitrogen occupies the axial position (Fig. 1A). In this case the ternary sorption complex is situated parallel to the hydrocarbonaceous interface layer and a mobile ligand of D-configuration has the possibility of additional hydrophobic interactions between its α-radical and the sorbent surface. We believe that this structure is responsible for the chiral recognition of mobile ligands with D-enantiomers forming stabler sorption complexes. The only exception is aspartic acid, which shows the opposite elution order of enantiomers. It is logical to suggest that the β-carboxy group of L-aspartic acid forms a hydrogen bond with the imidazole group of the fixed ligand, thus gaining an advantage over the D-isomer, for which only weak interactions with the hydrophobic surface are possible.

However, most fixed ligands seem to coordinate the copper ion according to a "diamine-type" interaction (Fig. 1B) with the carboxy group in the axial position. This situation corresponds to the (*S*)-configuration of the asymmetric α-nitrogen.

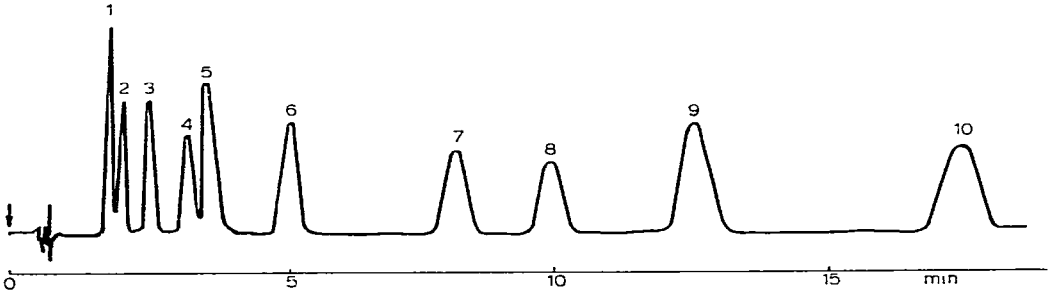

Fig. 2. Resolution of five racemic amino acids on Zorbax C_8 (10 μm) coated with C_{10}-L-His. Column, 250 × 4.6 mm I.D. Eluent, 10^{-4} *M* copper acetate in water. Flow-rate, 2 ml/min. 1 = L-Ala; 2 = D-Ala; 3 = L-Pro; 4 = D-Pro; 5 = L-Abu; 6 = D-Abu; 7 = L-Ival; 8 = D-Ival; 9 = L-Tyr; 10 = D-Tyr.

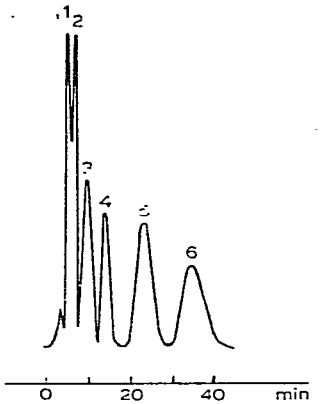

Fig. 3. Resolution of three racemic amino acids on LiChrosorb RP-18. Column, 80 × 4.6 mm I.D. Flow-rate, 0.5 ml/min. Other conditions as in Fig. 2. 1 = L-Abu; 2 = D-Abu; 3 = L-Val; 4 = D-Val; 5 and 6 = Ph-Ser.

Doubtless the diamine chelate contributes considerably to the retention of amino acid solutes (via ternary sorption complexes), but it does not enhence chiral recognition. α-Amino groups of the two amino acid ligands in such a ternary complex can occupy both the *cis*- and *trans*-positions in the copper coordination square because the carboxy group of the C_{10}-L-His ligand is no longer capable of directing into a *trans*-position the equally charged carboxy group of the mobile ligand, as was the case with the "glycine-type" complexes. At the *trans*-structure of the coordination square plane, hydrophobic interactions with the sorbent favour the binding of the L-solutes, and at the *cis*-structure the binding of D-isomers. Enantioselective effects in the "diamine-type" sorption complexes thus cannot be very high and, moreover, they may even display an opposite sign to the enantioselectivity of the above "glycine-type" sorption complexes.

Situation B possibly occurs more frequently than situation A (Fig. 1). In the

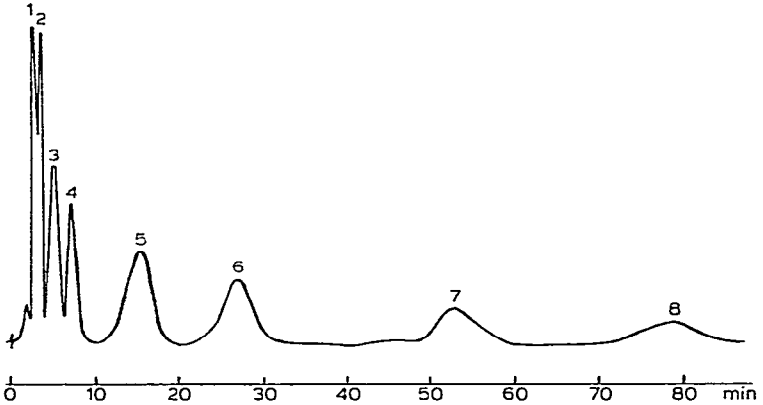

Fig. 4. Resolution of four racemic amino acids. Conditions as in Fig. 3. 1 = L-Abu; 2 = D-Abu; 3 = L-Val; 4 = D-Val; 5 = L-Nleu; 6 = D-Nleu; 7 = L-Phe; 8 = D-Phe.

former structure, both the six-membered chelate ring and the imidazole ring are situated nearly parallel to the surface of the sorbent.

A combination of the above two structures of sorption complexes may provide a basis for a sufficient retention of unmodified amino acids on a C_{10}-L-His-coated packing and for decreasing the enantioselectivity to a level most convenient for analytical separations (Figs. 2–4).

ACKNOWLEDGEMENTS

We are indebted to Drs. R. A. Eyre and J. A. Baxter of Scientific Glass Engineering for kindly supplying chromatographic columns.

REFERENCES

1 Yu. A. Zolotarev, N. F. Myasoedov, V. I. Penkina, O. R. Petrenik and V. A. Davankov, *J. Chromatogr.*, 207 (1981) 63.
2 V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia*, 13 (1980) 677.
3 P. E. Hare and E. Gil-Av, *Science*, 204 (1979) 1226.
4 N. LePage, W. Lindner, G. Davies, D. E. Seitz and B. L. Karger, *Anal. Chem.*, 51 (1979) 433.
5 E. Oelrich, H. Preusch and E. Wilhelm, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 269.
6 Y. Tapuhi, N. Miller and B. L. Karger, *J. Chromatogr.*, 205 (1981) 325.
7 C. Gilon, R. Leshem and E. Grushka, *J. Chromatogr.*, 203 (1981) 365.
8 C. Gilon, R. Leghem and E. Grushka, *Anal. Chem.*, 52 (1980) 1206.
9 C. Gilon, R. Leghem, J. Tapuhi and E. Grushka, *J. Amer. Chem. Soc.*, 101 (1979) 7612.
10 V. A. Davankov, *Advan. Chromatogr.*, 18 (1980) 139.
11 G. Gübitz, W. Jellenz and W. Santi, *J. Chromatogr.*, 203 (1981) 377.
12 J. Boué, R. Audebert and C. Quivoron, *J. Chromatogr.*, 204 (1981) 185.
13 V. A. Davankov, S. V. Rogozhin, A. A. Kurganov and L. Ya. Zuchkova, *J. Inorg. Nucl. Chem.*, 37 (1975) 369.