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STRUCTURE–RESOLUTION RELATIONSHIP

I. THE EFFECT OF THE ALKYLAMIDE SIDE-CHAIN OF ASPARTYL DERIVATIVES ON THE RESOLUTION OF AMINO ACID ENANTIOMERS

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SUMMARY

The resolution of amino acid enantiomers in liquid chromatography using chiral eluents is examined in light of the three "points" association model. The resolution is found to be a function of the alkyl chain length of the resolving agent, which is an alkyl amide derivative of aspartic acid complexed with copper(II). For example, mobile phases with L-aspartylethylamide copper(II) chiral reagent gave lower resolution than with L-aspartyl-*n*-hexylamide copper(II). The pH also influences the resolution. In general, k' values and selectivity factors increase with increasing pH. A mobile phase containing a derivative of glutamic acid could not resolve enantiomers. Analysis of the results shows that the three "points" contact is between the α-carboxy and α-amino groups of the amino acid and the copper(II) and the side-chain of the amino acid and the alkylamide moiety of the aspartyl derivative.

INTRODUCTION

The separation of amino acid enantiomers by liquid chromatography in general, and reversed-phase chromatography in particular, is becoming more commonplace. Two recent reviews[1,2] show the versatility and potential of enantiomeric resolution via high-performance liquid chromatography. The key to direct chromatographic separation of enantiomers is the formation of diastereomers *in situ* during the run. To form diastereomers chromatographically, two options are available: a chiral resolving agent can be either in the stationary phase or in the mobile phase. The first option is more frequently used (*e.g.* refs. 3–6), especially in the form of ligand-exchange chromatography (*e.g.* refs. 7 and 8). Only recently, the use of chiral mobile phases was reported[9–14].

The use of chiral mobile phases is attractive for a number of reasons, not the

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least of which is the possibility of deciphering the resolution mechanism. The present paper will describe an approach aimed at achieving such a goal.

In 1952, Dalgliesh[15] advanced the concept of the three "points" contact in order to explain enantiomeric separation. This concept states that for a unique spatial arrangement, a chiral molecule must be associated with another chiral moiety via, at least, three "points". Thus, diastereomers are formed by fixed positions in space, which are stereoselective. The bonds in these diastereomers, it must be stressed, need not be covalent. The interaction "points" in the diastereomers are of more labile nature, *i.e.*, hydrogen bonds, hydrophobic interactions, electrostatic interactions, charge transfer, etc. The necessity for the three "points" contact was demonstrated again and examined experimentally, by, among others, Feibush and Gil-Av[16], Beitler and Feibush[17] and by Pirkle and Sikkenga[18]. Weinstein *et al.*[19] have recently determined crystal structures of chiral secondary amines in order to explain chiral recognition. The importance of spatial arrangements is well apparent from their work.

In our previous paper on enantiomeric resolution of amino acids[14], we have speculated that, in the system where the chiral reagent is the copper complex of an L-aspartic acid derivative, the three "points" of association are made between the α-carboxy and α-amino acid of the solute and the copper(II), and the hydrophobic interactions between the side-chain of the solute and the alkylamide moiety of the resolving agent. To prove this speculation a set of experiments was designed and will be discussed here.

EXPERIMENTAL

Apparatus

The experimental set-up was described in a previous publication[14]. Briefly, a Spectra Physics Model SP 8000 liquid chromatograph with a variable-wavelength detector was used. Detection was carried out at 230 nm. The mobile phase flow-rate was 2 ml/min at 34°C. The ODS column was 25 cm × 4.1 mm I.D.

Chemicals

All amino acids were obtained from Sigma (St. Louis, MO, U.S.A.). Various amines used in preparing the resolving agents were obtained from various sources, and distilled prior to reaction. Water was triple-distilled in our laboratory. Spectroscopic grade methanol was purchased from Bio-Lab (Jerusalem, Israel).

Synthesis of chiral reagents

For the work described here the following compounds were synthesized; L-aspartylethylamide (AspEth), L-aspartyl-*n*-butylamide (AspnBut), L-aspartyl-*n*-hexylamide (AspnHex), L-aspartyl-*n*-octylamide (AspnOct) and L-glutamylcyclohexylamide (GlucHex). The synthesis of all the chiral reagents followed the procedure in ref. 14. Table I lists the synthetic data for the protected and unprotected aspartic acid and glutamic acid derivatives prepared in this study. Although only the unprotected derivatives were used, the data concerning the protected precursors are listed in Table I, for the sake of completeness and future syntheses. Information concerning L-aspartylcyclohexylamide (AspcHex) is given in ref. 14.

TABLE I

SYNTHESIS INFORMATION CONCERNING THE CHIRAL REAGENTS

*Compound**	*Yield (%)*	*M.p. (°C)*	*Formula*	*Calculated*			*Found*		
				C	*H*	*N*	*C*	*H*	*N*
OBzl | Z–Asp–X									
X = $NHCH_2CH_3$	96	103–104	$C_{21}H_{24}N_2O_5$	65.61	6.29	7.29	65.40	6.46	7.73
$NH(CH_2)_3CH_3$	95	90–92	$C_{23}H_{28}N_2O_5,H_2O$	64.17	7.02	6.51	64.27	6.74	6.82
$NH(CH_2)_5CH_3$	98	76–77	$C_{25}H_{32}N_2O_5$	68.16	7.32	6.36	68.21	7.08	6.09
$NH(CH_2)_7CH_3$	88	93–94	$C_{27}H_{36}N_2O_5$	69.21	7.74	5.98	69.24	7.61	5.73
Asp–X									
X = $NHCH_2CH_3$ (AspEth)	87	196–197	$C_6H_{12}N_2O_3, 0.25H_2O$	43.76	7.65	17.00	44.10	7.97	16.77
$NH(CH_2)_3CH_3$ (AspnBut)	92	216–218	$C_8H_{16}N_2O_3,H_2O$	46.59	8.80	13.58	46.34	9.18	13.81
$NH(CH_2)_5CH_3$ (AspnHex)	90	209–211	$C_{10}H_{20}N_2O_3, 0.25H_2O$	54.52	9.15	12.72	54.30	9.33	12.61
$NH(CH_2)_7CH_3$ (AspnOct)	96	198–200	$C_{12}H_{24}N_2O_3$	58.99	9.90	11.47	58.95	10.17	11.67
OBzl | Z–Glu–*c*-C_6H_{11}	89	138–139	$C_{25}H_{30}N_2O_5$	68.47	6.90	6.39	68.13	7.16	6.73
Glu–*c*-C_6H_{11} (GlucHex)	92	176–179	$C_{11}H_{20}N_2O_3, 0.5H_2O$	55.70	8.86	11.81	55.86	9.02	11.75

* Materials were found to be pure by thin-layer chromatography on silica gel using three different mobile phases: chloroform–methanol–acetic acid (85:10:5), chloroform–methanol (1:1), *n*-butanol–acetic acid–water (4:1:1).

Procedure

The concentration of the copper(II) or nickel(II) complexes of the chiral reagents is given in the Results section. The complexes were made by dissolving the appropriate amounts of analytical grade $CuCl_2$ or $NiCl_2$ and the aspartates or glutamate in distilled water or phosphate buffers.

Once a mobile phase containing the required amount of the chiral reagent was prepared, it was passed through the column until the detector base-line was stable. This took *ca.* 1 h at a flow-rate of 2 ml/min. It was assumed that at this point the system was equilibrated, and the amino acids were then injected in mixtures or individually.

RESULTS AND DISCUSSION

To accomplish enantiomeric separation of amino acids, we used the copper(II) complexes of L-aspartyl-L-phenylalanine methyl ester or of L-aspartyl-cyclohexylamide (AspcHex) as the chiral reagent, in aqueous mobile phases, in conjunction with reversed-phase columns[13,14]. The rationale for the choice of these reagents lies in the fact that they form, with metal ions, optically active complexes containing, most likely, a six-membered ring involving the β-carboxy and α-amino groups of the aspartyl residue. Such a six-membered ring is less stable than the five-membered ring, formed by α-carboxy and α-amino groups of amino acids. Thus, in a solution containing $(AspcHex)_2Cu(II)$ the amino acid solutes can replace one aspartyl group to give a ternary complex (AspcHex)(amino acid)Cu(II). This species, which is made up of one

six-membered and one five-membered ring is essential not only for the separation, but also for the detection of the amino acids at 230 nm. In addition, molecular models seem to indicate that hydrophobic interactions, between the solutes' side-chain and the aspartyl derivative, determine not only the retention times, but also the selectivity of the enantiomeric resolution[14]. That is, the hydrophobic interactions form the third "point" contact and are, therefore, essential for separation to occur. Fig. 1 shows the configuration of the ternary complexes. Changing the magnitude of these interactions can therefore unravel part of the resolution mechanism which takes place in the column.

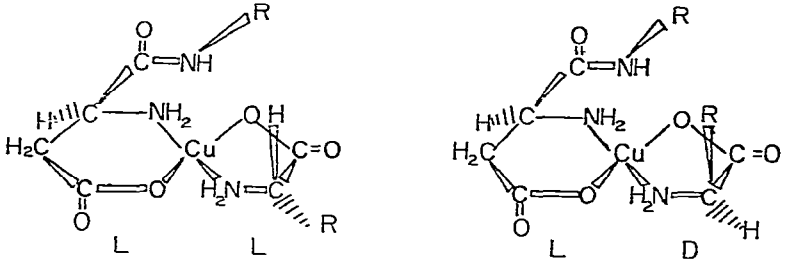

Fig. 1. Configuration of the ternary complexes.

Initially, we studied the effect of branching in the alkylamide moiety of the aspartyl reagent. Table II shows the capacity ratios and α values of three pairs of enantiomers with (AspcHex)Cu(II) and with (AspnHex)Cu(II) in the mobile phase. It is seen that the α values, as well as the capacity ratios, are larger when the bulkier AspcHex is the resolving agent. More interesting, however, is the relative change in the k' values of the L-isomers *versus* the D-isomers. These changes, termed $\%\Delta$(L) or $\%\Delta$(D), are also given in Table II; $\%\Delta$ is defined as:

$$\%\Delta = \frac{(k' \text{ with AspcHex} - k' \text{ with AspnHex})\ 100}{k' \text{ with AspnHex}}$$

The decrease in k' values of the D-isomers is greater than those of the L-isomers in the cases studied here. In a previous study[14], it was shown that hydrophobic interactions between the side-chain of the solutes and the alkylamide moiety of the

TABLE II

CAPACITY RATIOS AND SELECTIVITY FACTORS (α) OF SEVERAL AMINO ACIDS WITH TWO DIFFERENT CHIRAL REAGENTS

Concentration of aspartyl derivative, $6 \cdot 10^{-4}$ *M*; concentration of Cu(II), $3 \cdot 10^{-4}$ *M*; phosphate buffer (0.05 *M* NaH_2PO_4), pH 5.

Amino acid	*AspcHex*			*AspnHex*				
	k'(L)	k'(D)	α	k'(L)	k'(D)	α	$\%\Delta$(L)*	$\%\Delta$(D)*
Proline	0.57	3.2	5.6	0.55	2.4	4.4	3.6	32
Tyrosine	5.5	9.5	1.7	5.1	7.4	1.5	7.8	28
Valine	1.00	1.73	1.73	0.91	1.55	1.7	9.6	12

* Relative change in the capacity ratio.

chiral reagents are feasible with the D-amino acids but not with the L-isomers. This explained the fact that the D-isomers were retained longer. The data in Table II can also be understood on the basis of hydrophobic interactions. With the less bulky AspnHex, these interactions are weaker; thus the ternary complex is less stable and the retention of the D-isomer in this system is diminished. The L-isomers, since their retention is less dependent on intramolecular hydrophobic interaction, do not show large differences in the k' values between the two systems. Table II, however, seems to indicate that when the solutes have a bulky hydrophobic side-chain, the change in the retention of both isomers is rather small, and the selectivity of AspcHex or AspnHex is about the same. This last point needs to be studied further.

To demonstrate further the importance of hydrophobic interactions between solutes and chiral reagents in determining the selectivity, we have measured α values using several resolving agents with different lengths of the alkylamide side-chain, *i.e.* AspEth, AspnBut and AspnHex. The results are given in Table III. AspEth, as might be expected, is the least effective for the separation. Improvement in the resolution is drastic when AspnBut is used as the resolving agent, and less drastic when AspnHex is employed. Fig. 2 is a graphic representation of some of the data. A leveling-off effect is suggested in the figure. Unfortunately, longer chain alkylamide aspartyl derivatives could not be studied under these conditions owing to solubility problems. However, in a later section, we shall discuss the behaviour of AspnOct using different operating conditions.

TABLE III

CAPACITY RATIOS AND SELECTIVITY FACTORS OF SEVERAL AMINO ACIDS WITH DIFFERENT CHIRAL REAGENTS

Concentration of aspartyl derivatives, $6 \cdot 10^{-4}$ *M*; concentration of Cu(II), $3 \cdot 10^{-4}$ *M*; phosphate buffer, pH 5.0.

Amino acid	*AspnEth*			*AspnBut*			*AspnHex*		
	k'(L)	k'(D)	α	k'(L)	k'(D)	α	k'(L)	k'(D)	α
Proline	0.31	0.34	1.09	0.50	1.89	3.8	0.55	2.4	4.4
Tyrosine	1.58	1.71	1.08	3.81	5.69	1.49	5.11	7.39	1.45
Leucine	2.53*	2.94*	1.16*	2.63	3.95	1.50	3.37	5.89	1.75
DOPA	2.08*	2.41*	1.16*	2.39	3.26	1.36	3.25	4.42	1.36
Methionine	—	—	—	1.93	2.51	1.30	2.43	3.39	1.39

* Results obtained with phosphate buffer at pH 6.

Closer inspection of Table III and Fig. 2 shows that the change in the capacity ratios of the D-isomers depends more strongly on the nature of the chiral reagent. This is again in agreement with our contention that hydrophobic interactions between the amino acid solutes and the aspartyl derivatives are essential for the resolutions.

Linear relationships between log k' or log α and the carbon number of the solute are well known. It is indicative of a group contribution to the distribution process. It was hoped that a similar behaviour would be observed when log α or log k' of the amino acids was plotted against the carbon number of the aspartyl derivative alkylamide side-chain. Such is not the case here, and the change is more drastic when going from ethyl to butyl than from butyl to hexyl. However, the values of $\Delta \log k$ for

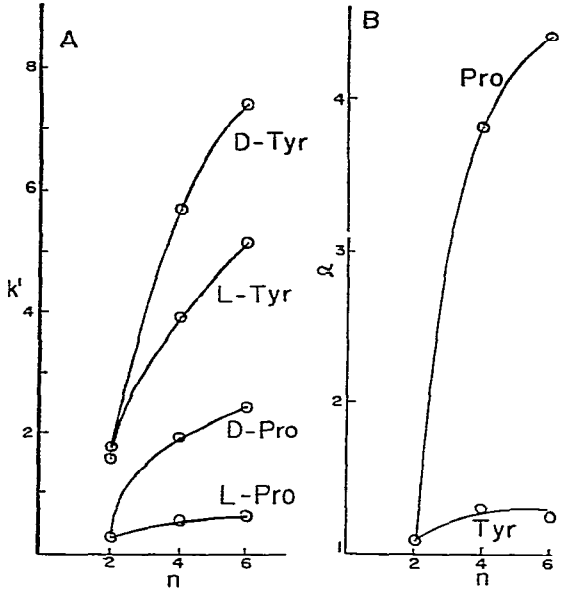

Fig. 2. The dependence of k' and of α on the carbon number of the alkylamide side-chain.

either D- or L-amino acids between the AspnBut and AspnHex systems are roughly constant having an average value of 0.3. It may be concluded therefore, that while the enantiomeric resolution is a strong function of the hydrophobicity of the chiral reagents, it does not depend in a simple manner on the number of methylene groups in the alkylamide moiety of the aspartyl derivatives. The data in Table III and Fig. 2 point to an upper limit of α, under given operating conditions. Above a certain chain length, although the retention changes, the selectivity is not affected. The retention increases because the (AspnAlk)(amino acid)Cu(II) complex becomes more hydrophobic. The selectivity is determined, however, by the stability of the ternary complex which is influenced by intramolecular hydrophobic interactions, *e.g.* the third "point" contact. These should not change much once the alkylamide side-chain of the aspartyl group is longer than a certain value.

Further proof of the above argument is suggested by the data in Table IV, where capacity ratios and α values are compared for AspnHex and AspnOct resolving agents. The data in Tables III and IV should be compared with care since the mobile phase containing the AspnOct was made up with 10% v/v methanol in a phosphate buffer in order to overcome the low solubility of the reagent. Moreover, even with 10% methanol, the solution containing $6 \cdot 10^{-4}$ *M* AspnOct and $3 \cdot 10^{-4}$ *M* copper(II) was cloudy and had to be filtered. Thus, the final concentration of the chiral reagent might have been less than that indicated above. Nonetheless, Table IV shows that the α values obtained with either reagent are quite close.

Effect of the pH

Since the stability of metal–amino acid complexes is pH-dependent, so must be the capacity ratios and the selectivities. Table V shows that such is the case. The capacity ratios as well as α increase with the pH. Again, the increase in retention of

TABLE IV

COMPARISON OF k' VALUES AND α FACTORS OF SEVERAL AMINO ACIDS WITH EITHER AspnHex OR AspnOct COPPER COMPLEX RESOLVING REAGENT

Concentration of (AspnHexCu(II) as in Table III; concentration of (AspnOct)Cu(II), see text; phosphate buffer, pH 4.

Amino acid	*AspnHex*			*AspnOct**		
	$k'(L)$	$k'(D)$	α	$k'(L)$	$k'(D)$	α
DOPA	1.53	1.89	1.23	1.68	1.99	1.18
Tyrosine	2.53	3.33	1.32	2.43	3.16	1.30
Methionine	1.14	1.55	1.36	1.37	1.81	1.32
Valine	0.52	0.80	1.55	0.53	0.81	1.53
Leucine	1.60	2.40	1.50	1.70	2.70	1.58

* Mobile phase contained 10% methanol; see text.

TABLE V

EFFECT OF pH ON k' AND α

Mobile phase containing $6 \cdot 10^{-4}$ *M* AspnHex and $3 \cdot 10^{-4}$ *M* Cu(II); 0.05 *M* phosphate buffer.

Amino acid	*pH 4*			*pH 5*		
	$k'(L)$	$k'(D)$	α	$k'(L)$	$k'(D)$	α
DOPA	1.53	1.89	1.23	3.25	4.42	1.36
Tyrosine	2.53	3.33	1.32	5.11	7.39	1.45
Valine	0.52	0.80	1.55	0.91	1.55	1.70
Proline	0.32	0.79	2.45	0.55	2.43	4.41
Leucine	1.60	2.40	1.50	3.37	5.89	1.75
Histidine	0.31		—	1.42		—
Alanine	0		—	0.22		—
Threonine	0.13		—	0.49		—

the D-isomer is greater than that of the L-isomer. Similar results are shown in Table VI where the pH range and chiral reagent used are different than those in Table V. The last three enantiomers in Table VI were run only at pH 6, since from previous work, it was known that they could not be resolved at pH less than *ca.* 6. For rough comparison, the k' and α values at pH 5 in Table V can be used. The most interesting points

TABLE VI

EFFECT OF pH ON k' AND α

Mobile phase containing $6 \cdot 10^{-4}$ *M* AspcHex and $3 \cdot 10^{-4}$ *M* Cu(II); 0.05 *M* phosphate buffer.

Amino acid	*pH 5*			*pH 6*		
	$k'(L)$	$k'(D)$	α	$k'(L)$	$k'(D)$	α
Proline	0.57	3.20	5.6	1.06	5.38	5.1
Tyrosine	5.54	9.49	1.7	10.2	18.1	1.8
Valine	0.99	1.73	1.75	1.79	4.21	2.35
Histidine	—	—	—	5.68	4.68	0.77
Threonine	—	—	—	1.02	0.94	0.92
Alanine	—	—	—	0.38	0.47	1.24

in Table VI are the enantiomeric differentiation of histidine, threonine and alanine, and the reversal of the elution orders of the enantiomeric isomers of His and Thr. Histidine is known to form tridentate complexes with copper(II). The hydroxyl group in the threonine may bind weakly to copper(II), thus also forming a tridentate complex. This perhaps is the reason for the fact that $k'(\text{D}) < k'(\text{L})$ for these amino acids.

The increase in k' and α as the pH is increased is in agreement with the recent work of Yamauchi *et al.*[20], where it was shown that ternary amino acid complexes with copper(II) are more stable at pH greater than 6. The results of LePage *et al.*[10] should also be mentioned here since the resolution achieved by them occurred at pH 9. In fact, at the pH values reported here they could not resolve enantiomers.

The results of the pH studies indicate that better separations could be obtained at high pH. However, the use of phosphate buffers prevented us from running basic solutions. We are now looking at other buffers and different cations which will allow operation at higher pH values.

Effects of chiral center and size of ring

The discussion above concerned one of the three "points", the one not directly related to the asymmetric center of the resolving agent. To verify the importance of the asymmetric center; *i.e.*, the other two contact points, GlucHex was prepared. This reagent has an optically active center, but instead of a β-carboxy, it possesses a γ-carboxy group. The rationale for preparing this reagent is as follows. If copper(II) complexes to the resolving agent via the α-amino and the amide moiety, then the enantiomeric resolution will not be affected much by changing from AspcHex to GlucHex. If, on the other hand, AspcHex complexes with copper(II) via the β-carboxy and α-amino groups, forming a six-membered ring, and if this ring is responsible for two "points" contact, then changing the chiral reagent to GlucHex would drastically change there solution. Copper(II) bonded by the α-amino and γ-carboxy forms a seven-membered ring, a very weak complex. When a mobile phase containing $6 \cdot 10^{-4}$ *M* GlucHex, $3 \cdot 10^{-4}$ *M* copper(II) and a phosphate buffer (0.05 *M*) at pH 4.7 was used, enantiomeric resolutions were not observed. This result indicates that the copper(II) in the aspartyl derivative is most likely complexed to the β-carboxy and α-amino groups. The six-membered ring thus formed, is stable enough to present a suitable geometry for the amino acid solutes with which to complex in a stereoselective conformation.

Another significant point, in addition to the lack of enantiomeric separation, is the very low retention values which we observed with the (GlucHex)Cu(II). For example, the capacity ratio of tyrosine is *ca.* 1.75 with the above reagent whereas it was 5.1 for the L-isomer and 7.4 for the D-isomer, when (AspcHex)Cu(II) was added to the mobile phase. This fact again seems to rule out the existence of the ternary complex (GlucHex)(amino acid)Cu(II), necessary for the resolution.

Experiments were carried out with a mobile phase containing $6 \cdot 10^{-4}$ *M* β-alanine, $3 \cdot 10^{-4}$ *M* copper(II) in a phosphate buffer (0.05 *M*) at pH 5. This mobile phase is not chiral, of course, and no resolution is expected. Indeed, none was observed. The capacity ratios of the amino acid were small and roughly equivalent to the values obtained with mobile phases containing just copper(II). The lack of enantiomeric resolution and low k' values are due not only to the absence of the chiral center in the mobile phase but also to very weak, if any, intramolecular hydrophobic interactions

in the (β-Ala)(amino acid)Cu(II) complex. In fact, it is not clear to us whether such a ternary complex even exists.

Effect of other metal cations

Ligand-exchange chromatography was used effectively with several metal cations. Lindner *et al.*[9] have shown that enantiomeric resolution can be achieved with chiral complexes containing different metal ions. Since in the present series of studies, copper(II) was used not only for achieving the resolution, but also for detection, we decided to check the feasibility of using ions other than copper. We report here some initial results with nickel(II).

Table VII shows a comparison of k' and α values between systems containing (AspcHex)Cu(II) and (AspcHex)Ni(II). The data in Table VII were obtained with a UV detector operated at 230 nm. The ternary complex (AspcHex)(amino acid)Ni(II) absorbs in this region, although its molar absorptivity seems to be, as might be expected, smaller than that of the copper(II) complex. The (AspcHex)Ni(II) reagent does give enantiomeric resolution. The resolving power is copper(II) > nickel(II), and this order is related to the fact that nickel(II) forms weaker complexes with amino acids. The resolving mechanism is most likely similar to that discussed with the copper (II) system.

TABLE VII

EFFECT OF THE METAL ION ON THE RACEMIC RESOLUTION

Mobile phase contained $6 \cdot 10^{-4}$ *M* AspcHex and $3 \cdot 10^{-4}$ *M* of either metal ion; phosphate buffer (0.05 *M*), pH 5.

Amino acid	*(AspcHex)Cu(II)*			*(AspcHex)Ni(II)*		
	$k'(L)$	$k'(D)$	α	$k'(L)$	$k'(D)$	α
Proline	0.57	3.20	5.6	0.26	0.62	2.36
Valine	0.99	1.73	1.75	0.36	0.56	1.55
Tyrosine	5.54	9.5	1.71	1.92	3.04	1.58

Lindner *et al.*[9] could not resolve hydrophobic amino acid enantiomers when the nickel(II) complex of their triamine was used as chiral reagent. In the present system, we had little difficulty in resolving hydrophobic amino acids, as well as polar ones, at pH values below those reported by Lindner *et al.*[9]. The retention orders of the enantiomers in the (AspcHex)Ni(II) system are the same as with the (AspcHex)-Cu(II) system, including the elution of D-Thr before L-Thr.

TABLE VIII

EFFECT OF pH IN THE (AspcHex)Ni(II) SYSTEM

Same concentrations of reagents as in Table VII; phosphate buffer (0.05 *M*).

Amino acid	*pH 5*			*pH 7*		
	$k'(L)$	$k'(D)$	α	$k'(L)$	$k'(D)$	α
Valine	0.36	0.56	1.55	1.45	3.42	2.36
Proline	0.26	0.62	2.36	0.74	2.86	3.86

With nickel(II), high pH values could be employed and Table VIII shows a sample comparison at two pH values. The trend of the data is the same as was found with the (AspcHex)Cu(II) system, *i.e.*, k' values and α increase with increasing pH. This fact again leads us to believe that the chiral recognition process is similar in both systems.

CONCLUSIONS

Racemic resolution can be accomplished with chiral eluents, provided that the chiral reagents meet certain criteria. The optically active reagent in the mobile phase must form stable diastereomers with racemic solutes. The reagent described here fulfils this requirement. The complex of L-aspartic acid derivatives with copper allows enantiomeric solutes to form stereo-oriented complexes with the copper(II). The stereo-orientation enables only one of the isomers to interact with the alkylamide side-chain of the aspartyl derivatives. These then define the three "points" contact: two associated with the copper(II), and the third with hydrophobic interaction between the R group of the amino acids and the alkylamide moiety of the aspartyl derivatives. The behaviour of the α values as a function of the length of the aspartyl alkylamide side-chain, and the lack of resolution with GlucHex, are consistent with the interaction model suggested above. The dependence on the pH further strengthens the arguments made.

A comparison of our results with those of Lindner *et al.*[9] is of interest. Using a chiral zinc–triamine derivative, they found large α values for the alanines, no resolution of the prolines, and $k'(\text{D}) > k'(\text{L})$ for the histidines. In addition, resolutions of enantiomers occurred only at very high pH, and amino acids were injected as dansyl derivatives. It is clear then that the three "points" association in their system is different than ours. Lindner *et al.*[9] have shown that the dansyl-amino acids could complex the zinc via the carboxy and a negatively charged sulfonamide group. These are two "points". The third association "point", however, is not as clear. The dansyl group as well as the amino acid side-chain can interact with the triamine moiety. From the observations that, among the hydrophobic amino acids, the α value for the alanines is the greatest, and that in general the α values are larger for bulky side-chains, we can assume that both groups take part in determining the stereoselectivity. The importance of the amino acid side-chain is best seen in the α values of the leucines and *allo*-leucines, and in the k' values of the aspartic acids or the asparagines *versus* the glutamic acids or the glutamines. Based on the arguments presented in this paper, we would predict that incorporating a charge-transfer group at the chiral center of the triamine should increase the selectivity of the system.

Among the questions which remain unanswered is whether the ternary complex (AspnAlk)(amino acid)Cu(II) is formed in the mobile phase, and then the whole complex undergoes partition, or whether the (AspnAlk)Cu(II) reagent is adsorbed on the ODS and forms a "dynamic" ligand-exchange system. Experiments are now being carried out to determine the predominant mechanism.

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