Table IV. Distances (Å) and Angles (deg) Corrected for Rigid-Body Motion

Bond distances		Bond angles	
C^{α}–C	1.537	C–C^{α}–N	110.0
C^{α}–N	1.497	C–C^{α}–C^{β}	111.2
C^{α}–C^{β}	1.534	C–C^{α}–H^{α}	108.4
C^{α}–H^{α}	1.091	N–C^{α}–C^{β}	109.8
C–O^1	1.249	N–C^{α}–H^{α}	107.0
C–O^2	1.266	C^{β}–C^{α}–H^{α}	110.4
N–H^1	1.032	C^{α}–C–O^1	118.3
N–H^2	1.050	C^{α}–C–O^2	116.0
N–H^3	1.034	O^1–C–O^2	125.7
C^{β}–$H^{\beta 1}$	1.096	C^{α}–N–H^1	111.2
C^{β}–$H^{\beta 2}$	1.097	C^{α}–N–H^2	109.3
C^{β}–$H^{\beta 3}$	1.096	C^{α}–N–H^3	108.9
		H^1–N–H^2	108.3
		H^1–N–H^3	110.8
		H^2–N–H^3	108.3
		C^{α}–C^{β}–$H^{\beta 1}$	110.1
		C^{α}–C^{β}–$H^{\beta 2}$	110.3
		C^{α}–C^{β}–$H^{\beta 3}$	110.1
		$H^{\beta 1}$–C^{β}–$H^{\beta 2}$	108.5
		$H^{\beta 1}$–C^{β}–$H^{\beta 3}$	108.6
		$H^{\beta 2}$–C^{β}–$H^{\beta 3}$	109.1

with a mean-square translation along the principal axis C^{α}–N of 0.011 (4) Å^2 and a mean-square rotation around the axis of 0.0106 (13) rad^2. The effective translations for the screw tensor are again small. The corrected distances and angles are given in Table IV.

An estimate of the librational frequencies ν and the barrier heights V_0 can now be obtained for the two groups. A harmonic oscillator approximation to an n-fold cosine-hindered rotor with $n = 3$ was used.[8] The potential function was described as $V(\alpha) = {}^1/_2V_0(1 - \cos n\alpha) \approx {}^1/_4V_0n^2\alpha^2$, where α is the angle of rotation around the principal axis. The mean-square rotation is then given by $\langle\alpha^2\rangle = h/(8\pi^2I\nu)\coth(h\nu/2KT)$, where I is the moment of inertia of the rotor and the frequency ν is given by $\nu = (n/2\pi)(V_0/(2I))^{1/2}$. For the methyl group we found $V_0 = 5.6$ kcal/mol and $\nu = 309$ cm^{-1}, and for the ammonium group we found $V_0 = 20$ kcal/mol and $\nu = 600$ cm^{-1}. The precision of the barrier heights is about 20% for the methyl group and about 35% for the ammonium group. The effect of the hydrogen bonds in raising the ammonium group barrier is dramatic.

Torsional angles are given in Table V. IUPAC–IUB conventions[13] have been used. The difference between the angles ψ^1 and ψ^2 is 180°, showing the group C^{α}–C–O1–O2 to be planar.

Table V. Torsion Angles (deg)

$\phi^{1\,a}$	58.3 (2)
ϕ^2	177.8 (1)
ϕ^3	−64.0 (2)
$\chi^{1\,b}$	57.6 (2)
χ^2	177.3 (2)
χ^3	−62.2 (2)
$\psi^{1\,c}$	−18.6 (1)
ψ^2	161.5 (1)

[a] ϕ^i is the torsional angle C–C^{α}–N–H^i. [b] χ^i is the torsional angle N–C^{α}–C^{β}–$H^{\beta i}$. [c] ψ^i is the torsional angle N–C^{α}–C–O^i.

Acknowledgment. One of us (M. S. L.) gratefully acknowledges the support from Statens Naturvidenskabelige Forskningsråd, Copenhagen, Denmark.

(13) IUPAC–IUB Commission on Biochemical Nomenclature, *J. Mol. Biol.*, **52**, 1 (1970).

Partial Resolution of Amino Acids by Column Chromatography on a Polystyrene Resin Containing an Optically Active Copper(II) Complex[1]

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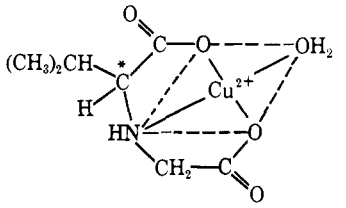
Abstract: A labile metal complex, (*N*-carboxymethyl-L-valine)copper(II), which had been previously shown to coordinate L-amino acids more strongly than their D enantiomers, has been chemically bound to a styrene–0.8% divinylbenzene copolymer. The resulting ligand-exchange resin has been used chromatographically to partially resolve several optically active amino acids. In all cases, the D enantiomers eluted first, and the degree of resolution increased with an increase in the bulkiness of the side chain on the α-carbon of the amino acid.

Chromatographic resolutions of optically active amino acids have been attempted on a wide variety of asymmetric sorbents.[4,5] By far the best resolutions have been achieved by gas chromatography of volatile amino acid derivatives,[6] but this technique is not easily adapted to resolutions on a preparative scale. In principle, such preparative resolutions could be carried out by liquid chromatography, but in practice they have been only partially successful. After the work

(1) Presented at the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., April 1, 1971.
(2) National Science Foundation Trainee, 1968–1969.
(3) Fellow of the Alfred P. Sloan Foundation, 1970–1972.
(4) D. R. Buss and T. Vermeulen, *Ind. Eng. Chem.*, **60** (8), 12 (1968).
(5) S. V. Rogozhin and V. A. Davankov, *Russ. Chem. Rev.*, **37**, 565 (1968).
(6) See, for example, J. C. Dabrowiak and D. W. Cooke, *Anal. Chem.*, **43**, 791 (1971); J. A. Corbin, J. E. Rhoad, and L. B. Rogers, *ibid.*, **43**, 327 (1971), and references therein.

reported in this paper was completed, Rogozhin and Davankov[7] reported the complete resolution of D,L-proline on a sorbent containing an L-proline complex of copper(II) bound to a styrene–divinylbenzene copolymer. Prior to this work, labile, optically active metal complexes had not been used to resolve amino acids.

Previously we reported[8] that the complex (*N*-carboxymethyl-L-valine)copper(II), Cu(L-ValMA)



exhibits stereoselectivity in its complexation of optically active amino acids and their esters. Stability constants, *K*, for the reaction

$$\text{Cu(L-ValMA)} + \text{A}^- \rightleftharpoons \text{Cu(L-ValMA)(A)}^- \qquad (1)$$

were generally larger for the L-amino acidates (A^-) than for the D enantiomers. This was found for the amino acidates of leucine, phenylalanine, alanine, and serine. The stability constants for D- and L-valine, however, were reversed. We have repeated the stability constant determinations for valine and find that the earlier results are in error. The new data indicate that the stability constant for L-valine is the same or slightly larger than that of the D enantiomer.

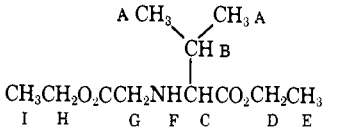
Since Cu(II) complexes are labile and the equilibrium given in eq 1 is established very rapidly, it seemed possible that the stereoselectivity of this reaction could be used to resolve optically active amino acids. In the present communication, we report the synthesis of a styrene–divinylbenzene copolymer containing Cu(L-ValMA) groups and its use in the partial resolution of enantiomeric amino acids.

Experimental Section

Materials. Two chloromethylated styrene–divinylbenzene copolymers (50–100 mesh) were used in the asymmetric resin syntheses. One of these (PS–0.8% DVB) contained 0.8% divinylbenzene and 15.7% Cl (4.4 mmol of Cl/g of copolymer), and the other (PS–1.8% DVB) contained 1.8% divinylbenzene and 2.6% Cl (6.1 mmol of Cl/g of copolymer). Optical rotations were measured on a Jasco ORD/UV-5 spectrophotometer.

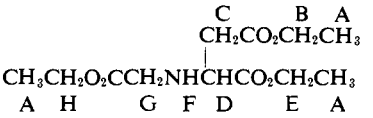
Preparation of *N*-Carboxymethyl-L-valine. Following a preparation outlined by Leach,[9] 1.55 g (13.2 mmol) of L-Val and 1.84 g (13.2 mmol) of bromoacetic acid were dissolved separately in a minimum amount of H_2O by adding 7 *M* NaOH until the pH rose to 11. The neutralization of the bromoacetic acid was carried out at 0°. The two solutions were then mixed together at 50° with constant stirring. To maintain a pH of 11, more 7 *M* NaOH was added as the reaction proceeded. When no further NaOH was required (less than 2 hr), the reaction was stopped. The reaction solution containing the disodium salt of *N*-carboxymethyl-L-valine, Na_2(L-ValMA), and NaBr was used directly in the resin synthesis.

Preparation of *N*-Carboxymethyl-L-valine Diethyl Ester (Et_2-L-ValMA). Esterification of 20.0 g of L-Val was accomplished by bubbling dry HCl through an ethanol suspension of the amino acid and refluxing for 4 hr.[10] The ethanol was removed under a water aspirator vacuum, and the resulting oil was dissolved in 25 ml of 1 *M* HCl. Potassium carbonate was added to this solution until CO_2 effervescence stopped. The product was extracted with three 30-ml portions of ethyl acetate. After drying the solution over $MgSO_4$, followed by filtering, the ethyl acetate was removed under a water aspirator vacuum. The product, L-ethyl valinate (80% yield), was then mixed with one-half as many moles of ethyl bromoacetate at room temperature with constant stirring for 4 hr. Then 25 ml of water was added to extract the excess L-ethyl valinate as the hydrobromide, which could be recovered by neutralization and extraction as described above. The *N*-carboxymethyl-L-valine diethyl ester, Et_2-L-ValMA, may be further purified by distillation at 0.1 mm, although much of the product is lost due to polymerization. The compound was characterized by a proton nmr spectrum of the neat sample with a tetramethylsilane internal standard.



The chemical shifts (τ scale) together with the multiplicities given in parentheses follow: A, 9.02 (2); B, 8.13 (multiplet); C, 7.02 (2); D, 5.87 (4); E, 8.79 (3); F, 8.02 (1); G, 6.69 (1); H, 5.85 (4); I, 8.77 (3).

A similar procedure was used to prepare *N*-carboxymethyl-L-aspartic acid triethyl ester, Et_3-L-AspMA, from L-aspartic acid and ethyl bromoacetate.



Its nmr spectrum showed resonances as follows: A, 8.78 (3); B, 5.92 (4); C, 7.41 (2); D, 6.36 (3); E, 5.88 (4); F, 7.62 (1); G, 6.58 (1); H, 5.87 (4).

Preparation of the Styrene–Divinylbenzene Copolymers Containing the *N*-Carboxymethyl-L-valine (L-ValMA) or *N*-Carboxymethyl-L-aspartic Acid (L-AspMA) Ligands. A mixture of 15.0 g (66 mmol of Cl) of the chloromethylated styrene–divinylbenzene (PS–0.8% DVB), 22.2 ml of predistilled dimethyl sulfide (5:1 molar excess), 75 ml of 2-propanol, and 60 ml of water was stirred at 25° for 96 hr.[11] Then 176 mmol of Na_2(L-ValMA) containing NaBr dissolved in 215 ml of H_2O was added along with 118 ml of 2-propanol. The entire mixture was refluxed for 48 hr. The copolymer was then filtered to remove excess reactants and refluxed again for 4 hr with 4 *M* NH_4OH to remove any remaining dimethyl sulfide. The resin was filtered and washed with 1 *M* HCl, then 1 *M* NaOH, and finally water. It was then equilibrated with 57 mmol of Cu^{2+}, and excess Cu^{2+} was filtered from the resin and titrated with EDTA using murexide as the indicator.[12] Subtraction of this quantity of Cu^{2+} from the amount added gave the amount of copper left on the resin. Typically, this quantity was between 20% and 28% of the initial number of moles of Cl in the starting chloro-methylated copolymer. The Cu^{2+} capacity of the Na_2(L-ValMA) resin was thus approximately 0.54 mmol/g.

Alternately, following another procedure,[13] 20.0 g (88 mmol of Cl) chloromethylated PS–0.8% DVB was stirred for 36 hr at reflux in a solution of 120 ml of acetone and 23.5 g (150 mmol) of NaI. The copolymer was washed free of excess salt with water, then treated for 5 more days under the same conditions. The resin was washed with water, then acetone, then dried at 100°. The gain in weight of the copolymer indicated an exchange of 94% of the Cl for I. A similar yield was obtained from a sample of chloromethylated PS–1.8% DVB.

The iodomethylated PS–0.8% DVB (3.0 g, 7.4 mmol of I) was stirred at 70° for 9 days with 10 ml of acetonitrile and 4.87 g (22.1 mmol) of Et_2-L-ValMA. The resin was then washed free of excess ester with ethanol and refluxed in a 1 *M* solution of NaOH in 95% ethanol for 24 hr. Analysis using Cu^{2+} as described above showed a yield of 91%. A similar procedure produced yields of only 62% for the reaction of the same ester with the iodomethylated

(7) S. V. Rogozhin and V. A. Davankov, *Chem. Commun.*, 490 (1971).
(8) B. E. Leach and R. J. Angelici, *J. Amer. Chem. Soc.*, **91**, 6296 (1969).
(9) B. E. Leach and R. J. Angelici, *Inorg. Chem.*, **8**, 907 (1969).
(10) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, Wiley, New York, N. Y., 1961, p 926.
(11) C. W. Roberts and D. H. Haigh, *J. Org. Chem.*, **27**, 3375 (1962).
(12) H. A. Flaschka, "EDTA Titrations, An Introduction to Theory and Practice," Pergamon Press, Elmsford, N. Y., 1959.
(13) R. F. Hirsch, E. Gancher, and F. R. Russo, *Talanta*, **17**, 483 (1970).

PS–1.8% DVB. Unfortunately, this resin also broke down to a fine powder during the hydrolysis of the ester.

An analogous reaction of Et_3-L-AspMA and iodomethylated PS–1.8% DVB produced yields generally of approximately 90%, although a few as low as 78% were noted. Yields decreased markedly when amounts greater than 5 g of halomethylated copolymer were used.

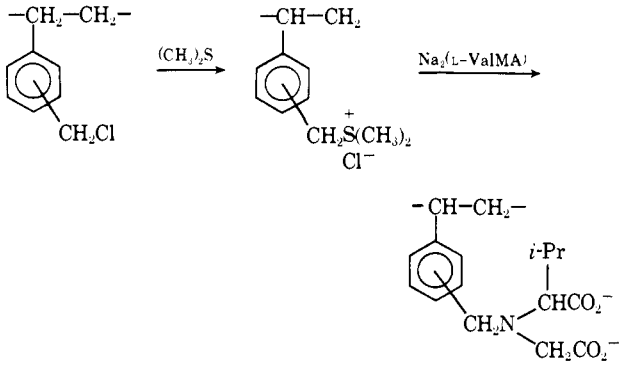
Construction and Operation of the Column. Except where indicated otherwise, the resolution studies were carried out in the following manner. A 25-g sample of L-ValMA-substituted PS–0.8% DVB copolymer (13.3 mmol of Cu^{2+} total capacity) in the Na^+ form was equilibrated with 9.5 mmol of Cu^{2+} and then packed into a 1-cm diameter column. The length of the bed was about 70 cm and was supported by a coarse glass frit. Below this column, and attached to it, was a similar column, 10 cm long, which contained the same resin in the Na^+ form (*i.e.*, no Cu^{2+}) to retain any Cu^{2+} leaking off the primary column. The effluent from the short column was passed into a fraction collector.

Approximately 4.2 mmol of a racemic amino acid sample was dissolved in 3–5 ml of H_2O by adding NaOH until the pH was 11.1. This solution was loaded into the column, followed by 2 ml of water, and then elution with 0.324 *M* ethylenediamine was begun. Fractions of 2.7 ml were collected at a flow rate of 0.2 ml/min; between 30 and 40 fractions contained amino acid. Ninhydrin analyses[14] and optical rotatory dispersion measurements were used to calculate the amount of D isomer in each fraction. The rotatory measurements were made at 240–245 nm on solutions made up of 1 ml of sample and 1 ml of 5 *N* HCl. After each run, the columns were regenerated by washing with 4 *M* HCl, followed by 4 *M* NaOH and H_2O, and then reequilibrating with Cu^{2+}.

In addition to the above resolution conditions, the eluent concentration, Cu^{2+} concentration, flow rate, and pH of the amino acid sample were varied to establish conditions for the maximum resolution of D,L-valine. More than 100 chromatographic runs over a period of 1.5 years were conducted on the resin with no observable loss in resolving power.

Results and Discussion

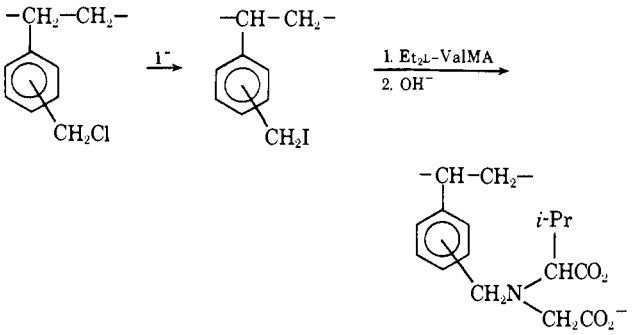
Synthesis of Ligand-Exchange Resin. The introduction of the ligand L-ValMA into the chloromethylated styrene–divinylbenzene copolymers could be accomplished by either of two routes. The first involves conversion of the chloride to a dimethylsulfonium salt, which is more compatible with the 2-propanol–water



solvent needed to dissolve the disodium salt of L-ValMA. Using a chloromethylated styrene–0.8% divinylbenzene copolymer containing 4.4 mmol of Cl/g of copolymer, a ligand-exchange resin containing approximately 0.5 mmol of Na_2(L-ValMA)/g of this resin was obtained. It was this resin that was used in the amino acid resolution studies. When $HN(CH_2CO_2Na)_2$ was used in place of Na_2(L-ValMA), the yield of imino diacetate, Na_2IMDA, incorporation was significantly higher (1.3 mmol of Na_2IMDA/g). It thus appears that the bulky isopropyl side chain in L-ValMA is primarily responsible for the lower yield of the asymmetric resin.

(14) J. R. Spies, *Methods Enzymol.*, **3**, 468 (1957).

The second route required Cl^- displacement by I^- followed by reaction in acetonitrile with the diethyl ester of L-ValMA.



The ester groups were then hydrolyzed in alcoholic NaOH to give the desired resin. The I^- exchange reaction proceeded in greater than 90% yield for both the 0.8% and 1.8% DVB-containing copolymers. The subsequent reaction with Et_2-L-ValMA proceeded in 91% yield with the iodomethylated resin containing 0.8% DVB but in only 62% yield with that containing 1.8% DVB. Thus, as is often observed,[15] the reactivity of the copolymer decreases as the degree of crosslinking (*i.e.*, percentage of DVB) increases. During the ester hydrolysis step of the 1.8% DVB resin preparation, the resin broke down to a fine powder. Under gravity flow, this resin gave flow rates too slow to be useful in the chromatographic investigations.

The introduction of the L-AspMA group into the 1.8% DVB resin by reaction with Et_3-L-AspMA proceeded in higher yield (~90%) than was observed for Et_2-L-ValMA. Unfortunately, the Cu(L-AspMA) resin swelled to such an extent when eluted with ethylenediamine that it sealed off the solution flow down the column; hence the resin could not be used in the resolution studies.

Resolution of Amino Acids. The chromatographic studies were carried out on a column of L-ValMA-substituted PS–0.8% DVB with a total Cu^{2+} capacity of 13.3 mmol. To the column was added a solution (pH 11.1) of the sodium salt of the D,L-amino acid (4.2 mmol). The amino acidate (A^-) presumably binds to the Cu^{2+} on the resin to form the complex Cu(L-ValMA)$(A)^-$, eq 1. The amino acidate was eluted by ligand displacement chromatography[16] using ethylenediamine as the displacing ligand. The higher stability constant for ethylenediamine coordination ($\log K = 10.5$) to Cu^{2+} *vs.* that for amino acid coordination (*e.g.*, $\log K = 8.5$ for alanine)[17] suggests that ethylenediamine will essentially quantitatively displace A^- from the Cu(L-ValMA)$(A)^-$ complex. Equilibrium studies[8] of reaction 1 indicate that if A^- is of the L configuration, it will displace D-A^- from Cu(L-ValMA)$(A)^-$ on the resin. Thus, as the ethylenediamine flows down the column, the D isomer will concentrate toward the front of the amino acid band. In ligand displacement chromatography,[16] this band is not resolved into D and L peaks; the D isomer simply concentrates at the front and the L enantiomer at the back (Figure 1). During a

(15) R. M. Wheaton and M. J. Hatch, *Ion Exch.*, **2**, 191 (1969).
(16) F. Helfferich, "Ion Exchange," McGraw-Hill, New York, N. Y., 1962, pp 437, 445.
(17) L. G. Sillén and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. 17 (1964).

Table I. Percentage of D-Amino Acid in Fractions Obtained by Chromatography on Cu(L-ValMA)) Resin

Amino acid	% D isomer in each group							
	1	2	3	4	5	6	7	8
Isoleucine	75	66	59	53	47	42	34	30
Valine	70	61	55	52	48	44	42	31
Norvaline	65	49	48	48	48	49	49	49
α-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

run, a small amount of Cu^{2+} eluted with the amino acid; this was removed by a short column of the resin in the sodium form. The removal of Cu^{2+} was necessary because of its interference with the rotation measurements at 245 nm.

To examine the factors that affect the extent of resolution, the separation of D,L-valine was studied under a variety of column conditions. First, the amount of Cu^{2+} bound on the resin was varied from 8 to 13 mmol. Since the resin had a total capacity of 13 mmol, this represents 60–100% saturation of sites with Cu^{2+}. Optimum resolutions were achieved when the resin contained 9.5–11 mmol of Cu^{2+}. Below 9.5 mmol, the resolutions declined sharply. Above 11 mmol, there was a slight decline, but the amount of Cu^{2+} eluting from the column increased. In subsequent studies 9.5 mmol of Cu^{2+} was used because it provided maximum resolution with minimum Cu^{2+} elution. The amount (4.2 mmol, 0.5 g for valine) of amino acid placed on the column was sufficient to saturate the upper 45% of the column as Cu(L-ValMA)-$(A)^-$.

Second, the flow rate of 0.32 *M* ethylenediamine was reduced below the 0.2 ml/min rate used in most of the studies. As expected, slower flow rates did increase resolution; thus the first one-eighth of the D,L-valine eluting from the column contained 73% D-valine at 0.1 ml/min as compared to 70% at 0.2 ml/min. The 0.2 ml/min was chosen as a matter of convenience since it required only 12 hr (overnight) to complete a run.

The dependence on the pH of the D,L-valine sample solution was evaluated on the column when saturated with Cu^{2+} (*i.e.*, 13 mmol). At pH 10.0, the first one-eighth of the valine eluting contained 52% D isomer; at pH 10.5, this fraction was 55% D and at pH 11.0, 58% D. Above pH 11.1, where the valine is completely in its anionic form, the resolution should not be affected by raising the pH further. Hence, 11.1 was the pH used in the other studies.

Figure 1 shows the grams of valine (curve A) and the per cent of the amino acid which is the D isomer (curve B) in each 2.7-ml fraction eluting from the column operated under conditions given in the Experimental Section. The early fractions contained up to 80% D- and 20% L-valine, while the terminal fractions contained 75% L and 25% D. Similar curves were obtained with the other amino acids. Table I gives a quantitative comparison of the resolutions in these cases. Fractions from each run were arranged into eight groups, with each group containing one-eighth of the amino acid eluted. Group 1 contains the first one-eighth of amino acid; group 2 contains the second one-eighth, etc. The percentage of D isomer in each group for each amino acid was calculated and is listed in Table I. With all the amino acids, the D isomer eluted first, which is consistent with the equilibrium studies[8] showing that L-amino acid complexation by Cu(L-ValMA) is favored over that of the D enantiomer.

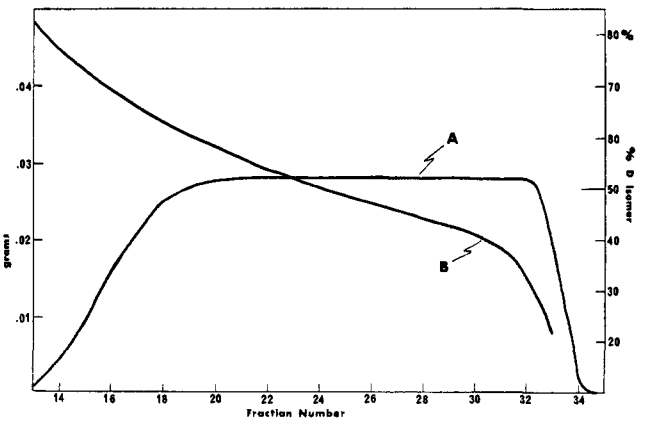


Figure 1. Resolution of DL-valine on Cu(L-ValMA) resin: grams of valine eluted (A) and percentage of D-valine eluted (B).

The extent of resolution of the different amino acids is perhaps best indicated by the per cent of D isomer in group 1. This value decreases as the bulkiness of the side chain (R) in the amino acid, $^+NH_3CH(R)CO_2^-$, decreases

$$-CH(CH_3)CH_2CH_3 > -CH(CH_3)_2 > -CH_2CH_2CH_3 > -CH_2CH_3 > -CH_3$$

Although the structure of the mixed complex, Cu(L-ValMA)$(A)^-$, on the resin is unknown, these results suggest that there is steric repulsion between the R group on the amino acid and the isopropyl side chain of the L-ValMA ligand.

It should be noted that with norvaline and α-aminobutyric acid, the resolution was reasonably good in the first group but decreased significantly in subsequent groups. This is apparently due to the observed shrinking and subsequent channeling of the resin when sorbing these amino acids. Such channeling was so extensive with leucine, phenylalanine, methionine, and threonine that no separations were achieved in these cases. Although channeling was not observed with aspartic and glutamic acids, these amino acids also showed no resolution on the column.

Together with the resolution of D,L-proline reported by Rogozhin and Davankov,[5] these results show that at least the partial resolution of relatively large amounts of several amino acids is possible on sorbents containing labile, optically active metal complexes. Increasing the length of the columns would almost certainly improve upon the resolutions reported here. Finally, the elution of the D-amino acids ahead of the L enantiomers in all cases studied on the Cu(L-ValMA) resin suggests that the resin may be useful in the assignment of absolute configurations to new amino acids.

Acknowledgment. The partial support of this research by the U. S. Public Health Service (Grant No. GM-12626) and the Iowa State University Research Foundation is greatly appreciated. We also thank Professors Jack E. Powell and James S. Fritz for helpful discussions. We are grateful to Dr. Robert D. Hansen of the Dow Chemical Company for providing the chloromethylated copolymers.