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A study of stereoselective amino acid complexes of copper (II) and nickel (II)

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A study of stereoselective amino acid complexes of copper(II) and nickel(II)

by

Richard Vern Snyder

A Dissertation Submitted to the
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TABLE OF CONTENTS

INTRODUCTION

The relative number of chemical studies reported in the recent literature dealing with biological or model biological systems has been increasing. One particular topic in this increased interest is metal ion chemistry because metal ions can play a critical role in the chemical activity of certain enzymes. In these systems the metal ion is generally located at the active site of the enzyme, and without it there would be lower or no enzymatic activity. Yet little is known concerning the interactions of metal ions and the basic units of enzymes, the amino acids. Also, little is understood of the stereoselectivity of these enzymes. That is, many enzymes will show no activity toward the antipode of a compound which itself is a substrate of the enzyme. Possible stereoselectivity has been investigated in labile metal-amino acid systems, but for most systems studied so far the negative results outnumber the positive results.

Bennett (1) thought he had found a simple system which exhibited stereoselectivity in 1959. He performed a series of titrations on 2:1 mixtures of either L-asparagine (L-$AspNH_2$) and copper(II) or DL-asparagine and copper(II). A comparison of the stability constants (defined as the equilibrium constant for Equation (1)) calculated from the data for each

$$Cu^{2+} + 2AspNH_2^- \rightleftharpoons [Cu(AspNH_2)_2] \qquad (1)$$

type of titration indicated that [Cu(L-$AspNH_2$)$_2$] or [Cu(D-$AspNH_2$)$_2$] was favored over [Cu(L-$AspNH_2$)(D-$AspNH_2$)].

In 1965 Wiegers *et al.* published a report (2) on some potentiometrically determined stability constants which cast doubt on Bennett's conclusions. Jellinek and coworkers reported that titrations of 2:1 mixtures of aspartic acid (Asp), glutamic acid (Glu), asparagine, or glutamine ($GluNH_2$) with either cobalt(II), nickel(II), or copper(II) gave virtually identical stability constants without regard to whether the amino acids were optically pure or racemic. Since asparagine and copper(II) had been used in Bennett's study, he and Jellinek disagree on the stereoselectivity of asparagine.

Gillard *et al.* also investigated the possibility of stereoselectivity by both potentiometric and electronic spectral methods (3). Titrations of 2:1 mixtures of the L-, D-, or DL-amino acids alanine, phenylalanine, valine, or proline with copper(II) gave identical formation curves for any one particular amino acid. This indicated that for one amino acid the stability constants for each of the possible bis complexes were the same; thus no selectivity was observed. Visible electronic spectra also failed to show any evidence for a preferential formation of either bis(D-, L-, or DL-amino acidato)copper(II).

Wellman *et al.* attempted to evaluate the interaction between amino acids in a bis(amino acidato)copper(II) complex

using optical rotatory dispersion (ORD)(4). The results showed little to no interaction because little variation was seen in the d-d transitions in the various solutions.

In 1971 Sharpe and Irving (5) studied the stability constants of diaminobutanetetraacetic acid with eleven different metal ions. This potentially hexadentate molecule exists as the d, l, or meso form due to two chiral centers, which means that it is not totally analogous to two separate amino acids having one chiral center each. The results of the titrations showed that in all cases the d and l forms of the acid showed larger stability constants by 1.5 to 2.5 log units than the meso form.

Barnes and Pettit studied the thermodynamics of various possible combinations of asparagine, glutamic acid, aspartic acid, or histidine (Hist) with copper(II), nickel(II) or zinc(II) (6,7). They measured the ΔH for the 2:1 reactions of the d-, l-, or dl-amino acids and metal ions in solution. Their results indicated that $[Cu(AspNH_2)_2]$ shows no stereoselectivity, which is in agreement with Jellinek et al. and contrary to Bennett's findings. They also concluded no selectivity for $[Cu(Glu)_2^{2-}]$ and $[Ni(Glu)_2^{2-}]$. The complex $[Cu(Asp)_2^{2-}]$ showed low selectivity, but definite selectivity was found for histidine complexes of copper(II), nickel(II), and zinc(II). The nickel(II) and zinc(II) complexes preferred a composition of [M(D-Hist)(L-Hist)](mixed), while the

copper(II) complex preferred to have only one enantiomer present in a given complex (optically pure) [Cu(D-Hist)$_2$] or [Cu(L-Hist)$_2$].

Histidine has been used by several other workers, and it has proven to be one common amino acid which consistently exhibits stereoselectivity. McDonald and Phillips (8) investigated cobalt(II) complexes of histidine using proton nuclear magnetic resonance. The shifts induced by the paramagnetic cobalt ion were different for the mixed complex than for the optically pure complexes. By observing the relative areas of the separate peaks, they concluded that the mixed complex was favored over the optically pure ones. Thus, the cobalt(II) complexes of histidine exhibit a selectivity similar to nickel(II) and zinc(II) and opposite of copper(II).

Two more potentiometric studies on metal-histidine complexes corroborated the earlier findings and presented some new ones. Ritsma et al. (9) determined stability constants for the histidine complexes of cobalt(II) and nickel(II). In both cases the mixed complexes were favored over the optically pure ones. Morris and Martin (10) also found this for nickel(II) and cobalt(II), but in the case of copper(II) the opposite preference was seen. That is, [Cu(D-Hist)$_2$] or [Cu(L-Hist)$_2$] was preferred over [Cu(D-Hist)(L-Hist)]. It is interesting to note that although Bennett's work is considered doubtful because it differs with two subsequent reports, his claim that

the mixed complexes of copper(II) are less stable is in agreement with the work of Morris and Martin. The conclusions from the studies of Martin, Pettit, Phillips, Jellinek, and their coworkers are all in agreement concerning histidine.

Hix and Jones (11) determined stability constants for the reaction D- or L-methyl histidinate plus [Ni(D-Hist)$^+$] or [Ni(L-Hist)$^+$] and found no difference for any of the four possible combinations of ester and complex. However, they did find that the esters hydrolyzed faster when the histidine and ester were of the same chirality rather than when they were of opposite chiralities.

In 1969 Leach and Angelici (12) reported an amino acid derivative whose copper(II) complexes exhibited a general preference for L-amino acids. A set of potentiometrically determined stability constants indicated that copper(II) complexes of N-carboxymethyl-L-valine, [Cu(N-Cm-L-Val)], formed stronger complexes with L-leucine, L-phenylalanine, L-alanine, L-serine, and D-valine than with the respective antipodes. Previously there had been no reports of a ligand exhibiting such a general stereoselectivity, although it appears now that N-benzyl-L-proline has a similar characteristic (13). Thus it was decided to pursue the chemistry of this particular L-valine compound and its analogs.

The studies began by attempting to use N-carboxymethyl-L-valine to resolve amino acids. There were several possible

techniques for resolutions of amino acids from which to select. DeLigny et al. had reported on a system to resolve amino acids with cellulose as the stereospecific agent using paper chromatography. Operating at 2°C with 90% methanol as the solvent, the cellulose strips could separate D- and L-cystine. However, this technique would be unsuitable in the present situation because incorporation of N-carboxymethyl-L-valine into a paper seems unlikely (14).

Partial resolution of the N-benzoyl derivatives of alanine, phenylalanine, leucine, and methionine by column chromatography was reported by Humbel et al. (15). Using an iron(III) complex of D-N-(hydroxy-2-ethyl)propylenediamine-triacetic acid which had been loaded on an anion exchange resin, the workers were able to enrich racemic N-benzoylamino acid solutions in the L isomer. That is, the D-N-benzoyl-amino acids were retained on the column longer than the L isomers. Although the separations were not extensive, the possibility of the technique working was demonstrated. However, [Cu(N-Cm-L-Val)] is neutral and would not attach itself to the column.

The more conventional technique of resolving racemates by precipitating the less soluble of a diastereomeric pair had been used by Dwyer and Halpern (16). However, as a variation they used transition metal complexes. They prepared and resolved the cis-dinitrobis(ethylenediamine)cobalt(III) ion and the oxalatobis-(ethylenediamine)cobalt(III) ion and then used

the D isomers to resolve the benzoyl, phthalyl, tosyl, or formyl derivatives of fifteen different amino acids. This method gave isomers which were pure to one part in five hundred. However, these complexes were inert rather than being labile as in the copper(II) case.

One example of another general method of resolving is given by Corbin et al. Their paper (17) reports on the use of solid dipeptides which serve as the stationary phase in gas chromatographic resolutions of volatile derivatives of amino acids. The technique is well developed, but it uses small quantities of amino acid and fails to use metal complexes.

A group of Russian workers performed a complete separation of a racemic amino acid mixture using column chromatography. Davankov and Rogozhin (18,19) substituted L-proline onto an iodomethylated polystyrene resin, and a column packed with this resin and saturated with copper(II) retained D-proline and allowed L-proline to be eluted from the resin using water. Ammonia then displaced the D-proline giving a reported complete separation. A system similar to this one had been decided upon before the publication of the Russians' work because it seemed to be the most promising method for resolving amino acids using copper(II) and N-carboxymethyl-L-valine.

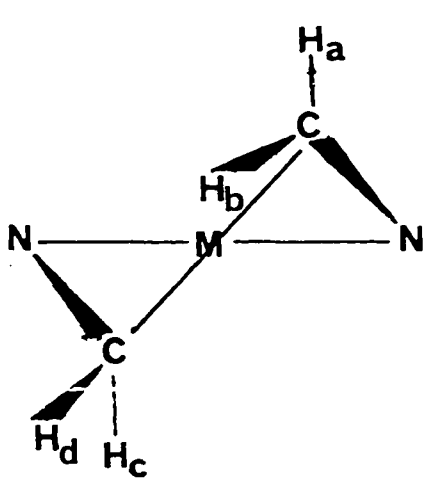
The attempted resolutions employing column chromatography were followed by a determination of the solution

equilibria constants involving copper(II), amino acids, and N-carboxymethylamino acid derivatives using potentiometric methods. Studies of this type are common; a complete account of the methods is given by Rossotti and Rossotti (20).

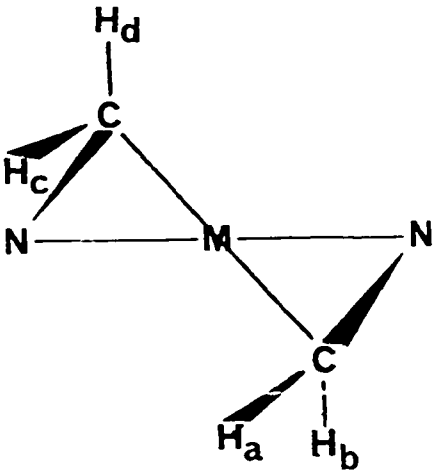
The final study of the system employed paramagnetically shifted proton nuclear magnetic resonance. Background for the technique is contained in two review articles and the references cited therein (21,22). A less rigorous and more easily understood introductory article was written by Keller and Schwarzhans (23). Early reports on the nmr spectra of amino acid complexes of nickel(II) and cobalt(II) were generally limited to qualitative descriptions of the origins of the observed shifts and assignment of peaks (24,25). The shift in the resonance position for a given proton is caused by the localization of some unpaired electron density at that proton. If the unbalanced electron density aligns with the external magnetic field, then the proton resonates at a lower field. If the net electron spin aligns against the magnetic field, the proton resonance moves upfield. Pratt and Smith noted in general that amine protons of amino acids move upfield about 175 to 220 parts per million (ppm), while other protons on the amino acids move downfield from 5 to 180 ppm, although there are exceptions. A later report by Pratt and Smith (26) covered derivatives of iminodiacetic acid (IMDA). In this study the <u>cis</u> and <u>trans</u> isomers of $[Ni(IMDA)_2^{2-}]$ were reported to give

separate resonances which indicated that exchange is slow on the nmr time scale and that relative isomer concentrations of complexes could be measured directly.

A significant advancement in the understanding and interpretation of the nmr spectra of polydentate chelated nickel(II) complexes was made by Ho and Reilley (27,28), Ho et al. (29), Erickson et al. (30,31). Their model for the system pictured the chelate rings as quickly inverting between the two possible conformations k and k' first described by Corey and Bailar (32). (See I and II.)



k
(I)



k'
(II)

This inversion is fast on the nmr time scale, and thus the observed resonance is a weighted average of the chemical shift of the two possible conformations. Each proton has a chemical

shift representative of its average environment. In more useful terms, the chemical shift of a proton is related to its axial-equatorial nature. The proton more equatorial of two otherwise equivalent protons will resonate at lower field than the more axial one. That is, more electron density reaches the proton that is closer to being in the plane of the chelate ring. Thus Reilley et al. were able to describe the relative ring geometries of several polyamines, aminopolycarboxylic acids, and simple amino acids.

The fairly direct interpretation of the chemical shifts arising from nickel(II) complexes is in part due to the absence of any pseudo contact shifts; rather only contact shifts are seen (33). The contact shift of a proton is dependent on the distance from the delocalizing center (or centers) and its relative orientation to the plane of the chelate ring of which it is a part. Protons which are further along a carbon chain from the atom which is bonded to the metal will experience a smaller contact shift, and protons in the chelate plane are equatorial and will be shifted further downfield relative to protons which are out of the plane (axial). These pure contact shifts can only arise from transition metals where there is a nondegenerate ground state. This is a necessary requirement, but not a sufficient one.

If there are pseudo contact contributions to the observed contact shifts, then the interpretation of the spectra is much

more involved. Pseudo contact shifts can arise in complexes where there is a degenerate ground state. It is sometimes referred to as a dipolar shift and arises when there is a preferred orientation of the complex in a magnetic field (34). The pseudo contact shift is dependent on two factors: 1) the length of the vector from the metal ion to the proton being observed, and 2) the angle this vector makes with the molecular axis (33). The first factor is similar to the two dependences of the contact shift. The difficulty arises from the second factor concerning the molecular axis because the size and direction (upfield or downfield) of the pseudo contact shift depend on the location of the axis, and they change as it changes. As of now only axially symmetrical systems have been interpreted with any degree of success (35,36).

Cobalt(II) complexes exhibit pseudo contact as well as contact shifts (34). Milner and Pratt (24) and Pratt and Smith (37) reported the shifts for a series of amino acidato and amino-polycarboxylato complexes, some of which exhibited geometrical isomerization. However, the interpretation of the conformational nature of the ligands could not be made as in the case of the analogous nickel(II) complexes.

As was noted earlier, McDonald and Phillips (8) used paramagnetic shifts to determine relative amounts of diastereomeric complexes of cobalt(II) and histidine. Since no reports of this type have been made for nickel(II), it may well be the

dipolar shifts which lead to separate resonances for mixed and optically pure complexes.

The objective of the research reported here was to determine if N-carboxymethyl-L-valine could be used to separate racemic amino acid mixtures by column chromatography. Once this purpose had been fulfilled, the investigation turned towards an understanding of the chemical causes of the stereoselectivity. Stability constants were determined for several combinations of copper(II), amino acids, and amino acid derivatives with the intention that trends within the series of stability constants might lead to an explanation of the observed selectivity. Finally, it was hoped that the models put forth to explain the selectivities could be substantiated using the data from paramagnetic nuclear resonance studies.

EXPERIMENTAL

Materials

All the amino acids used in these studies were purchased from either Aldrich Chemical Company, Mann Research Laboratories, or Eastman Chemical Company and were used without further purification. In addition, $SnCl_4$, $NaBH_4$, $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, NaI, $Cu(NO_3)_2 \cdot 3H_2O$, NaOH, NH_4HCO_3, $(NH_4)_2CO_3$ were analytical grade and were used directly from the bottle. Potassium nitrate, potassium hydrogen phthalate, and disodium ethylenediaminetetraacetic acid were dried at 100°C before being used. The bromoacetic acid was practical grade, the chloromethylmethyl ether was 97% pure, the ethylenediamine was C. P. grade, the ethyl bromoacetate was 98% pure, and all were used directly. The benzaldehyde was N. F. grade, and it was distilled under nitrogen at atmospheric pressure and stored in a stoppered flask under nitrogen at 0°C. Methyl sulfide, 98%, was distilled under a flow of nitrogen at atmospheric pressure. The nitrogen flow was bubbled through acidified permanganate as it left the distillation apparatus to oxidize the volatile sulfides. The XAD-2 macroreticular resin was supplied by Rohm and Haas. The two chloromethylated styrene-divinylbenzene copolymers were supplied by the Dow Chemical Company. One of these contained 0.8%-divinylbenzene and 15.7% chlorine, while the other was 1.8% in divinylbenzene and 27% in chlorine.

Preparation of Amino Acid Esters

The ethyl ester of valine was prepared by bubbling dry hydrogen chloride through an absolute alcohol suspension of 20.0g (170 mmoles) of L-valine (38). After the alcohol was saturated with HCl, the mixture was heated at reflux for four hours. The ethanol was removed under water aspirator vacuum. The resulting oil was dissolved in 25 ml of 1 N HCl and stirred while being neutralized with an excess of potassium carbonate. After the effervescence ceased, the product was extracted with three 30 ml portions of ethyl acetate. The ethyl acetate solution was dried over magnesium sulfate overnight, filtered, and finally evaporated under a water aspirator vacuum to remove the solvent. The product, ethyl L-valinate (Et-L-Val) (III) was characterized by nmr in $CDCl_3$ solution (see Table 1). Diethyl L-aspartate (Et_2-L-Asp) (IV), diethyl N-carboxymethyl-L-valinate (Et_2-N-Cm-L-Val) (V), and triethyl N-carboxymethyl-L-asparate (Et_3-N-Cm-L-Asp) (VI) were prepared in a similar manner except that for the L-aspartic acid ester the ethanol solution was not heated and stood for only two hours. Yields were generally about 80%, although the triester was closer to 70%. Further purification (if necessary) was accomplished by vacuum distillation.

Preparation of N-carboxymethylamino Acid Esters

Although these were also prepared by esterification of the parent N-carboxymethylamino acids, the following procedure proved to be more convenient (39). To 14.5 g (100 mmoles) of ethyl L-valinate, 8.85 g (50 mmoles) of ethyl bromoacetate were added, and they were stirred together for two hours. Then 25 ml of water was added to dissolve the hydrobromide salt of the ethyl L-valinate which could be recovered later using potassium carbonate as described in the previous section. Ethyl acetate was used to facilitate the separation of the product ester (diethyl N-carboxymethyl-L-valinate) (V) from the water. Drying the ethyl acetate solution over magnesium sulfate, filtering, and removing the solvent under water aspirator vacuum produced the desired product. Vacuum distillation purified the product if needed, but much of it was lost due to polymerization at the high temperatures necessary (about 120°C). Triethyl N-carboxymethyl-L-aspartate (VI) could be prepared by the same process, and both products were characterized by nmr in $CDCl_3$ solution (see Table 1).

Preparation of N-carboxymethylamino Acids

Following a known procedure (40), 50 mmoles of L-aspartic acid and 50 mmoles of bromoacetic acid were neutralized separately with 7 M NaOH to a pH of 11 (the bromoacetic acid was done at 0°C). These two solutions were then stirred

together at 50°C with additional 7 M NaOH as was needed to maintain a pH of 11 for about 90 minutes. If the product were to be used in a resin synthesis, no attempt to remove the inorganic salts was made. If the product were to be used in potentiometric titrations, 4 M HCl was added until the pH reached 2. The solution was then loaded onto a 2.5 x 80 cm column of Dowex 50W-X4 (100-200 mesh) cation exchange resin in the H^+ form and eluted with 240 ml of water (one column volume). The column was then eluted with 0.50 M ammonia at a flow rate of about 1 ml per minute, and 15 ml fractions were collected. Any anions passed directly through the column, cations and the product adhered to the resin, and the ammonia displaced the product while leaving the cations behind. Fractions which contained the desired product, N-carboxymethyl-L-aspartic acid (N-Cm-L-Asp) (VII), had a pH between 2 and 4, so these were saved, mixed, and reduced in volume under water aspirator vacuum until a precipitate began to form. Addition of absolute ethanol equal to three times the volume of water produced the first crop of crystals. These were filtered, washed with absolute ethanol, ether, and dried under vacuum. Addition of ether to the filtrate produced more product. The total yield was about 80%.

The analogous derivatives of D-valine (D-Val) (VIII), L-valine (L-Val) (IX), L-serine (L-Ser) (X), L-glutamic acid (L-Glu) (XI), and L-isoleucine (L-Ileu) (XII) were prepared in

a similar manner. In the purification of the less soluble derivative N-carboxymethyl-L-glutamic acid, the ammonia concentration was reduced to 0.25 M, while for N-carboxymethyl-L-valine, -D-valine, -L-serine, and -L-isoleucine 0.75 M ammonia was used.

Characterization of these products was done by nmr and elemental analysis (see Tables 1 and 2). The nmr spectra were determined in D_2O solutions to which sodium metal had been added to produce a basic medium. This increased the solubility of the samples to a more convenient level by neutralizing the acidic protons.

Preparation of N-benzyl-N-carboxymethylamino Acids

Following a procedure to prepare N-benzylamino acids (41), 5.3 g (50 mmoles) of benzaldehyde was added to a continuously stirred solution of 6.55 g (50 mmoles) of L-leucine and 25 ml of 2 M NaOH. After 15 minutes the formation of the Schiff base was complete as the mixture became homogeneous; it was then cooled on an ice bath. While maintaining a temperature below 15°C, 0.57 g (15 mmoles) of sodium borohydride was added in small portions over a 15 minute period, and then the stirring was continued for another 30 minutes. Using the same conditions as before, the additions of benzaldehyde and sodium borohydride were repeated and followed by two hours of stirring. To precipitate the product, N-benzyl-L-leucine, 4 M HCl was

added until the pH reached 7. The product was then filtered, washed with a small amount of cold water, and dried under vacuum overnight. The yield was 91% based on leucine.

The N-benzyl-L-leucine was converted to the desired product by dissolving 10.41 g (49.1 mmoles) of it in water by adding 1 M NaOH until the pH reached 11 and then adding 6.83 g (49.1 mmoles) of bromoacetic acid which had been dissolved and neutralized at 0°C with 7 M NaOH. The mixture was heated at 60°C and stirred for 2.75 hours while base was added to maintain a pH of 11. The product, N-benzyl-N-carboxymethyl-L-leucine (N-Bz-N-Cm-L-Leu) (XIII), precipitated as the hemihydrate upon addition of 4 M HCl until the pH reached 2. After allowing the product to sit at a pH of 2 for 3 hours, it was filtered, washed with water, and dried under vacuum overnight. Addition of ethanol to the filtrate produced a small amount of additional product. The total yield was 94%. The analogous compound N-benzyl-N-carboxymethyl-L-alanine (N-Bz-N-Cm-L-Ala) (XIV) was prepared similarly as the hemihydrate. Both products were characterized by nmr and elemental analysis (see Tables 1 and 2).

Because of the solubility of disodium iminodiacetate, N-benzyliminodiacetic acid could be prepared in a different manner. A mixture of 9.75 g (50 mmoles) of disodium iminodiacetic acid (monohydrate), 100 ml acetone, and 135 ml of water were stirred until the solid had dissolved. More acetone

was added slowly until an oil started to form. Then 8.55 g (50 mmoles) of benzyl bromide was added along with 4.14 g (30 mmoles) of potassium carbonate. This mixture was heated at reflux for four hours during which time the benzyl bromide dissolved. The volume of solution was reduced by one-half under water aspirator vacuum, and 4 M HCl was added until the pH reached 1.5 and CO_2 effervescence had ceased. The solution was then loaded onto a cation exchange column, eluted with 0.5 M NH_3, and the ten fractions (about 150 ml) collected before the appearance of the ammonia were reduced together under water aspirator vacuum until crystals began to form. The addition of three volumes of ethanol produced more product, N-benzyliminodiacetic acid (XV), which was filtered and then washed with ethanol and ether. The product was characterized by nmr (See Table 1).

Table 1. Proton nuclear magnetic resonance data

Compound	Proton	Chemical shift[a]	Multiplicity
b a $CH(CH_3)_2$ | $NH_2CHCO_2CH_2CH_3$ (III) p c j k	a	0.89	doub. of doub.
	b	1.88	doub. of hept.
	c	3.15	doublet
	j	4.13	quartet
	k	1.23	triplet
	p	1.62	singlet

[a]The chemical shift is given in parts per million downfield from sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

Table 1 (Continued)

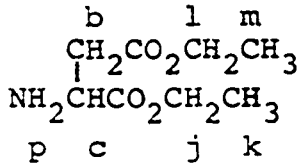
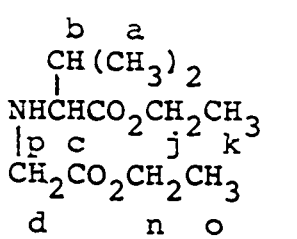
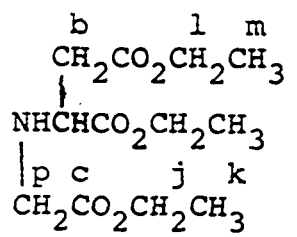
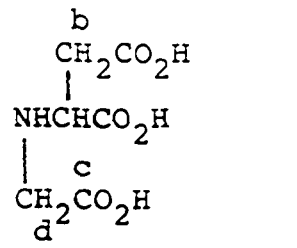
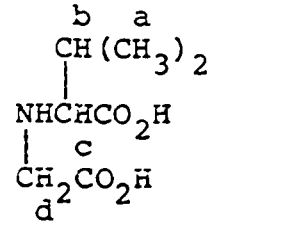
Compound		Proton	Chemical shift[a]	Multiplicity
	(IV)	b	2.68	doublet
		c	3.76	triplet
		j	4.14	quartet
		k	1.22	triplet
		l	4.12	quartet
		m	1.22	triplet
		p	2.28	singlet
	(V)	a	0.96	doublet
		b	1.95	doub. of hept.
		c	3.04	doublet
		d	3.35	singlet
		j	4.16	quartet
		k	1.25	triplet
		n	4.19	quartet
		o	1.27	triplet
		p	2.04	singlet
	(VI)	b	2.66	doublet
		c	3.64	triplet
		d	3.43	singlet
		j	4.12	quartet
		k	1.22	triplet
		l	4.10	quartet
		m	1.22	triplet
		n	4.13	quartet
		o	1.22	triplet
		p	2.39	singlet
	(VII)	b	2.35	three peaks
		c	3.30	quartet
		d	3.09	singlet
	(VIII) (IX)	a	1.67	doublet
		b	2.18	multiplet
		c	3.51	doublet
		d	3.61	singlet

Table 1 (Continued)

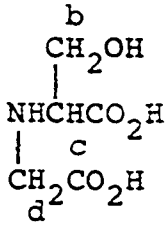
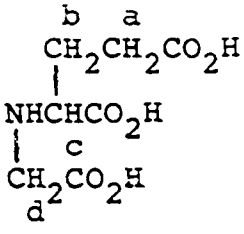
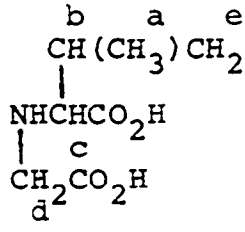
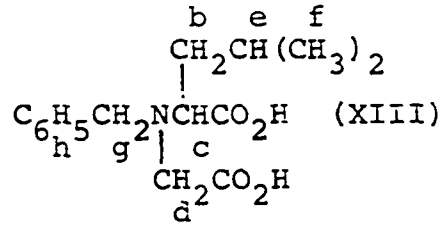
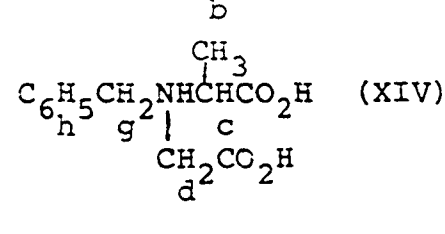
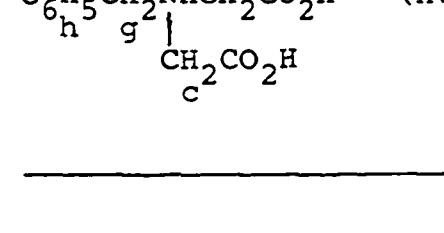
Compound	Proton	Chemical shift[a]	Multiplicity
 (X)	b	3.85	singlet
	c	ca. 3.96	obscured
	d	3.97	singlet
 (XI)	a	2.35	multiplet
	b	2.35	multiplet
	c	3.70	triplet
	d	3.63	singlet
 (XII)	a	1.01	doublet
	b	2.00	multiplet
	c	ca. 3.85	obscured
	d	3.80	singlet
	e	1.41	multiplet
	f	0.97	triplet
 (XIII)	b	1.48	multiplet
	c	3.28	multiplet
	d	3.13	doublet
	e	1.48	multiplet
	f	0.85	doublet
	g	3.82	doublet
	h	7.28	singlet
 (XIV)	b	1.23	doublet
	c	3.37	multiplet
	d	3.15	singlet
	g	3.70	singlet
	h	7.33	singlet
 (XV)	c	3.85	singlet
	g	4.32	singlet
	h	7.39	singlet

Table 2. Analytical data

Compound		Source of %	%C	%H	%N
N-Cm-L-Asp	(VII)	Calculated	37.66	4.74	7.32
		Found	37.59	4.84	7.15
N-Cm-D-Val	(VIII)	Calculated	47.88	7.46	7.98
		Found	47.90	7.54	8.00
N-Cm-L-Val	(IX)	Calculated	47.88	7.46	7.98
		Found	47.67	7.36	8.21
N-Cm-L-Ser	(X)	Calculated	36.61	5.53	8.54
		Found	36.47	5.73	9.16
N-Cm-L-Glu	(XI)	Calculated	40.98	5.40	6.83
		Found	40.84	5.48	6.86
N-Cm-L-Ileu	(XII)	Calculated	50.23	7.90	7.32
		Found	50.74	7.65	7.21
N-Bz-N-Cm-L-Leu	(XIII)	Calculated	62.48	7.34	4.86
		Found	62.54	7.29	5.02
N-Bz-N-Cm-L-Ala	(XIV)	Calculated	58.52	6.14	5.69
		Found	58.68	6.34	5.40

Preparation of Substituted Chloromethylated Styrene-Divinylbenzene Copolymers

Following a procedure given by Roberts and Haigh (42), 15.0 g (66 mmoles of Cl) of the chloromethylated styrene-0.8%-divinylbenzene copolymer (PS-0.8%-DVB) was stirred with 22 ml of distilled dimethyl sulfide (5:1 molar excess of sulfide), 75 ml of 2-propanol, and 60 ml of water at room temperature for 96 hours. At this time a solution of 118 ml of 2-propanol, 215 ml of water, and 176 mmoles of disodium N-carboxymethyl-L-

valine (3:1 molar excess over the resin and containing sodium bromide from the preparation) was added and the whole mixture refluxed for 48 hours. The resin was then filtered to remove excess reactants followed by a second reflux with 4 M ammonia to remove any sulfides. The resin was again filtered, washed successively with 1 M HCl, 1 M NaOH, and finally water. To determine the yield of the reaction and the capacity of the resin, 57 mmoles of a copper(II) nitrate solution was equilibrated with the resin, and the excess copper(II) washed off with water. The amount of excess copper(II) was determined by diluting it to 100 ml and titrating a 10 ml aliquot of it with 0.1 M EDTA using murexide as the indicator (43). Subtraction of this amount of copper from the initial amount of copper(II) added gave the capacity of the resin. Generally this was from 20% to 28% of the initial number of moles of chlorine. This same procedure was used to prepare iminodiacetic acid substituted copolymers, but in this case the yields were as high as 56%.

As an alternate method, the chloromethyl groups could be converted to iodomethyl groups and then reacted with amino acid esters (44). Specifically, 20 g (78 mmoles of Cl) of chloromethylated styrene-0.8%-divinylbenzene copolymer was stirred for 36 hours at reflux in a solution of 120 ml of acetone and 23.5 g (150 mmoles) of sodium iodide. The copolymer was then washed with water to remove halide salts,

then with acetone to remove the water, and then treated with a sodium iodide-acetone solution as before for five more days. This reflux period was followed by filtration, water washings, acetone washings, and then drying at 100°C for four hours. The gain in weight indicated 94% of the chlorine atoms had exchanged for iodines. Using a chloromethylated styrene-1.8%-divinylbenzene copolymer (27% in chlorine), an exchange yield of 89% was obtained. However, several subsequent attempts produced lower yields than this.

The addition of the amino acid derivative was accomplished by heating a mixture of 3.0 g (7.4 mmoles of I) of the iodide-exchanged styrene-0.8%-divinylbenzene copolymer, 10 ml of acetonitrile, and 4.87 g (22.1 mmoles) of diethyl N-carboxymethyl-L-valinate for 9 days at 70°C. The resin was then washed free of excess black-red reactants with ethanol and then refluxed for 24 hours in a 1 N NaOH-95% ethanol solution. The copper capacity of the product indicated a yield of 91%. The same procedure using iodomethylated styrene-1.8%-divinylbenzene copolymer produced only a 62% yield, and the resin produced a finer mesh after the ethanol hydrolysis.

N-carboxymethyl-L-aspartic acid was substituted on a resin in a similar manner using triethyl N-carboxymethyl-L-aspartate. This reaction gave yields generally around 90%, but a few as low as 78% were found. Most resin preparations were run in small batches of about 5 g or less of resin;

larger ones seemed to reduce the yields.

Preparation of Substituted Chloromethylated Macroreticular Resin

To clean the resin as it came from the manufacturer, 10 g of XAD-2 were successively washed with 2 N HCl, methanol, and acetone, and then dried at 100°C. The beads were then mixed in a round bottom flask fitted with a condensor with 54.6 g (680 mmoles) of chloromethylmethyl ether and 7.81 g (30 mmoles) of anhydrous tin tetrachloride. The mixture was stirred in a 70°C oil bath for two hours. The beads were then filtered, washed with an 80% dioxane-water mixture, and then methanol. Chemical analysis of the product gave 11.08% chlorine, a value which compared well with reported values (44).

An iodide exchange was performed on this XAD-2 resin as described previously, but by analysis only 56% of the chlorines exchanged for iodines (16.65 I by analysis). This resin was reacted with diethyl iminodiacetate in acetonitrile as described previously, and only a 31% yield resulted. A preparation using diethyl N-carboxymethyl-L-valinate produced an 11% yield.

Construction and Operation of the Column

The column and associated equipment on which most of the separations were attempted had the following description. The

column itself was a 1.0 x 100 cm glass tube with a coarse glass frit at the bottom. Below this the tube narrowed quickly to 3/16" where a 1/8" x 3" piece of Tygon tube was connected. A screw clamp on the tube regulated the flow rate. Attached to the other end of this tube was a shorter column (1.0 cm x 10 cm) also fitted with a coarse glass frit. An 18/9 ball and socket joint placed near the top made it possible to easily remove and replace the resin inside if necessary. The bottom of the shorter column was constructed similarly to the longer column and was attached by 0.7 mm i.d. Teflon tubing to an LKB 3404B Siphon Stand. This was connected to an LKB 3403B Controller which regulated the movement of an LKB 3406A Turntable. The siphon on the Siphon Stand would deliver a set volume to a test tube hung from the Turntable. Before a sample would leave the siphon, the Turntable advanced bringing a new tube just below the siphon outlet. The eluting solution was stored in a 500 ml separatory funnel connected by tubing to the top of the column. The funnel had a pressure head of 5 gallons of water at about 3 feet above the top of the resin bed.

Resolution studies were carried out in the following manner. A 25 g sample (dry weight) of N-carboxymethyl-L-valine-substituted chloromethylated styrene-0.8%-divinylbenzene copolymer (with a 13.3 mmole Cu(II) capacity) in the sodium ion form was equilibrated with 9.5 mmoles of copper(II) nitrate

and packed into the larger column forming about a 70 cm bed. The smaller column was filled with the same type of resin (with a slightly higher capacity) in the sodium ion form to trap any copper(II) leaking from the larger column. Then about 4.2 mmoles of a racemic amino acid was dissolved in 3 to 5 ml of water by adding 4 M NaOH until the pH reached 11.1 as measured by a Beckman Zeromatic SS-3 pH Meter. This solution was loaded on the column followed by 2 ml of water and then 0.324 M ethylenediamine. Fractions of 2.7 ml each were collected at a flow rate of 0.2 ml/min; 30 to 40 fractions contained amino acids. The amino acid concentration of each fraction was measured by ninhydrin (45, 46), and the rotatory measurements were made by diluting 1 ml of sample from the fractions with 1 ml of 5 M HCl and measuring the rotation at either 240 or 245 nm on a Jasco ORD/UV-5 spectrophotometer. From these data the amount of D isomer in each fraction was calculated. After each run the resin was washed with 4 M HCl, then 4 M NaOH, then water, and then removed from the column and reequilibrated with copper(II). The resin in the short column was treated similarly omitting the final copper equilibration.

Potentiometric Titrations

The titrations necessary to determine proton and metal stability constants were performed at 25°C with constant

stirring and a nitrogen flush in a 70 ml water-jacketed container. Carbonate-free base (stored in plastic bottles so that the air could be squeezed out) was added in equal increments of 0.05 or 0.10 ml from a Kimax 3197 5.00 ml buret calibrated to the nearest 0.01 ml. The pH of the solution was measured after each addition of base with a Beckman Zeromatic SS-3 pH Meter and its associated glass and calomel electrodes. Before each titration the meter was standardized versus two of three standard buffers (either 4.01 and 7.00 or 7.00 and 10.00) depending on the pH range to be spanned. Titration of a known concentration of nitric acid with a known concentration of sodium hydroxide after standardization of the meter showed less than 0.03 pH units difference between measured and calculated pH, so it was assumed that the meter read hydrogen ion concentration directly between pH 2 and 10.

For determining metal ion stability constants, equal molar quantities of $Cu(NO_3)_2$ and optically pure samples of either N-benzyl-N-carboxymethyl- or N-carboxymethylamino acids (as either a solution or a solid) together with sufficient 0.200 M KNO_3 and doubly distilled water to make 40.00 ml of solution and an ionic strength of 0.10 M (based on copper, potassium, and nitrate ions assuming no initial complex formation) were mixed together to prepare titrating solutions. Initial copper and amino acid concentrations were typically 7×10^{-3} M. Using 0.16 M NaOH, the solution was titrated until all

replaceable protons were neutralized. This generally occurred before a pH of 7 was reached. Then one equivalent of either the D or L isomer of an amino acid was added as a 0.1 M solution, and the titration continued. For determining proton stability constants, the solutions were prepared and titrated similarly, except that no $Cu(NO_3)_2$ was present. The concentration of most solutions used to prepare the titrating solutions were either calculated from the weight of the compound dissolved (as in the case of amino acids and amino acid derivatives) or was determined by titrimetric standardization (as in the case of NaOH using potassium hydrogen phthalate or $Cu(NO_3)_2$ using ethylenediaminetetraacetic acid (43)). However, N-benzyl-N-carboxymethyl-L-leucine and N-carboxymethyl-L-glutamic acid were too sparingly soluble to prepare solutions of a convenient concentration, and so they were weighed as a solid before each titration. Solutions of N-benzyl-N-carboxymethyl-L-alanine and N-carboxymethyl-L-aspartic acid were prepared at less than 0.1 M concentrations, generally about 0.025 M.

Data Treatment

Data from the titration of an amino acid (HA) or an amino acid derivative (H_mAD) with NaOH in the absence of copper(II) were used to calculate a set of proton stability constants for one of the two following types of reactions:

$$H + H_nAD \underset{}{\overset{K}{\rightleftharpoons}} H_{n+1}AD \qquad (2)$$

or

$$H + A \overset{K}{\rightleftharpoons} HA \qquad (3)$$

where the charges have been omitted for convenience. Up to four such equilibria might be considered for one derivative (m=4), but in practice the values of the equilibrium constants (K) differed sufficiently so that some equilibria could be considered independently while others needed only to be considered in groups of two.

An equation relating the proton equilibrium constants to the experimentally measured variables was derived in the following manner. The step-wise proton stability constants (totaling m in number) were defined as

$$K_n = [ADH_n]/[ADH_{n-1}][H] \qquad (4)$$

where ADH_{n-1} is the conjugate base of ADH_n; AD_t was defined as the total concentration of AD present in solution, i.e.

$$AD_t = \sum_{n=0}^{m} [ADH_n] \qquad (5)$$

and H_t was defined as the total titratable hydrogen concentration present in solution, i.e.

$$H_t = H + \sum_{n=0}^{m} n[ADH_n]. \qquad (6)$$

Equations 5 and 6 were then rearranged to give the following:

$$AD_t = [AD](1 + \sum_{n=1}^{m} \beta_n H^n), \tag{7}$$

where

$$\beta_n = \prod_{i=1}^{n} K_i \tag{8}$$

and

$$H_t - H = [AD](\sum_{n=1}^{m} n\beta_n H^n). \tag{9}$$

Equation 7 was divided by Equation 9

$$AD_t/H_t - H = 1 + \sum_{n=1}^{m} \beta_n H^n / \sum_{n=1}^{m} n\beta_n H^n \tag{10}$$

and this was rearranged to

$$H_t - H + \sum_{n=1}^{m} \beta_n H^n [(H_t - H) - nAD_t] = 0. \tag{11}$$

Each point in a titration produced enough calculated and measured data to substitute for all the terms in Equation 11 except the β_n's. These equations were solved in groups of one or two equations with the aid of an IBM 360. The β's thus calculated were used to calculate the individual K's,

$$K_n = \beta_n/\beta_{n-1}. \tag{12}$$

When dealing with metal ion stability constants, it was always possible to consider only one equilibrium at a time, either Equation 13 or 14.

$$Cu + AD \overset{K_f}{\rightleftharpoons} Cu(AD) \qquad (13)$$

$$Cu(AD) + A \overset{K_x}{\rightleftharpoons} Cu(AD)(A) \qquad (14)$$

An equation relating measured variables to the stability constant K_f was developed in the following manner. The same notation as before was used, plus Cu_t was defined as the total copper concentration

$$Cu_t = Cu + Cu(AD) \qquad (15)$$

Two expressions for the average number of ligands per copper(II) were written in terms of Cu_t and A_t.

$$\bar{n} = Cu(AD)/Cu_t = [Cu][AD]K_f/\{[Cu] + [Cu(AD)]\} =$$

$$[AD]K_f/\{1 + [AD]K_f\} \qquad (16)$$

$$\bar{n} = AD_t - AD(1 + \sum_{n=0}^{m} \beta_n H^n)/Cu_t \qquad (17)$$

Equations 16 and 17 were set equal to each other, rearranged, and solved for K_f to give

$$K_f = [AD_t - AD(1 + \sum_{n=1}^{m} \beta_n H^n)]/A[Cu_t - AD_t + AD(1 + \sum_{n=1}^{m} \beta_n H^n)] \qquad (18)$$

The values for AD were obtained from a rearrangement of Equation 7:

$$AD = AD_t/(1 + \sum_{n=1}^{m} \beta_n H^n) \qquad (19)$$

Each point in a titration yielded enough data to substitute for all the terms in Equation 18. Thus each titration gave a set of stability constants whose average was reported by the computer. Substitution of [Cu(AD)] for [Cu] and [Cu(AD)(A)] for [Cu(AD)] in Equations 15 through 19 gave an expression for the calculation of K_x.

The computer calculated a mean and a standard deviation for each stability constant. Four titrations were performed for each combination of metal, derivative, and amino acid; and the four stability constants were averaged. The standard deviation of the four stability constants was also calculated. Calculations were performed on only the middle 60% of a titration because the first and last 20% were discarded as unreliable.

Whenever a titration proceeded above pH 7, base was required to titrate the water. This consumption of base was corrected for by adding extra base to the usual 0.05 ml increment. The amount to be added was determined by titrating 40.00 ml of 0.100 M KNO_3 with 0.01 ml aliquots of the standard base and recording the pH produced by each addition of base. Thus, whenever a normal titration reached a pH above 7, the

extra base it needed was dictated by the KNO_3 titration.

Preparation of NMR Samples

The following procedure was used to prepare samples of nickel(II) complexes for nmr studies. A 1:1 molar ratio of 5×10^{-4} moles of an N-carboxymethylamino acid and an amino acid was weighed out in a beaker. An equal amount of $Ni(NO_3)_2$ was added as a 0.5 M solution. While stirring the mixture, enough 1.0 M NaOH was added to neutralize all of the acid protons. As much of the water as possible was removed by water aspirator vacuum at 50°C. The sample was dissolved in 0.8 ml D_2O to make about a 0.5 M solution. This sample was then delivered into a 5 mm nmr tube and capped. Before running the spectrum, sodium 2,2-dimethyl-2-silapentane-5-sulfonate was added as a standard.

Because of the possible oxygen susceptibility of cobalt(II) complexes of amino acids, all solutions were prepared in a nitrogen atmosphere. The solids were weighed out in small screw-capped vials with the lids on loosely and then placed in a vacuum desiccator along with any apparatus which would come in contact with the cobalt samples. The desiccator was evacuated, and then prepurified nitrogen gas was bled in. Everything was then transferred to a dry bag which was then flushed with nitrogen. Deoxygenated cobalt(II) (0.5 M) and NaOH (1.0 M) solutions were then added to the vials

by syringes to prepare the desired complexes. With the caps loosened, the vials were transferred back to the desiccator and dried under vacuum. An infrared lamp was used to speed the evaporation process. Once dry, the samples were dissolved in 0.5 ml of D_2O in the dry bag. Drying and dissolution (this time to 0.8 ml) were repeated. Finally, a small amount of sodium 2,2-dimethyl-2-silapentane-5-sulfonate was added, and the solutions were placed in nmr tubes and capped.

Operation of the Varian Associates' HA-100

Each paramagnetic sample was recorded using two different recorders and recording techniques. The first of these used the instrument in HR mode and its recorder. To switch to HR from HA mode the automatic Y gradient on the Field Homogeneity Unit (VK3531B), the Frequency Counter (V4315), and the lock, spectrum amplitude, sweep mode, and manual oscillator on the Internal Reference Controller (V4354A) were all turned off. The Horizontal-External Sensitivity on the oscilloscope was set from 200 μ sec/cm to 1 v/cm. The attenuation on the Fixed Frequency Unit (V4311) was set at 54 d b, and the mode on the Mode Selector Panel (V4391) was turned to HR. Finally, the fine and coarse dials on the Linear Sweep Unit (V4352A) were set to their maximum readings. In order to bring TMS to the center of the scope, the magnetic field was decreased using the Slow Sweep Unit (V3507) set at 2 x 100. The sample was

then placed in the Probe (V4333A), the attenuation was set up at 34 db, the Receiver Gain on the RF Unit was changed to 3, and the Probe was balanced by turning the red and blue paddles until a minimum reading on the meter on the RF Unit was achieved. The Function knob on the Integrator/Decoupler Unit (V3521A) was changed to "spectrum", the sweep rate on the Slow Sweep Unit was changed to 0.4 x 100, and the coarse adjustment on the Linear Sweep Unit was set to "off". The oscilloscope's vertical position adjustment was set to remove the trace from the scope. Using the Slow Sweep Unit, the field was scanned upfield until the last peak was passed, then the recorder sweep speed was set at 500 seconds, and finally both recorder and sweep were begun at the same time. The peak amplitude was adjusted by the output controls on the Integrator/Decoupler Unit. Calibration was achieved by using the sidebands at 2,500 Hz.

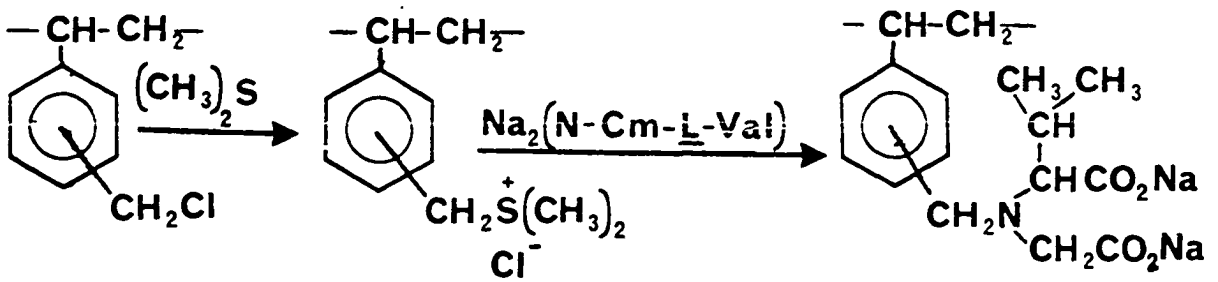
The second recorder was an external recorder with a d. c. source for its baseline. In order to use it the following changes were made once the instrument was in the HR mode. The cable at the back of the oscilloscope marked "vert" was disconnected. Jack J314 on the back of the RF Unit was disconnected and replaced with one which connected to the right-hand terminals on the oscilloscope. The external recorder (Varian Associates' G-10) was connected to the Recorder Output on the RF Unit, a Hewlett-Packard 4204A oscillator was

connected to the Modulation Input terminals on the RF Unit, the AC Sweep was disconnected, the Frequency Response dial on the RF Unit was turned to 2, and the Receiver Gain was set to 3. The Linear Sweep Unit was turned off, the trace on the oscilloscope was moved off the screen, and the Recorder Level was set as required to adjust the height of the spectra on the paper. As the Recorder Level was increased and/or the phase angle adjusted, the paddles on the Probe were adjusted to keep the recorder on scale. Once the detector phase was set properly, the spectrum was run by sweeping with the Slow Sweep Unit at 0.6 x 100. Calibration was done by the sideband technique using the external oscillator set at 3,000 Hz. After running the spectra, the instrument was returned to normal HA mode and locked on TMS.

RESULTS

Column Chromatography

One major problem in attempting to substitute an amino acid onto a polystyrene-divinylbenzene copolymer is solubility. The copolymer is permeable to solvents such as methylene chloride or dioxane, while the amino acids to be substituted on the copolymer are water soluble. The problem can be alleviated either by altering the copolymer to make it more hydrophilic or by altering the amino acids to make them more hydrophobic. Both approaches to the problem were used, and the second one generally gave higher yields of the desired product. Under the first method, the chloromethyl groups of the copolymer were reacted with dimethyl sulfide in an alcohol-water solvent to give a sulfonium ion product, which left the copolymer permeable to polar solvents. The amino acid group displaced the dimethyl sulfide by nucleophilic attack at the benzyl carbon. The main disadvantage of this method is the



(20)

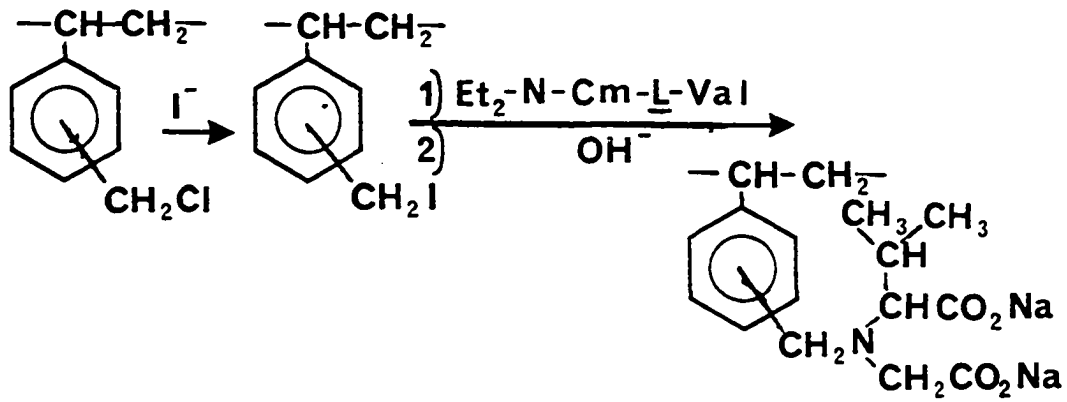
high pH of the solution which must be maintained in order to guarantee deprotonation of the nitrogen of the amino acids. The concentration of hydroxide at this pH is sufficient for it to successfully compete with the amino acid at the reaction site. Thus a sizeable portion of the chloromethyl functions were substituted by hydroxyl groups instead of amines. This successful competition is reflected in the low yields as disodium N-carboxymethyl-L-valinate reacted with the sulfonium ion to give at best a 28% yield. Disodium iminodiacetate produced a maximum yield of 58%, probably reflecting a lower steric crowding compared to the valine derivative because the isopropyl side chain is replaced by hydrogen.

The alternate method is to derivatize the amino acid such that it is compatible with organic solvents. The ester derivatives were chosen because they are fairly simple to synthesize, but Rogozhin et al. employed the amide derivatives of amino acids in the synthesis of substituted polystyrene-divinylbenzene copolymers (47). They reacted leucinamide in aqueous dioxane at 40°C for an optimum yield of substituted copolymer.

It is advantageous for good yields to convert the chloromethyl groups into iodomethyl groups since iodine is a superior leaving group compared to chlorine. Both Hirsch et al. (44) and Korshak et al. (48) employed this procedure, and the substitution of iodine for chlorine has been effected

in this work under similar conditions. The Russians (47) also found that the substitution of iodine for chlorine before reaction with the amino acid amide or ester is unnecessary. The same yields were obtained by adding sodium iodide to the reaction mixture of copolymer and amino acid ester or amide as when the exchange was carried out separately.

After the treatment of the copolymer with sodium iodide and then amino acid ester, hydrolysis of the esters in NaOH-ethanol (95%) gives the desired product.



(21)

The yields for the halogen exchange were greater than 90% for both the 0.8%- and the 1.8%-divinylbenzene copolymer. The reaction of the 1.8%-divinylbenzene copolymer with diethyl N-carboxymethyl-L-valinate gave a 62% yield while the same reaction with 0.8%-divinylbenzene copolymer produced a 91% yield. The higher percentage of divinylbenzene (cross linkage) reduces the reactivity of the copolymer. The particles of the 1.8%-divinylbenzene copolymer broke down during the hydrolysis step, and a column packed with this resin refused to flow under gravity pressures. Reactions

between the iodomethylated polystyrene-1.8%-divinylbenzene copolymer and triethyl N-carboxymethyl-L-aspartate gave 90% yields generally, but when packed in a column the resin expanded to such an extent during elutions with ethylenediamine that the column sealed itself off. Thus, even though the resin produced by the sulfonium route had a lower density of active sites, it was the only one which could be used due to the flow characteristics of the ones produced using the esters.

In an attempt to avoid the difficulties encountered by using gel resins, the synthesis of a column packing using a macroreticular resin was attempted. The XAD-2 resin was first chloromethylated in yields comparable to those reported (44), but an exchange of iodine for chlorine gave low yields. The reaction of the iodomethylated macroreticular resin with diethyl iminodiacetate or diethyl N-carboxymethyl-L-valinate gave poor yields. Hirsch et al. (44) reported yields in excess of 90% for XAD-1 and diethyl iminodiacetate, but those could not be duplicated using XAD-2. The poor yields eliminated any hope of using the resin.

Initially the separations attempted on the column were conducted differently than described in the Experimental section. At first, it was decided to use a buffered eluent system in order to control the pH of the column operation. An ammonium carbonate-ammonium bicarbonate buffer was used because it could easily be removed from the amino acids under

vacuum by applying heat. With the column saturated with copper(II), elution with ammonium bicarbonate produced carbon dioxide bubbles on the column causing it to channel. To avoid this, an ammonia solution was run through the column to prevent any coordination of ammonia from the buffer to the copper(II) which would release acid. After this treatment, the ammonium carbonate buffer could be used.

The racemic amino acid samples were added in a buffer solution. As the amino acid eluted from the column, the normal pale blue color of the eluent darkened slightly, so the progress of the amino acid could be followed by absorbance methods. However, the copper(II) made rotational measurements impossible because it absorbs so strongly in the ultraviolet that the instrument could not detect any light passing through the solution. To remedy this, a second column was added in tandem with the first and packed with the same resin (in the sodium form) as in the first column. The smaller column removed the copper(II) from the eluent.

Results on the early runs showed that there was a good deal of tailing by the amino acids and that raising the pH of the buffer from 8.25 to 10.0 sharpened the peaks, but it seemed to do nothing to improve the separations. In order to eliminate the tailing, the buffer was replaced by an ethylenediamine solution which forced the amino acid to elute ahead of it but did not change the separation. The ethylenediamine

forced the amino acid from the column because the stability constant of an amino acid such as glycine with copper(II) is less than that for ethylenediamine (log K_x = 8.62 (49) for glycine compared to 10.72 (50) for ethylenediamine). However, the subsequent stability constants for ethylenediamine are small enough (log K_2 = 9.30, K_3 = 0.1 (50)) that it does not remove a majority of the copper(II) from the column, although it does remove more than the amino acids as noted by the increased rate of accumulation of copper(II) on the second column towards the end of a run. The concentration of the ethylenediamine was varied, and 6.5 ml of it per 300 ml of water (0.32 M) was decided as an optimum concentration because it was concentrated enough to elute the column efficiently (within 12 hours) yet dilute enough not to concentrate too much amino acid into the last few fractions. When using other columns the concentration of ethylenediamine will be a function of the density of sites on the column and would need to be adjusted from the one used here.

After ethylenediamine became the eluting solution, the procedure was changed so that the amino acid was partially neutralized in solution (3-5 ml) and loaded onto the column. Variation of the extent of neutralization by varying the pH of the amino acid solution from 8.5 to 11.1 improved the separations. For instance, at pH 10.0 the first one-eighth of the valine eluting from the column was 52% D isomer while at pH 11.1 this fraction contained 58% D isomer.

One of the original operating assumptions concerning the column was that the vast majority of the copper(II) remained coordinated to the N-carboxymethyl-L-valine group attached to the resin. Comparison of the β_1 for [Cu(L-Val)$^+$] (log β_1 = 8.19 (3) or 7.98 (51)) to the β_1 for [Cu(N-Bz-IMDA)] (log β_1 = 9.88 (52) or 10.61 (53)) seems to bear this out: [Cu(N-Bz-IMDA)] is favored over [Cu(L-Val)$^+$], so the similar [Cu(N-Bz-N-Cm-L-Val)] should also be favored. However, if one compares the β_2 for [Cu(L-Val)$_2$] (log β_2 = 15.18 (3) or 14.71 (51)) to the β_2 for [Cu(N-Bz-IMDA)(L-Val)$^-$] (log β_2 = 15.82 or 15.09, calculated from β_1 for [Cu(N-Bz-IMDA)] and K_2 for [Cu(N-Cm-L-Val)(L-Val)$^-$] (12) assuming that K_f for [Cu(N-Bz-IMDA)(L-Val)$^-$] and [Cu(N-Cm-L-Val)(L-Val)$^-$] are similar), it is obvious that the complexes [Cu(L-Val)$_2$] and [Cu(N-Cm-L-Val)(L-Val)$^-$] have similar stabilities at similar concentrations. Thus it would be possible for the neutral complex [Cu(L-Val)$_2$] to form and move through the column interacting less with the asymmetric ligand bound to the resin. Since bis(amino acidato)copper(II) complexes show no stereoselectivity in general, the relative concentrations of [Cu(L-Val)$_2$], [Cu(D-Val)$_2$], and [Cu(L-Val)(D-Val)] would be governed by statistics alone and exhibit no separation of D from L. This conclusion is qualitatively supported by the observation that the top of the column loses some of the blue copper color when the amino acid solution was passed through it. In order to reduce the formation of

[$Cu(Val)_2$], the copper(II) on the resin was reduced below saturation. The amount of copper(II) was varied from 60% to 100% of saturation, and the optimum separation was achieved when 9.5 to 11.0 mmoles of copper(II) were equilibrated with the resin (13.3 mmoles capacity) before packing the column. The subsequent column runs were made with 9.5 mmoles of copper(II), or about 72% of saturation.

Of the experimental problems faced in the column work, the flow characteristics of the different resins were the most difficult with which to deal. The swelling of the N-carboxymethyl-L-aspartic acid substituted polystyrene-1.8%-divinylbenzene copolymer and the loss of particle size of the N-carboxymethyl-L-valine substituted polystyrene-1.8%-divinylbenzene copolymer have already been mentioned. One other problem, the shrinking of the N-carboxymethyl-L-valine substituted polystyrene-0.8%-divinylbenzene copolymer, was encountered. When the amino acids norvaline, alpha-aminobutyric acid, leucine, phenylalanine, methionine, and threonine were eluted through the column, the resin contracted enough so that it channelled and significantly reduced the separations. In fact, only norvaline and alpha-aminobutyric acid showed any separations at all. The shrinking could be partially compensated for by vibrating the column during a run, effectively continually resettling the resin, but this was limited in its usefulness.

When separations were attempted as described in the experimental section and channelling did not occur, then the D isomer of the amino acid eluted before the L isomer in all cases. This is opposite to the order reported by Rogozhin and Davankov where L-proline eluted ahead of D-proline from an L-proline substituted resin (54). Figure 1 gives a graphic description of the flow and separation characteristics of the column operating under optimum conditions. Curve A traces the weight of valine in each fraction, and Curve B represents the percentage of D-valine in each fraction. Early fractions contained up to 80% D-valine while fractions towards the end contained up to 75% L-valine. The sharp drop in the weight of amino acid at fraction 32 (Curve A) is caused by the ethylenediamine forcing the last of the amino acid from the column. The ethylenediamine also accounts for the flat portion of Curve A. Without the ethylenediamine in the eluent the curve would be rounded, but the ethylenediamine forces the amino acid from the column at a constant rate and gives the flat curve.

A comparison of the extent of resolution of six different amino acids is presented in Table 3. After the ninhydrin and optical rotatory dispersion analyses had been measured on each fraction collected for one particular run, the analysis data were divided into eight approximately equal groups by weight, and the amount of D isomer in each of these groups was

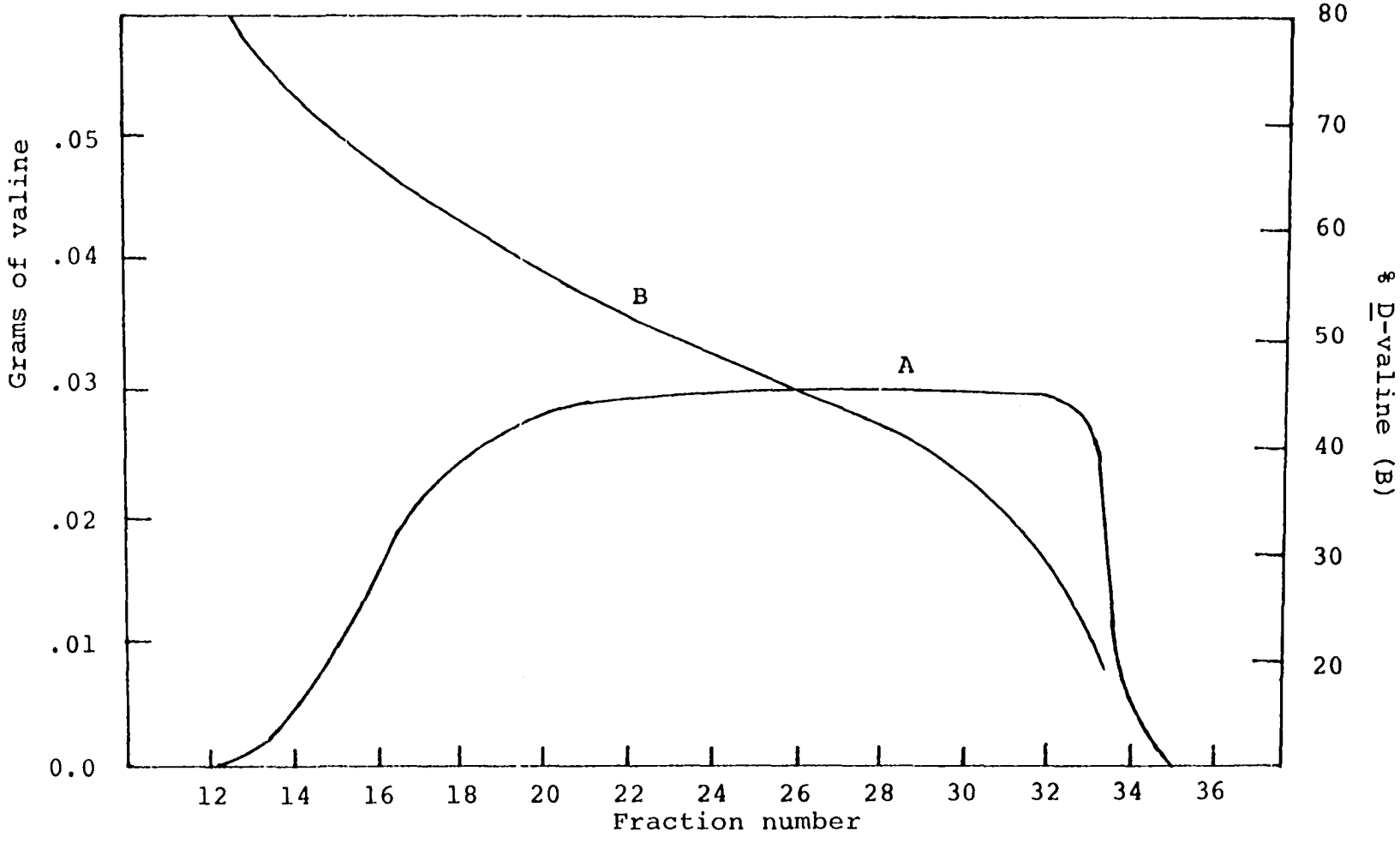


Figure 1. Graph of grams valine (A) or % D-valine (B) vs. fraction number

calculated. Column 1 (Group 1) represents the first one-eighth of the particular amino acid listed on the left of Table 3 to elute from the column, Column 2 (Group 2) represents the second one-eighth, etc.

Table 3. Percent of D-amino acid in each one-eighth of a chromatographic separation

Amino acid	Group 1	2	3	4	5	6	7	8
Isoleucine	75	66	59	53	47	42	34	30
Valine	70	61	58	52	48	44	42	31
Norvaline	65	49	48	48	48	49	49	49
Alpha-aminobutyric acid	63	52	50	49	47	47	48	46
Alanine	62	58	56	51	50	50	47	35
Proline	60	56	52	52	50	48	48	42

A comparison of Group 1 for each of the amino acids listed indicates that (with the exception of proline) as the size of the alpha-substituent on the amino acid decreases, the amount of D-amino acid decreases. That is, the smaller the side chain, the poorer the separations. This has previously been interpreted as being indicative of a direct interaction of the alpha-side chains (55), but this is not a reasonable explanation as was noted earlier (12). A complete discussion of the interactions appears in the Discussion section.

Of the amino acids studied, aspartic acid and glutamic acid did not channel the resin and failed to show any separation. A possible explanation for this involves the bis(amino acidato)copper(II) species. It has already been shown that [Cu(L-Val)$_2$] is of comparable stability to [Cu(N-Cm-L-Val)(L-Val)$^-$]. If, in the cases of aspartic and glutamic acids, [Cu(Asp)$_2^{2-}$] or [Cu(Glu)$_2^{2-}$] is favored over [Cu(N-Cm-L-Val)(Asp)$^{2-}$] or [Cu(N-Cm-L-Val)(Glu)$^{2-}$], then the bis(amino acidato)copper(II) complexes would pass through the column without interacting with the asymmetric group. Stability constants for the pertinent reactions are not available, so this explanation for the lack of separations for aspartic and glutamic acids is speculative.

Stability Constants

The preparations of the N-carboxymethylamino acids followed the general method of Leach and Angelici (40), but the products were isolated using chromatography instead of crystallization. The N-carboxymethylamino acids bind to the cation exchange resin in the protonated form ($HO_2CCH_2\overset{+}{N}H_2CH(R)CO_2H$), but ammonia easily displaces them. The amount of product displaced per volume of the ammonia is determined by the concentration of the ammonia. If the concentration of ammonia is high, enough of the product may be displaced to exceed its solubility in water. The result is precipitation

on the column, noted by white areas just ahead of the ammonia front. Thus, a lower concentration (0.25 M) was used for the less soluble derivatives.

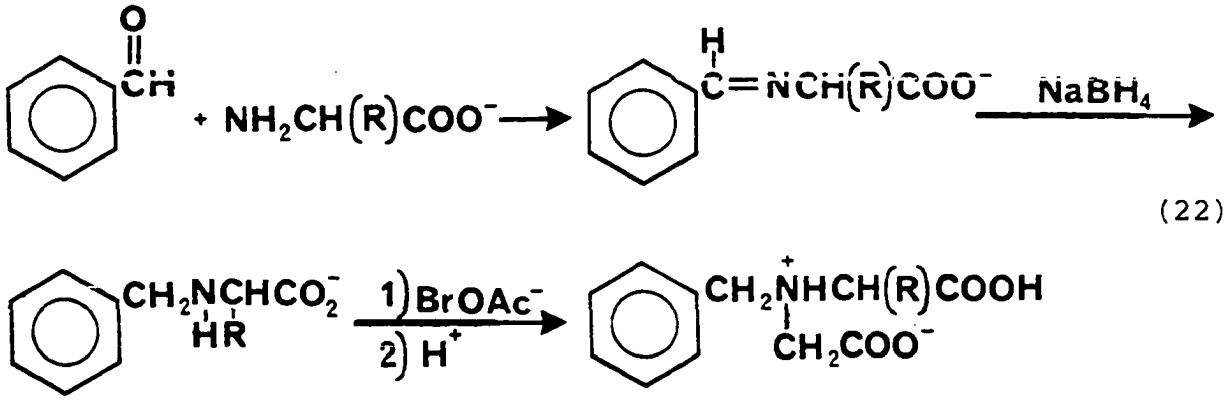
The column packing itself was 100-200 mesh, which gives an even flow. It is advantageous to use the X4 resins (4% crosslinkage) because higher crosslinked resins (X8) flow too slowly to be of much use in large amounts under gravity pressures. The X4 resins shrink a small amount when washed with HCl and subsequently expand as water is washed through them. This tends to pack the resin tighter and reduce the flow rate. To avoid this, the column was backwashed after each HCl and water wash.

The progress of the product on the column was followed both visually and potentiometrically. The product gave the column a lighter color than normal, while the ammonia gave it a darker color, and by these changes one could see the approximate location of the product. The pH measurements of the fractions located the product more precisely. After the pH dropped initially to 1 or less due to large amounts of hydrogen ions being displaced by sodium ions, it rose to around 5. Ideally it should have risen to 7, but a small amount (less than 5%) of the product eluted from the column with just water. As the majority of the product eluted, the pH dropped to between 2 and 4 after which it rose quickly when the ammonia came off. Any fraction which came after the

product started to elute and had a pH over 4 was either discarded or recycled because it probably contained ammonia.

Analysis of the product by titrating a weighed sample of it with a standard base indicated that up to 5% of the product could be in the sodium salt form instead of the acid form. This percentage could be reduced to less than 1% by a second run through the column, but this was unnecessary for the studies reported here.

The preparations of the N-benzyl-N-carboxymethylamino acids were accomplished in two steps. The first step was addition of benzaldehyde to the sodium salt of the amino acid in water to form the Schiff base and then reduction by sodium borohydride. Subsequently, the reaction of the N-benzylamino acid with bromoacetic acid in basic aqueous solution produced the product. This method was successful for alanine, phenylalanine,



(22)

and leucine, but it failed for valine. A reasonable explanation for this involves steric crowding by the substituent on the alpha-carbon of the amino acid because the size of the

alpha-group affects the reactivity of the nitrogen. When the alpha-substituent is hydrogen, one mole of the amino acid glycine reacts with two moles of benzaldehyde to form N,N-dibenzylglycine. With other amino acids where the substituent is methyl, isopropyl, isobutyl, or secondary-butyl (alanine, valine, leucine, or phenylalanine respectively), only one mole of benzaldehyde adds, implying that two large benzyl groups cannot easily crowd onto the nitrogen of an amino acid with the larger alpha-groups present. A study by Bowman compared the reactivities of various aldehydes and amino acids (56). He investigated the addition of aldehydes to amino acids through Schiff base formation and subsequent reduction by H_2 in the presence of palladized charcoal and found that only one mole of an aldehyde would add to valine under conditions that would result in two moles adding to glycine. He also found that once one branched aldehyde had been added to an amino acid, it was difficult to add any other aldehyde. Finally, his results showed in general valine to be less reactive than glycine, leucine, or phenylalanine. These results indicate that the size of an alpha-substituent affects the reactivity of an amino acid towards an aldehyde, and the isopropyl group on valine is the most effective in reducing the reactivity of the amino acid. Thus, the failure to produce N-benzyl-N-carboxymethyl-L-valine by the methods described here is probably due to steric interference by the isopropyl group.

This argument does not indicate that N-benzyl-N-carboxymethyl-L-valine cannot be synthesized, for it obviously was produced when the polystyrene resins were synthesized by both the sulfonium ion route and the ester route. Both of these routes were employed in attempted syntheses, and in some cases partial success was obtained. The most promising method was the reaction between diethyl N-carboxymethyl-L-valinate and benzyl bromide in acetonitrile. The desired product was formed in about 60% yield based on an nmr spectrum of the reaction mixture, but no obvious method for easily separating the starting ester, product, and side products was found. Thus, the investigation turned to other amino acids which were less crowded and hopefully more reactive. In retrospect, hydrolysis of the esters, both starting and product, followed by precipitation of the N-benzyl product with added HCl might have separated product and starting materials due to the relative insolubility of N-benzylamino acids. The amino acid could then be reesterified and used in another preparation. It is the ease of separation of the substituted polymers by filtration from the reactants and side products due to insolubility that made the resin synthesis much more practical than the preparation of N-benzyl derivatives of valine.

A list of the calculated proton stability constants along with some literature values for comparison is given in Table 4. There is general agreement between the calculated values and

Table 4. Log proton stability constants[a]

Compound	K_1	K_2	K_3	K_4	Ref.
Glycine	9.62	2.43			57[b]
IMDA	9.33	2.58			58
N-Bz-IMDA	8.90	2.24			59
N-Bz-IMDA	9.03	2.29	1.36		
N-Cm-L-Val	9.34	2.53	1.65		
L-Val	9.62	2.32			60[c]
N-Cm-L-Asp	9.82	3.80	2.44	1.65	
L-Asp	9.63	3.71	1.94		57[b]
N-Cm-L-Glu	9.28	4.16	2.49	1.56	
L-Glu	9.64	4.18	2.18		57[b]
N-Cm-L-Ser	8.89	2.54	1.70		
L-Ser	9.12	2.29			61[d]
N-Cm-L-Ileu	9.34	2.54	1.63		
L-Ileu	9.69	2.31			62
N-Bz-N-Cm-L-Ala	9.57	2.07	1.70		
N-Bz-N-Cm-L-Leu	9.09	2.14	1.70		
L-Val	9.63	2.39			
L-Thr	9.09	2.31			
L-Thr	9.03	2.29			61[d]
L-Leu	9.67	2.46			
L-Leu	9.69	2.36			62

[a] μ = 0.10 M KNO_3 and T = 25.0°C unless otherwise noted.

[b] μ = 0.10 M $NaClO_4$.

[c] μ = 0.01 M.

[d] μ = 0.20 M KNO_3.

those previously reported, except possibly in the case of N-benzyliminodiacetic acid. As was explained in the Experimental section, each reported stability constant is an average of more than seventy values. The standard deviations of these values for K_1 and K_2 are generally $\pm$.03 log units. However, the standard deviations for K_3 and K_4 may run as high as $\pm$.20 log units. This large variation is unavoidable for two reasons. First, K_3 and K_4 were calculated using the values for K_2 or K_3 respectively, so any uncertainty in these would be added into the calculations of K_3 and K_4. Second, K_3 and K_4 were calculated from data taken near a pH of 2.0 where the glass electrode is less dependable.

In all cases studied, substitution of an N-carboxymethyl group for a hydrogen on the amine function of an amino acid slightly decreases its base strength (log K_1 is smaller). This is most easily explained by an electron withdrawing effect by the N-carboxymethyl group on the nitrogen reducing its electron density and thus its attraction for a proton. At a first glance, the trend in the K_2's is just the opposite: the addition of an N-carboxymethyl group raises the apparent base strength of the acid function toward the addition of one proton (log K_2 is larger). However, in these molecules there are two acid functions which are chemically very similar, so the proton could reside on either one of them (excluding the alpha-carboxylates of N-carboxymethyl-L-aspartic and -L-glutamic acids). The result is that there are twice as many sites where

the proton may reside, so this will approximately double the stability constant (K_2). If one subtracts out log 2 (.3010) from each log K_2 value for the N-carboxymethylamino acids, it will be seen that the K_2's are now approximately equal to or a little smaller than the K_2's of the parent amino acids. Thus, the trend in the first acid proton stability constants (K_2's) is that they are equal to or less than the stability constant of the parent amino acid. There appears to be little effect of the N-carboxymethyl group upon the apparent acidity of the amino acid.

The values for K_3 (or K_4 for N-carboxymethyl-L-aspartic and -L-glutamic acids) indicate that there is no difference in the base strengths of the neutral molecules. This may be the actual case, but it is indefinite because the uncertainties in these values are too large for any minor variations in K_3 and K_4 to register a measurable change of any significance.

Titrations on 1:1 mixtures of copper(II) and N-carboxymethyl-L-valine, and 1:1:1 mixtures of copper(II), N-carboxymethyl-L-valine, and L-valine indicated that the two equilibria defined by Equations 13 and 14 could be considered separately. Justification for this is shown in Figure 2, a graph of pH *versus* volume of NaOH. Over the range of 2 to 3 equivalents of base added, the lower curve shows that L-valine adds to the previously formed [Cu(N-Cm-L-Val)] complex (formed from 0 to 2 equivalents) at a pH above the pH where copper(II)

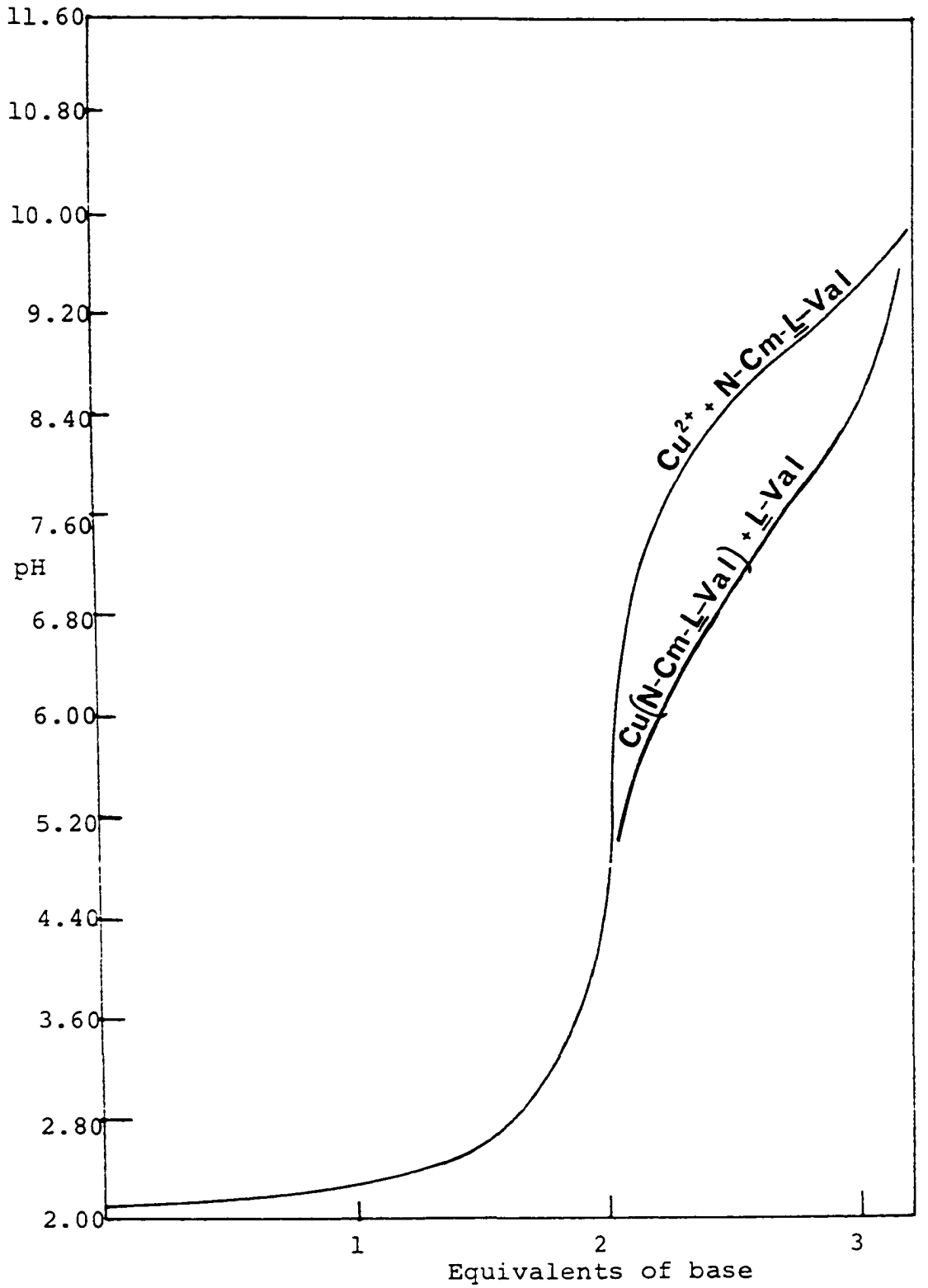


Figure 2. Titration curves of Cu^{2+} + N-Cm-L-Val and Cu(N-Cm-L-Val) + L-Val

and N-carboxymethyl-L-valine combine. Over the same range, the upper curve indicates hydroxo complex formation [Cu(N-Cm-L-Val)$(OH)^-$], but this occurs at a pH above the range where L-valine adds to [Cu(N-Cm-L-Val)]. Thus, it was not necessary to consider K_f and K_x simultaneously nor to consider hydroxo complexes in the calculations of K_f and K_x.

The calculated values of the stability constants for the reaction of copper(II) and either N-carboxymethyl- or N-benzyl-N-carboxymethylamino acids are listed in Table 5 along with some literature values. As with the proton stability constants, these are averages from four separate titrations, and the standard deviation for each value is listed in parentheses. Most of the derivatives listed exhibit similar constants, although N-benzyl-N-carboxymethyl-L-alanine exhibits an unexpectedly large constant. In general N-benzyl derivatives form less stable complexes than their parent group (see values in Table 5), but this is not found here as N-benzyliminodiacetic acid forms a slightly stronger complex than iminodiacetic acid.

The N-carboxymethyl derivatives of glutamic and aspartic acids exhibit an anomalous behavior complexed to copper(II) when compared to the other amino acid derivatives. This behavior is the formation of a protonated tridentate complex. In the case of the glutamic acid derivative, consistent stability constant values could only be obtained from the

Table 5. Log K_f values[a]

Compound	Log K_f	References
IMDA	10.63	63[b]
N-Bz-IMDA	9.88, 10.61	52, 53[c]
N-Bz-IMDA	10.73 (.03)	
Alanylglycine	5.44	64[d]
N-Bz-Alanylglycine	4.51	65[e]
Ethylenediamine	10.5	66
N-Bz-Ethylenediamine	9.43	67[f]
N-Cm-L-Ileu	10.68 (.06)	
N-Cm-L-Ser	10.93 (.06)	
N-Cm-L-Glu	10.56 (.21)	
N-Cm-L-Val	10.97 (.06)	
N-Bz-N-Cm-L-Leu	10.96 (.10)	
N-Bz-N-Cm-L-Ala	12.37 (.26)	

[a] μ = 0.10 M KNO_3 and T = 25.0°C unless otherwise noted; standard deviations of values given in parentheses.

[b] T = 20°C.

[c] μ = 0.10 M KCl.

[d] μ = 0.16 M KCl.

[e] μ = 0.12 M NaCl.

[f] μ = 0.1 M KCl, T = 20°C.

calculations when it was assumed that the carboxylate on the alpha-side chain remained protonated during complex formation with copper(II). This group ionizes only after the complex is formed, and it has a log K of 4.54 $\pm$ .09 (compared to 4.16

for the uncomplexed derivative). Thus, N-carboxymethyl-L-glutamic acid forms a tridentate complex with copper(II) initially, and the gamma-carboxylate becomes more basic in the complex than when the molecule was uncomplexed. In a similar situation, a protonated complex between copper(II) and nitrilotriacetic acid was reported (68). The stability constant was much lower than most aminopolycarboxylic acids (log K = 3.39), so it is probably the nitrogen which retains the proton.

The situation for N-carboxymethyl-L-aspartic acid is less convenient to handle. Neither treating the data as representing a tridentate nor a tetradentate ligand gave consistent stability constants. A reasonable explanation for this is that both the protonated (tridentate) and unprotonated (tetradentate) copper(II) complexes exist simultaneously in solution in equilibrium. A similar situation was noted by Wiegers *et al.* (2) in their study of copper(II) complexes of glutamic and aspartic acids. Similar to this work, their titration data failed to give consistent stability constants for either the tridentate or bidentate (protonated) complex. Thus they also concluded that both species exist together in solution.

The values in Table 6 are the stability constants for the reaction of either [Cu(N-Cm-D- or -L-amino acid)] or [Cu(N-Bz-N-Cm-L-amino acid)] with the D or L isomer of valine, threonine, or leucine. These amino acids were chosen because

Table 6. Log K_x values

Compound	Isomer	Valine	Threonine	Leucine
N-Cm-D-Val	L	5.51 (.03)	----------	----------
	D	5.57 (.02)	----------	----------
N-Cm-L-Ileu	L	5.32 (.00)	5.30 (.01)	5.46 (.03)
	D	5.31 (.01)	5.17 (.02)	5.35 (.01)
N-Cm-L-Ser	L	5.46 (.03)	5.27 (.04)	5.43 (.00)
	D	5.39 (.02)	5.25 (.06)	5.41 (.01)
N-Cm-L-Asp	L	4.24 (.02)	----------	4.23 (.02)
	D	4.39 (.03)	----------	4.37 (.02)
N-Cm-L-Val	L	5.34 (.05)	5.14 (.06)	5.44 (.01)
	D	5.30 (.02)	5.07 (.07)	5.35 (.03)
N-Cm-L-Glu	L	5.08 (.05)	----------	----------
	D	5.37 (.02)	----------	----------
N-Bz-N-Cm-L-Ala	L	5.53 (.01)	5.53 (.02)	5.63 (.03)
	D	5.45 (.03)	5.28 (.02)	5.63 (.03)
N-Bz-N-Cm-L-Leu	L	5.42 (.04)	5.28 (.04)	5.43 (.02)
	D	5.30 (.05)	5.09 (.04)	5.23 (.04)

the separations from the column work indicated that it is the larger alpha-substituent which exhibits the larger differences between D- and L-amino acids. The numbers in parentheses next to the stability constants are the standard deviations of those values. One will note that in some cases the difference between the stability constants for the D and L isomers for a particular amino acid is close to or less than the sum of the standard deviations. Thus one could conclude no difference exists rather than a small difference exists. However, in this study the consistency in the preference for the L isomer over the D isomer by the L derivatives of the neutral amino acids in both the questionable N-carboxymethyl derivatives and

the more certain N-benzyl-N-carboxymethyl derivatives increases the reliability of the conclusion that a difference does exist. The body of data acquires a reliability that an individual datum cannot.

The stability constants in Table 6 indicate that for the neutral amino acids (noncoordinating side chains) the copper(II) complexes of the N-carboxymethyl or N-benzyl-N-carboxymethyl derivatives form more stable complexes with amino acids of the same chirality rather than those of the opposite chirality. Specifically, L-valine, D-valine, L-isoleucine, L-serine, L-alanine, and L-leucine gave derivatives whose copper(II) complexes with valine, threonine, or leucine are more stable if the chiralities are the same rather than opposite. This is in general agreement with the work of Leach and Angelici (12), although they reported a preference by N-carboxymethyl-L-valine for D-valine. They also reported that the difference between the stability constants for the D and L isomer of a particular amino acid were about 0.41 to 0.81 log units. The data here disagree with those last two conclusions, and based upon consistency it is felt that the data reported here are correct and those data reported earlier are in error.

One definite exception to the general trend cited here is the N-benzyl derivative of proline. As concluded from both the column work (19) and stability constant work (69,13), [Cu(N-Bz-L-Pro)$^+$] coordinates D-proline, D-valine, and N-

benzyl-D-proline more strongly than the L-isomers. Proline is a unique amino acid with its ring spanning the nitrogen and alpha-carbon, so this may be the cause of its unexpected preferences.

Compared to the derivatives of the neutral amino acids, an opposite preference was observed by the N-carboxymethyl derivatives of L-aspartic and L-glutamic acids. That is, copper(II) complexes of N-carboxymethyl-L-aspartic or -L-glutamic acids coordinate D isomers more strongly than L isomers.

Stability constants for [Cu(N-Cm-L-Asp)$^-$] with valine and leucine differ from the other mixed constants reported in Table 6 by being about a factor of ten smaller. A probable cause for this would be electrostatic repulsion between the negative charges of the complex and the amino acidates. Complexes such as [Cu(N-Cm-L-Val)] are neutral, so the repulsion would be less. A similar effect is seen in the comparison of the stability constants for the addition of glycine to [Cu(NTA)$^-$] (log K = 5.36) or to [Cu(IMDA)](log k = 5.87)(70). Again the formally negative complex has a lower stability constant than the neutral one toward the same ligand.

The complex [Cu(N-Cm-L-Glu)$^-$] does not follow this reasoning in that it formally has a negative charge yet its mixed ligand complexes with amino acidates show stability constants larger than for [Cu(N-Cm-L-Asp)$^-$]. The weakly

coordinating nature of the side chain group has already been mentioned concerning the tridentate nature of this ligand. Thus, the negative charge on the side chain carboxylate may not be transferred to the metal as well as in [Cu(N-Cm-L-Asp)$^-$], so the repulsion is lower. There must be some positive interaction though between the side chain and the metal because of the similarities in selectivity between the two complexes.

A comparison of the extent of the differentiation between the L and D isomers by the derivatives is given in Table 7. For each combination of amino acid and derivative, the log K_x for the D isomer is subtracted from the log K_x for the L isomer, and then all these values for one derivative are averaged. These averages indicate that the N-benzyl derivatives prefer the L over the D isomers to a greater extent than the plain N-carboxymethyl derivatives. They also show that the N-carboxymethyl derivatives of neutral amino acids with bulky side chains differentiate between L and D better than less bulky groups (isoleucine > valine > serine). Finally, N-carboxymethyl-L-aspartic acid prefers D over L as well as N-benzyl-N-carboxymethyl-L-leucine prefers L over D. The value for the glutamic acid derivative represents only one difference and its position in the list may change with more data.

Table 7. Average values of log K_x(L) - log K_x(D)

Compound	log K_x(L) - log K_x(D)
N-Bz-N-Cm-L-Leu	+0.17
N-Bz-N-Cm-L-Ala	+0.08
N-Cm-L-Ileu	+0.08
N-Cm-L-Val	+0.07
N-Cm-L-Ser	+0.04
N-Cm-D-Val	-0.06
N-Cm-L-Asp	-0.15
N-Cm-L-Glu	-0.29

Proton Nuclear Magnetic Resonance Study of Paramagnetic Complexes

The compounds used in this study were already available either from commercial sources or from preparations described in the Experimental section. The nickel(II) complexes were prepared by adding the correct volume of a metal salt solution to previously weighed solids and neutralizing with standard NaOH. It was at this point that a solubility problem appeared. Solutions which were approximately 0.5 M in metal ion and amino acid formed a precipitate upon neutralization if the alpha-side chain were particularly hydrophobic. For instance, 1:1 mixtures of $Ni(NO_3)_2$ and phenylalanine or leucine precipitated immediately upon addition of sufficient base to

neutralize the amino acids, while valine, methionine, and isoleucine solutions precipitated over a time span of one to eight hours. Mixtures of glycine, alanine, serine, threonine, alpha-aminobutyric acid, or 2-methylalanine and $Ni(NO_3)_2$ remained in solution indefinitely. With the exception of IMDA, N-carboxymethyl-L-aspartic, and -L-glutamic acids, all the IMDA-like compounds (N-carboxymethyl derivatives) formed precipitates with $Ni(NO_3)_2$. Addition of a second ligand such as glycine prevented the formation of the precipitate long enough to prepare the solutions and obtain their spectra. It appears that complexes with a charge remain in solution longer than neutral ones.

The nature of the precipitates has not been thoroughly investigated, but they appear to be neutral complexes of nickel(II). The precipitate formed when 10^{-3} moles of $Ni(NO_3)_2$, L-valine, and NaOH were mixed together was mulled with Nujol or Fluorolube, and the infrared spectrum of each was run. The spectra obtained were more characteristic of the sodium salts of amino acids than of the zwitterion or hydrochloride forms (71). The precipitates were not wetted by water and appeared to be a polymeric material. This information plus the observations that charged complexes of this type tend to remain in solution and neutral ones precipitate lead to the tentative conclusion that it is the bis(amino acidato)nickel(II) or N-carboxymethylamino acidatonickel(II) complex which

precipitates. Similar precipitates were seen in the cobalt(II) solutions.

As was explained in the Experimental section, the spectra of the paramagnetic complexes were run by two different techniques. The first employed the usual HA-100 recorder and operated the instrument in an unlocked mode (HR). This method of operation had acceptable sensitivity, but the side bands which appeared as negative peaks could and did obliterate some small peaks. In order to locate these peaks if present, an external recorder was used to record the spectra. This eliminated the side bands, but since one amplifier was bypassed in the process, the sensitivity was lower. It proved difficult to locate some of the broad peaks among the noise especially at low fields using this method. Thus the two methods were used to complement each other.

During this study more than 200 spectra were recorded of various nickel(II) or cobalt(II) complexes of amino acids, their derivatives, and combinations of the two. The majority of these were nickel(II) complexes because, as was noted in the Introduction, cobalt(II) complex spectra are not easily related to structures as are the nickel(II) spectra. It is quite possible that once enough work has been done on cobalt-(II) complexes, then more can be said about structure. But for now, only the nickel(II) spectra offer insight into the structure of the complexes.

The contact shifts and peak assignments for a series of 1:1 nickel(II)-amino acid complexes are listed in Table 8 along with some reported values for comparison. These are not the observed shifts, but rather the observed shift minus the diamagnetic shift. In essence the contact shift indicates how far a particular proton has been shifted from its diamagnetic position. The diamagnetic positions were determined under basic conditions and taken from a publication of the TCU Research Foundation (72).

Table 8. Nickel(II) contact shifts of some amino acids[a]

Amino acid	α-H	β-H	γ-H	Reference
Glycine	-60	---	---	29
Glycine	-61	---	---	
Alanine	-43	-18	---	29
Alanine	N.O.	-18	---	
Alpha-amino-butyric acid	-30	-24,-14	-1.0	
Serine	-31	-16	---	
Threonine	-32	-29	-1.5	
2-Methylalanine	---	-16	---	29
Aspartic Acid	-140	+8.2	---	29
Aspartic Acid	-144	+8.1	---	

[a]Shifts are in parts per million (ppm).

In general the contact shifts reported here agree with those of Reilley et al., and the trends in the shifts are consistent with their interpretation. That is, the more equatorial a proton is relative to the chelate ring, the larger its contact shift. Thus, as the alpha-substituent gets larger, it should become more equatorial in nature and the alpha-proton more axial in nature. The resulting change in shifts should be more downfield and more upfield respectively. For example, the contact shifts of the alpha-protons for glycine, alanine, and alpha-aminobutyric acid are -60, -43, and -30 ppm, respectively, while the contact shift for the beta-protons of alanine and the average of the two beta-protons of alpha-aminobutyric acid are -18 and -19 ppm, respectively.

The fact that the contact shift for the beta-proton of threonine does not follow the trend set by alanine, serine, and alpha-aminobutyric acid leads into the well-investigated problem of possible coordination by the hydroxyl group of serine and threonine. A series of stability constants for serine and threonine with six different metal ions determined by Sharma (73) indicated that serine forms slightly more stable complexes than threonine. A similar study, which also included alpha-aminobutyric acid, concluded that the copper(II), nickel(II), zinc(II), and cobalt(II) complexes of serine and threonine are of similar stability, but, with the exception

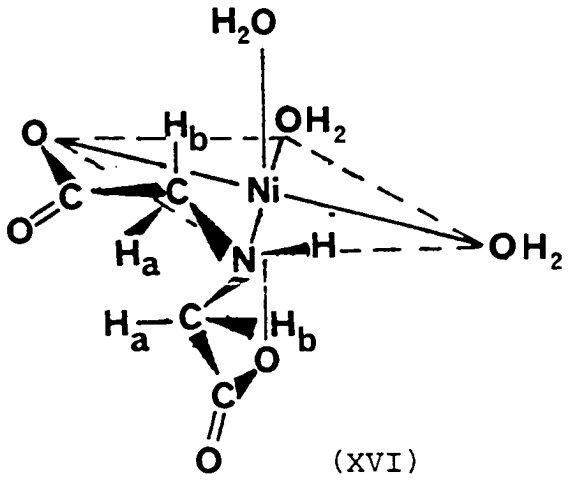
of copper(II), are more stable than the complexes of alpha-aminobutyric acid (74). Copper(II) complexes of serine, threonine and alpha-aminobutyric acid are of equivalent stability. Tsangaris and Martin used visible circular dichroism to investigate copper(II) complexes of aspartic acid, serine, and threonine (75). They concluded that aspartic acid and threonine show possible axial coordination but that serine does not.

The beta-proton of threonine resonates at a much lower field than expected from the position of its alpha-proton. Considering the contact shifts of the alpha- and beta-protons of alpha-aminobutyric acid and serine compared to the alpha-proton of threonine, the threonine beta-proton should exhibit a contact shift of about -18 ppm instead of -29 ppm. This downfield shift could indicate a delocalization of spin through the hydroxyl oxygen, thus implying partial coordination to the nickel. Serine appears to show no such behavior. The nmr data leads to a conclusion consistent with Tsangaris and Martin but possibly not with the stability constant work. However, if threonine coordinates only as strongly as a water molecule, then the stability constant data will not reflect the coordination through a larger constant. Thus, this work is not inconsistent with the results of Sharma, Gergely et al.

The uncertainties in the shifts vary depending on the position of the observed peaks. Those peaks which appear

within ten ppm of sodium 2,2-dimethyl-2-silapentane-5-sulfonate usually have peak widths of one ppm, while those further downfield may be much broader and have peak widths of twenty ppm. Estimating the position of these peaks is difficult so that the uncertainty in the position may be up to five ppm.

The contact shifts for a series of N-carboxymethylamino acids complexed with glycine to nickel(II) are listed in Table 9. The assignments were made using reasoning similar to Erickson et al. (30) and Pratt and Smith (26). When the IMDA chelate coordinates with its carboxylates cis (facial), the two protons on each methylene are inequivalent (H_a and H_b),



(XVI)

but one proton in each methylene has an equivalent one in the other methylene (see Structure (XVI)). This is a result of two of the protons (H_a) pointing towards each other (inside) and the other two (H_b) pointing away (outside). The protons pointing in have more equatorial nature than those pointing

out, and thus they should exhibit a larger contact shift. This is observed in [Ni(IMDA)] which gives two resonances, one near -90 ppm and one near -25 ppm.

Substitution of one of the protons gives an N-carboxymethyl derivative. Because of potential crowding if the added group is pointed in, the substituted group should prefer a position pointing out (an axial group), and this should eliminate one proton from the upfield resonance (H_b) and replace it with one or more new resonances. It is also unlikely that the two inside protons will remain equivalent, so a doubling of the downfield resonance could result. These effects were observed by Pratt and Smith (26) with N-carboxymethylalanine. The IMDA resonance near -25 ppm lost intensity and moved to -21 ppm; a new resonance occurred at -9 ppm (the methyl group of the alanine), and the resonance near -90 ppm split into two equal ones at -79 ppm and -100 ppm. Since one of the methylene protons of the N-carboxymethyl group moved upfield to -21 ppm, the other one would be expected to move downfield. Thus it is assigned as the -100 ppm peak. The alpha-proton of alanine is the -78 ppm peak. With the exception of N-carboxymethyl-L-aspartic acid where the N-carboxymethyl resonance failed to split, the same effects were observed in general for all the compounds in this study.

Table 9. Contact shifts of the N-carboxymethylamino acid ligand in [Ni(N-Cm-AA)(Gly)$^{1,2-}$] complexes[a]

Ligand	α-H	β-H	γ,δ-H	N-Cm	Reference
N-Cm-L-Asp	-145	+10.5, 6.5	---	-62	--[b]
N-Cm-L-Asp	-115	+10.3, 7.2	---	-54	
N-Cm-L-Val	-60	-11.6	-0.6, -1.4	-97, -12.8	
N-Cm-L-Glu	-89	-24	-6.9	-79, -36	--[b]
N-Cm-L-Glu	-85	-22	-6.6	-71, -45	
N-Bz-N-Cm-L-Ala	-61	- 6.1	---	-90, -11.9	
N-Cm-L-Ileu	-61	-10.3	-.30	-98, -15.1	
N-Cm-L-Ser	-89	- 7.5	---	-16 -98	
	N-Bz	o-H	m-H	p-H	
N-Bz-N-Cm-L-Ala	-5.3	∓.26	-1.67	-.24	

[a]Shifts in ppm.

[b]Complexes without glycine, i.e. [Ni(N-Cm-AA)$^-$].

The contact shifts in Table 9 allow some comparisons to be made between the N-carboxymethyl derivatives. Comparing the alpha-protons of the parent amino acids, the contact shifts decrease in the order aspartic acid > glutamic acid ∿ serine >> isoleucine ∿ alanine ∿ valine. This series indicates that the

alpha-protons become increasingly more axial on the average, and thus it follows that the alpha substituents become more equatorial on the average.

A comparison of the two methylene resonances of the N-carboxymethyl group for the same series indicate that they become less equivalent. The methylene protons of the aspartic acid derivative give one line, the glutamic acid derivative two lines separated by 26 ppm, and the other three derivatives two lines with a separation of about 85 ppm. Thus one proton is becoming more axial and the other more equatorial, which implies that the N-carboxymethyl ring is becoming more puckered. On the average this ring must be essentially planar in the aspartic acid case, but as the alpha-substituent on the amino acid moves more equatorial (becomes more hydrophobic), the N-carboxymethyl ring becomes more puckered.

As was noted earlier, some of the N-carboxymethyl derivatives had to be complexed to nickel(II) in the presence of glycine for solubility reasons, and the question of the effect of the glycine on the derivative ligand arises. This effect is believed to be minimal for two reasons. First of all, the observed shifts for separate glycine and IMDA complexes of nickel(II) are -65 and -96 and -26 respectively. Upon complexing both of them to the same nickel(II), the observed shifts are -62 and -19, -27, -88, and -97. There is a small effect on one of the IMDA rings as the shifts change from -26

and -96 ppm to -19 and -88 ppm, but the other ring remains much the same (-27 and -97 ppm). Secondly, a comparison of the shifts for a particular N-carboxymethylamino acid complexed with various amino acids or a comparison of the shifts for one particular amino acid complexed with various N-carboxymethyl-amino acids reveals virtually no variations. The resonances differ only slightly from complex to complex, and the amino acid resonances are close to those of the 1:1 complexes (see Table 8). Since little or no variation was observed, a list of those resonances will not be given.

Of all the complexes studied, only one exhibited any observable isomerization. This occurred in [Ni(N-Cm-L-Asp)(L-Ala)$^{2-}$] where a shoulder appeared on the downfield side of the methyl resonance of the alanine. The two peaks presumably correspond to the cis and trans isomers of the complex; the upfield peak is cis and the lower one is the trans isomer. This assignment was made on the basis of reasoning that will be developed in the Discussion section.

The assignment of the resonances of N-benzyl-N-carboxy-methyl-L-alanine were made by comparing the observed resonances and assignments of hexakis(benzylamine)nickel(II) tetra-fluoroboride made by Fitzgerald and Drago (76). They assigned the observed resonances at -5.83, -8.71, and -5.75 ppm as the ortho-, meta-, and para-protons respectively. The meta-proton appears further downfield than the ortho- or para-protons

because the pi system of the ring enters into the delocalization of the electrons from the metal, and typically this form of delocalization alternates the sign of the contact shift instead of retaining one sign. Thus the pi contribution at the ortho- and para-positions is negative and positive at the meta-position. On this basis, the meta-proton in [Ni(N-Bz-N-Cm-L-Ala)(Gly)$^-$] was assigned to be the peak observed at -9.00 ppm. On the basis of relative intensities, the ortho-proton was assigned as the -6.97 ppm peak and the para-proton as the -7.57 ppm peak. This is a reversal of the ortho-para ordering of Drago et al.; however, the two peaks differ by only 0.08 ppm in the benzylamine complex and only 0.60 ppm in the N-benzylamino acid case. Considering that the solvent of Drago et al. was organic and that water was used in this study, the inconsistency is not considered to be serious.

One final comment should be made comparing the spectra of nickel(II) versus cobalt(II) complexes. Although the cobalt(II) spectra cannot easily be interpreted in terms of structure, the peaks are much sharper than the nickel(II) complexes. Whereas only one example of isomerization was seen for nickel-(II), the better resolution of cobalt(II) offers the possibility of observing diastereomers in equilibrium as did McDonald and Phillips in [Co(Hist)$_2$] (8).

DISCUSSION

The previous sections of this dissertation have attempted to present facts in the forms of a brief review from the current literature on particular subjects, experimental procedures, and experimental results. An attempt was also made to limit explanations and interpretations to generally accepted direct arguments of cause and effect. It will be the purpose of this section to discuss the experimental results further by interrelating the results of different techniques and by proposing a model for the system. By its nature this section will have more elements of speculation, but it will still be based on the results reported in the previous section.

A quantitative comparison can be made of the separations from the column chromatographic work and the stability constants. An equation relating the length of a column (expressed in band widths of the eluted substance) to the stability constants for the column towards the substance being eluted can be developed in the following manner. If α is designated as the separation factor, it is defined as

$$\alpha = (\bar{L})(D)/(L)(\bar{D}) \qquad (23)$$

for optical isomers where the bar means that isomer is bound to the resin. Thus, α will be greater than one if L is preferred over D. One can define

$$\varepsilon = \alpha - 1 \tag{24}$$

and the number of band widths ν is related to it by:

$$\nu = 1 + \varepsilon N_o/\varepsilon \tag{25}$$

where N_o is the mole fraction in the original mixture of the substance which elutes first (77). If ν equals four, and if a complete separation of two substances is to be achieved, then the column will need to be four times the length of the column packing originally occupied when the mixture was first loaded onto the column.

In terms of a column which contains the copper(II) complex of N-benzyl-N-carboxymethyl-L-valine group as the optically active site and DL-valine as the substance loaded on the column, the separation factor takes on the form

$$\alpha = [Cu(N\text{-}Bz\text{-}N\text{-}Cm\text{-}L\text{-}Val)(L\text{-}Val)][D\text{-}Val]/$$
$$[Cu(N\text{-}Bz\text{-}N\text{-}Cm\text{-}L\text{-}Val)(D\text{-}Val)][L\text{-}Val]. \tag{26}$$

If this expression is multiplied in the numerator and denominator by [Cu], the following results:

$$\alpha = [Cu(N\text{-}Bz\text{-}N\text{-}Cm\text{-}L\text{-}Val)(L\text{-}Val)][D\text{-}Val][Cu]/$$
$$[Cu][L\text{-}Val][Cu(N\text{-}Bz\text{-}N\text{-}Cm\text{-}L\text{-}Val)(D\text{-}Val)] \tag{27}$$

which is nothing more than

$$\alpha = K_L/K_D. \tag{28}$$

Substituting (28) into (24) and that result into (25), one obtains

$$\nu = 1 + N_o(K_L/K_D - 1)/(K_L/K_D - 1). \qquad (29)$$

Thus, if one knows the stability constant for the interaction of each species in solution with the resin phase, it is possible to calculate the theoretical number of band widths through which the original band needs to be washed to achieve a separation.

Since N-benzyl-N-carboxymethyl-L-valine was not synthesized, it is impossible to make a calculation directly comparing the column separations and stability constants. However, using the stability constants for D- and L-valine reacting with [Cu(N-Bz-N-Cm-L-Leu)], one can make an approximate comparison. Taking the appropriate constants from Table 6 ($\log K_L = 5.42$, $\log K_D = 5.30$) and an initial mole fraction of 0.50 (N_o), one must elute through at least 3.62 band widths. The original band widths were generally 4.2 mmoles, so a column of 15.2 mmoles would be needed at a minimum. Since the column was only 13.3 mmoles long, it is obvious that no complete separations would have been achieved. Although it is negative agreement, no complete separations were ever achieved which does agree with the calculated figures.

Using another approach, one can calculate an effective separation factor from the separation achieved for a complete

column run. Using the valine data from Table 3, the first half of the run contained 60% D-valine and 40% L-valine, which yields a separation factor of 2.25. Since the column is 13.3 mmoles long and one band width is 4.2 mmoles, the number of band widths is 3.15. The actual separation factor is related to the apparent one by

$$(\alpha)^{3.15} = 2.25. \qquad (30)$$

Solving for α, the actual separation factor is 1.30. The separation factor calculated from the stability constants is 1.32. Considering the approximations that were made, i.e. the substitution of [Cu(N-Bz-N-Cm-L-Leu)] for [Cu(N-Bz-N-Cm-L-Val)] and the assumption that the solution equilibria approach the resin equilibria, there is good (possibly surprising) agreement between the column work and the stability constants.

Now that the agreement between two of the methods has been hopefully established, a model will be proposed through which the results from each method may be interrelated. The model in this case will be a proposed structure for the copper(II) and nickel(II) complexes which are involved in the studies. Through this proposed structure the consistency between the three experimental methods and their results can be established.

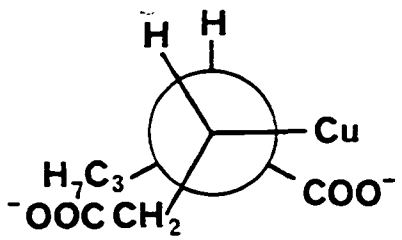
The model was developed mainly from the stability constant work, so much of the data used to establish the structures

come from those experiments. The amino acid derivatives with noncoordinating side chains most likely coordinate to copper-(II) in a meridional fashion due to the Jahn-Teller distortion of that ion. Models of this complex show that an amino acid entering the coordination sphere by coordinating the amine group in the plane and the carboxylate axially will experience an essentially symmetrical environment because the two asymmetric centers (carbon and nitrogen) are too far away for any interaction. However, if one of the carboxylate groups were to move from a meridional position to an axial one leaving two uncoordinated sites in the copper(II) plane, then an amino acid entering with its amine group cis to the amine group of the derivative would be close enough for some interaction.

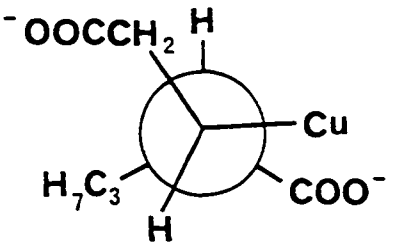
There is some evidence available to indicate that the postulation of an rearrangement is justified. A model of the meridional isomer indicates that the substituents on the alpha-carbons will be eclipsed, while the facial isomer allows the groups to avoid each other through puckering in the rings. This may help to explain the observation by Legg and Cooke that the facial isomer of [Co(dien)(IMDA)$^+$] is favored over the meridional one by 2 to 1 (78). Thus it appears that the facial isomer would be preferred were it not for the distorted metal ion, so the rearrangement seems to be feasible.

There are no data to indicate whether it is the N-carboxymethyl or the amino acid carboxylate which shifts from

meridional to axial positions, and in fact either one could. But whichever one it is that makes the shift, it has only one of the two axial positions to move to. This is a result of the nitrogen being asymmetric. There are two possible conformations of the nitrogen, and these are shown in the Newman projections (XVII) and (XVIII). Viewing from the nitrogen towards the asymmetric carbon, Structure (XVIII) has less



(XVII)



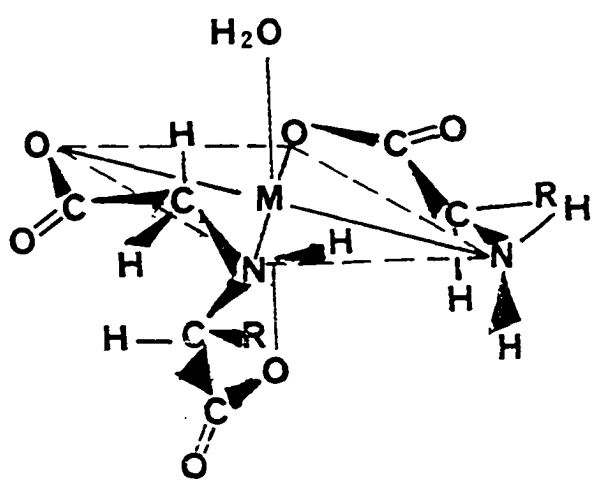
(XVIII)

steric crowding than (XVII) because the protons are near large groups instead of each other. Thus, one of the two possible conformations of the nitrogen is preferred, and the result is that each of the two carboxylates has but one axial position to move to because it would require an inversion of the nitrogen to reach the other axial position. The usual facile inversion of nitrogen is impossible with it coordinated to the copper(II) ion.

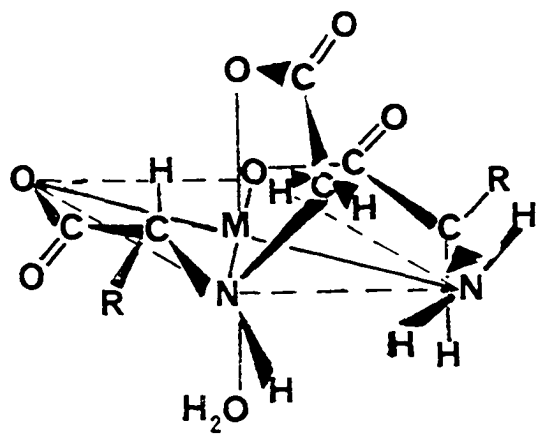
The amino group of the incoming amino acid will coordinate either *cis* or *trans* to the nitrogen of the derivative, and from available data it appears that either one may occur depending on the system and conditions. Freeman *et al.* (79) reported a *cis* structure for $[Cu(Gly)_2(H_2O)]$, but the *trans* isomer can also exist for this complex as well as for $[Cu(Gly)_2(H_2O)_2]$ (80). A similar situation occurs for the copper(II)-alanine system. Gillard *et al.* reported that the *trans* isomer of $[Cu(Ala)_2]$ forms initially (81), but the *cis* isomer forms if the *trans* is shaken in water implying that the *cis* may be thermodynamically more stable (82). Only the *cis* isomer has been reported for $[Cu(L\text{-}Ser)_2]$ (83), and in the mixed complex [Cu(Hist)(Thr)] the two amino acids are *cis* (84).

Gillard and Laurie attempted to determine the *cis*-*trans* nature of some bis(amino acidato)copper(II) complexes in solution by comparing their solid state and solution optical rotatory curves (85). The identical curves for $[Cu(L\text{-}Ileu)_2]$, $[Cu(L\text{-}Val)_2]$, and $[Cu(L\text{-}Tyr)_2]$ in both solid state and solution led them to conclude that the structures were the same. They then cited a crystal structure of $[Cu(L\text{-}Ala)_2]$ (86) which showed *trans* nitrogens. Thus, it was concluded that the other three complexes were *trans* also. However, a crystal structure by Weeks *et al.* (87) on $[Cu(Ileu)_2]$ reported the structure as *cis*, casting doubt on the conclusions by Gillard *et al.*

No clear-cut consensus on the isomeric nature of the complexes exists, but for the benefit of the discussion to follow the cis structure will be assumed; it is at least as probable as the trans. The two proposed structures for [Cu(N-Cm-L-amino acid)(L-amino acid)$^-$] are shown in Structures (XIX) and (XX). There are two proposed structures because the question of which carboxylate shifts has not been answered.



(XIX)



(XX)

The structures for the corresponding nickel(II) complexes could be developed in a similar manner with the exception that requiring a change from meridional to facial is unnecessary because the nickel(II) complex most likely forms the facial isomer initially upon coordination of the amino acid

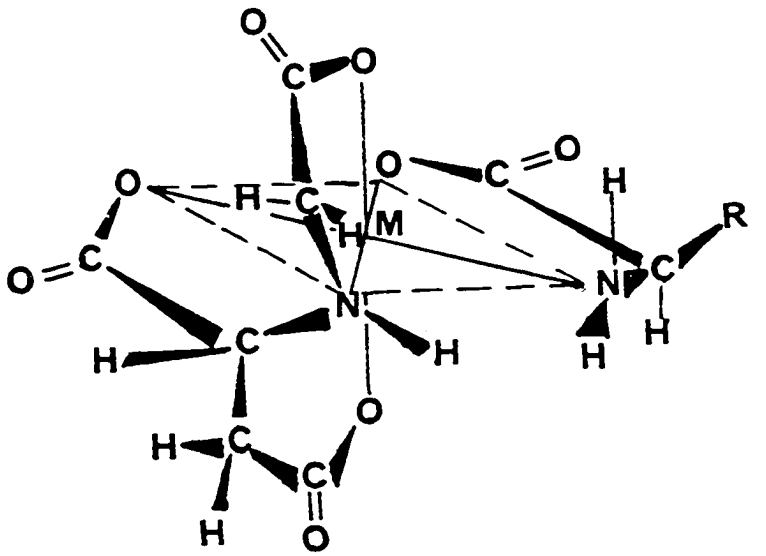
derivative. The problem of cis or trans nitrogens is no better answered for nickel(II) than for copper(II) as both [Ni(Hist)$_2$ $(H_2O)_2$] (88) and [Ni(L-Ser)$_2(H_2O)_2$] (89) are cis, yet [Ni(Gly)$_2(H_2O)_2$] is trans (90). Thus, the Structures (XIX) and (XX) could apply equally well to nickel(II).

The proposed site for the interaction between the two ligands is the amine protons. When the chiralities of the amino acid and the amino acid derivative are the same and the bulky groups on each ligand take on an equatorial position, then the amine protons on the amino acid and on the derivative will be staggered. If the chiralities are opposite, then the amino acid chelate ring conformation will be enantiomeric relative to the previous case, and one of its amine protons will eclipse the amine proton of the derivative. Thus, there is more steric interference when the chiralities are opposite than when they are the same, and copper(II) and nickel(II) complexes of L-amino acid derivatives with noncoordinating side chains should prefer L-amino acids over D-amino acids. For copper(II) both the column work and the stability constants support this.

As was noted earlier, the N-carboxymethyl derivatives of L-aspartic acid and L-glutamic acid form copper(II) complexes which prefer D-amino acids over L-amino acids. This preference can be explained in terms of the model already developed. The main difference between those derivatives discussed first and

these of aspartic and glutamic acids is the coordinating ability of the side chain. Whereas the alpha-substituents of the neutral amino acids are hydrophobic and noncoordinating, the substituents of the acidic amino acids are hydrophilic and coordinating. The result is that neutral substituents will be equatorial as they tend to move away from the metal while acidic substituents will be axial as they move to coordinate to the metal. In terms of Corey and Bailar's terminology (32), the chelate ring of the amino acid changes from k to k'; that is, it inverts (see Structures (I) and (II)). As a result the position of the amine proton is changed as though the chirality of the alpha-carbon had inverted. The N-carboxymethyl derivative thus acts as though it were D instead of L, and thus its copper(II) complexes prefer D-amino acids over L-amino acids through the same interactions of the amine protons as described previously. The proposed structure for [Cu(N-Cm-L-Asp)(L-amino acid)$^{2-}$] is shown in Structure (XXI).

The reports of resolving DL-proline using a polystyrene-divinylbenzene copolymer (19) and determining the stability constants for the reaction of [Cu(N-Bz-L-Pro)$^{+}$] and valine (13) indicate that [Cu(N-Bz-L-Pro)$^{+}$] prefers to coordinate D-amino acids over L-amino acids. Although the arguments are not so clear cut as before, the previously developed model can be used to explain these results. In the previous cases the conformation of the chelate rings were fairly well defined

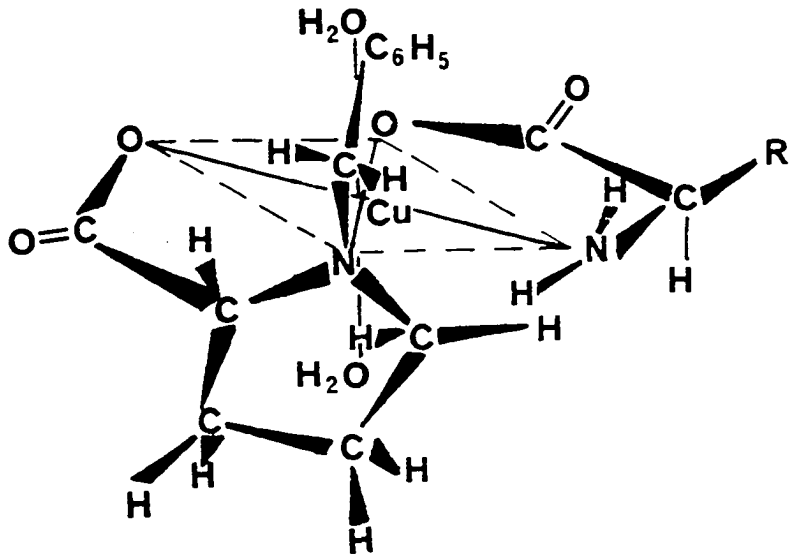


(XXI)

by the axial-equatorial nature of the alpha-substituent. This is not true with N-benzyl-L-proline complexes of copper(II). If the ligand adopts a conformation where the benzyl carbon is very axial relative to the proline chelate ring, then there will be steric interference between the amine protons of the amino acid coordinated cis to the derivative and protons in the cyclic group of proline when the chiralities are the same. However, if the benzyl carbon moves to a generally equatorial position, the ring has changed so that interference occurs when the chiralities are opposite. The first conformation produces a conclusion consistent with the observed data, while the second produces a conclusion opposite the observed results.

There is some indirect support for the structure where the benzyl carbon is axial rather than equatorial. From the nmr work of Ho and Reilley (27), the methyl protons on N-methylethylenediamine resonate at -135 ppm while the methylene protons on the N-ethyl group of N-ethylethylenediamine resonate at -70 and -75 ppm indicating that these protons are more axial than the N-methyl ones. Pratt and Smith (26) reported that the methyl protons on N-methyliminodiacetic acid resonate at -105 ppm, yet the methylene protons on the N-ethyl group of N-ethyliminodiacetic acid were found at -70 ppm. Finally, Fitzgerald and Drago (76) assigned the benzyl protons in $[Ni(benzylamine)_6(BF_4)_2]$ to the peak at -38.3 ppm, while the same protons in [Ni(N-Bz-N-Cm-L-Ala)(Gly)$^-$] were assigned to a peak at -9.00 ppm. The first two sets of shifts indicate that the carbons next to the nitrogen become more axial as the group becomes larger. The third set of shifts shows that the benzyl group is more axial when attached to a chelate ring rather than just an amine group. These conclusions from the shifts seem to support a structure where the large benzyl group is axial, and this conformation would predict a selectivity which agrees with the selectivity observed by Davankov and Rogozhin (18,19). The arguments are not conclusive because they have ignored bonding changes which can affect the chemical shifts by changing the amount of spin delocalized onto the chelate ring. A proposed structure consistent with these

arguments is given in (XXII) for [Cu(N-Bz-L-Pro)(L-amino acid)].



(XXII)

The discussion presented so far has treated the stereochemistry in terms of rigid structures and an either-or attitude. This is a simplification of the situation, for the chelate rings are not rigid, but rather are in constant motions which change their conformations. There is both theoretical and experimental evidence for the dynamic nature of the complexes. Ho and Reilley (28) postulated that the Ni(II) complex spectra they were observing were a weighted average of the shifts for two conformational extremes. They reasoned that the conversion from the k to k' forms was fast on the nmr time scale, and thus only an average of the environments of the k and k' forms was observed. To substantiate

this they recorded a series of spectra at various temperatures. The relative distribution of the k and k' forms should change as the temperature changes, and the observed shifts should change also. This was found; as the temperature was raised, the shifts of the protons tended to move toward the shift of the less-favored of the two conformations.

From a theoretical point of view, Gollogly et al. have investigated the chelate ring in an effort to calculate energies involved in its interactions. In one study (91) they estimated that 5 to 7 kcal/mole are needed to convert the k form to the k' form for ethylenediamine. This amount of energy is low enough that on the nmr time scale the rings are constantly flexing as it should take a barrier of 12 to 15 kcal/mole to keep the rings from inverting. Thus, Gollogly et al. support the conclusions of Reilley et al.

These studies require that the description of the complexes be revised to include the dynamic characteristics of the system. Thus, one should speak of a group being axial or equatorial a majority of the time realizing that it is very possible that it is equatorial or axial a part of the time.

In the Results section two lists were presented. One was a comparison of the extent of separating ability (Table 7), and the other was a list of alpha-proton contact shifts. A comparison of the two will show them fairly similar. The former orders the N-carboxymethyl derivatives as glutamic

acid > aspartic acid > serine > valine > isoleucine > N-benzylalanine, while the latter orders the same derivatives as aspartic acid > glutamic acid ∿ serine > isoleucine ∿ N-benzylalanine ∿ valine. It is no accident that they are essentially the same. Earlier it was noted that amino acids with larger alpha-substituents resolved to a greater extent. However, it has also been postulated that it is not the alpha-substituents which interact in the stereoselectivity, so there must be a link between the size of the alpha-substituent and the amine proton interactions. The link is provided by the above two lists: the decreasing differentiating ability for the opposite over the same chirality by the derivatives parallels the decrease in the equatorial nature of the alpha-proton. The change from equatorial to axial corresponds to an inversion of the amino acid chelate ring from the k' to k forms, and thus changes the optical preference for the amino acid.

The reason that the large alpha-substituent results in better separations is that the amino acid will remain in either the k or k' form for a larger percentage of the time than if the substituent is small. IMDA prefers neither the k or k' form, but rather populates both equally. Thus, the contact shifts for [Ni(IMDA)] of -21 and -91 ppm are indicative of an alpha-proton which is equally in the k and k' form on the average. An alpha-proton which resonates at fields

greater than -91 ppm is indicative of a ring in the k form, while one which resonates at a lower field than -91 ppm is in the k' form. Thus, the alpha-proton resonance of -60 ppm for [Ni(N-Cm-L-Val)] indicates that it should prefer L-amino acids (both rings k), while the -115 ppm for [Ni(N-Cm-L-Asp)$^-$] indicates it should prefer D-amino acids (both rings k'). This is of course supported by the experimental results for copper(II).

There appears to be an inconsistency for the N-benzyl-N-carboxymethyl-L-alanine results because it exhibits a much larger DL differentiation than would be expected on the basis of the size of the alpha-substituent. One would expect an N-carboxymethyl derivative with a simple methyl group to be a poor differentiator and give an alpha-proton resonance near -90 ppm. The better separation characteristics of the complex could be explained on the basis of the large benzyl group increasing the steric interference between derivative and amino acid (92). However, this does not account for the relatively high alpha-proton resonance. Therefore, it must be concluded that the benzyl group not only increases the steric interference, but that its size also prevents the ring from inverting from k to k' as often as it would without the benzyl group. This is confirmed by comparing the alpha-proton contact shift of N-carboxymethylalanine (-75 ppm) to the corresponding N-benzyl derivative (-61 ppm). Thus the ring

remains k for a longer time and can exhibit a preference for amino acids of similar chirality.

These conclusions concerning N-benzyl-N-carboxymethyl-L-alanine imply that the polystyrene-divinylbenzene copolymers do more than serve as a support for the copper complexes. The amino acid derivatives are linked to the resins through a benzyl linkage, and these inert groups facilitate the separations through the effects discussed in the above paragraph. This was also concluded by Davankov and Rogozhin in their work with L-proline substituted polystyrene resins (19).

It is now possible to explain the assignment of the cis and trans isomers to the two methyl resonances of [Ni(N-Cm-L-Asp)(L-Ala)$^{2-}$]. The L-alanine is not the preferred isomer, so the steric interactions of the cis amine protons should work to reduce the equatorial nature of the methyl group since maximum interference should occur with an equatorial methyl. The trans isomer would have no such interference, and so the methyl would be in its normal equatorial position. In comparing the methyl resonances, one would expect the trans isomer to show the lower field peak while the cis isomer would give a resonance slightly upfield due to its reduced equatorial nature. Thus, the main peak is assigned to the cis isomer and the shoulder on the low field side is assigned to the trans isomer. In agreement with the crystal structures, it appears that both cis and trans isomers are possible, and fortunately

for some of the arguments presented here the cis is predominant over the trans.

Since this type of isomerization was not observed in any other spectra, one may wish to conclude that no trans isomers exist in these others. This is a dangerous conclusion because the resolution may have been too poor to see the isomers. Even in the case cited here, the less favored isomer appeared as a shoulder on the main peak. So, both isomers probably exist in the other cases, but the peaks were too poorly resolved to observe them.

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