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A Study of Model Complexes of Products Expected from N-Terminal Hydrolysis of Polypeptides Containing Trifunctional Amino Acids. Tetraminecobalt(III) Complexes of Glutamic and Aspartic Acids

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Isomers of the complexes $Co(en)_2(L\text{-}aa)^+$ (aa = aspartate and glutamate) and $Co(en)_2(L\text{-}aa)^{2+}$ (Haa = protonated aspartate and glutamate), as well as the model complex $Co(NH_3)_4(L\text{-}asp)^+$, have been characterized in terms of their visible, proton magnetic resonance, and circular dichroism spectra. The diastereoisomers of each complex were separated chromatographically. In all complexes the amino acids were found to coordinate only through the five-membered glycinate ring as demonstrated through selective isotopic exchange of the coordinated glycinate methine proton for deuterium. These results suggest that it may be possible to use a tetraminecobalt(III) complex to hydrolyze peptides N-terminal in these amino acids since coordination through the larger six- (asp) and seven-membered (glu) rings is not anticipated.

Introduction

We are investigating chelate systems of cobalt(III) complexes of aliphatic polyamines and trifunctional amino acids because of our interest in the nature of the interaction between peptides containing these amino acids and metal ions. In the investigation reported here we have focused our attention on the interaction of amino acids of this kind with a metal ion where only two ligation sites are available to the amino acid. Because of its stability and relatively simple stereochemisty the *cis*-diacidobis(ethylenediamine)cobalt(III) system was selected as a model for these complexes. This particular system has been widely used to study the coordination of the simpler amino acids. The stereochemistries of complexes of the type $Co(en)_2aa^{2+}$ where aa = alanine or phenylalanine,[1,2] leucine,[1-3] isoleucine,[2] valine,[2,3] and proline[4] have been investigated in detail. For these complexes the only possible chelate which can form is the five-membered ring resulting from the incorporation of the glycine unit of the amino acids. Complexes of the amino acids serine and threonine which contain the weakly coordinating hydroxyl group have also been examined.[5] As expected only the glycinate ring formed for each of these amino acids.

The introduction of a third, strong ligating group into the amino acid might be expected to lead to several modes of coordination when only two sites are available. Kothari and Busch[6] found that cysteine coordinates through the amine and the sulfide in the complex $Co(en)_2cys^{2+}$ to form a five-membered ring. Gillard and coworkers[7] synthesized the complex $d(+)$-$[Co(en)_2(L\text{-}glu)]ClO_4$. A crystal structure revealed that the five-membered glycinate ring was formed by the glutamic acid. The dangling γ-carboxylate was hydrogen bonded to the amine protons of one of the ethylenediamines.

Collman and coworkers have used the complex hydroxoaquo(triethylenetetramine)cobalt(III) and hydroxoaquobis(ethylenediamine)cobalt(III) to achieve N-terminal hydrolysis of small peptides.[8] The hydrolysis step involved coordination of the amine function and the carbonyl oxygen of the peptide linkage belonging to the N-terminal amino acid, which was a simple bidentate amino acid such as glycine or alanine. However, if the amino acid were to contain a third group capable of coordination, then interference with hydrolysis might be expected if, for example, the N-terminal amino acid were to coordinate through the α-amino group and this third group. Because of our studies of metal ion hydrolysis of peptides containing trifunctional amino acids we undertook the investigation of the model system $Co(en)_2aa^{n+}$ (aa = trifunctional amino acid) with L-aspartic acid (asp) and L-glutamic acid (glu) to determine the mode of coordination of tridentate α-amino acids when only two sites are available to the acid. The tetraammine analog of L-aspartic acid was prepared as a model for the proton magnetic resonance and circular dichroism studies.

Experimental Section

The Synthesis and Separation of the Isomers of Aspartatobis(ethylenediamine)cobalt(III) Perchlorate.—Freshly prepared carbonatobis(ethylenediamine)cobalt(III) chloride[9] (2.75 g, 0.01 mol) was dissolved in 50 ml of water; 2 g of activated charcoal (Norit A, alkaline) was added, and the mixture was stirred and heated to 40° for 20 min. Then L-aspartic acid (1.33 g, 0.01 mol) was added to the stirred mixture. The temperature was raised to 70° and after the evolution of CO_2 ceased, the reaction was allowed to proceed an additional 30 min at 70° with stirring. After cooling, the carbon was removed by filtration through a fritted-glass funnel. The filtrate was diluted to 500 ml with water and loaded on a Dowex 50W-X4 cation-exchange column (100–200 mesh) at a rate of 1–2 ml/min. The capacity of the column was 1150 mequiv. The complex was eluted (flow rate *ca.* 1 ml/min) with 0.20 *M* sodium perchlorate, adjusted to a pH of 7.0 with Na_2CO_3. The complex separated cleanly into two red-orange bands. Each band was collected in *ca.* five to ten 600-ml fractions, evaporated to near dryness in an air stream, and excess $NaClO_4$ was filtered off. Finally, the solutions of complex were taken to complete dryness and absolute ethanol was added to dissolve the sodium perchlorate, leaving the yellow-orange complex. After filtration the solid complexes were stored overnight in absolute ethanol. The combined products yielded 83% of the complex based on the initial

(1) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964).
(2) D. A. Buckingham, L. Durham, and A. M. Sargeson, *Aust. J. Chem.*, **20**, 257 (1967).
(3) T. Yasui, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Jap.*, **39**, 2417 (1966).
(4) M. Saburi, M. Homma, and S. Yoshikawa, *Inorg. Chem.*, **8**, 367 (1969).
(5) S. K. Hall and B. E. Douglas, *ibid.*, **8**, 372 (1969).
(6) V. M. Kothari and D. H. Busch, *ibid.*, **8**, 2276 (1969).
(7) (a) J. H. Dunlop, R. D. Gillard, N. C. Payne, and G. B. Robertson, *Chem. Commun.*, 874 (1966); (b) J. H. Dunlop and R. D. Gillard, *J. Chem. Soc.*, 1469 (1967).
(8) D. A. Buckingham, J. P. Collman, D. A. R. Harper, and L. G. Marzilli, *J. Amer. Chem. Soc.*, **89**, 1082 (1967); D. A. Buckingham and J. P. Collman, *Inorg. Chem.*, **6**, 1803 (1967).
(9) G. W. Schlessinger, "Inorganic Laboratory Preparations," Chemical Publishing Co., New York, N. Y., 1962, p 230.

TABLE I
ANALYTICAL DATA FOR THE COBALT(III)–AMINO ACID COMPLEXES SYNTHESIZED

	% calcd			% found		
	C	H	N	C	H	N
Λ-[Co(en)$_2$(L-asp)]$ClO_4 \cdot H_2O$	22.47	5.42	16.38	22.50	5.54	16.12
Δ-[Co(en)$_2$(L-asp)]$ClO_4 \cdot H_2O$	22.47	5.42	16.38	22.46	5.63	16.26
Λ-[Co(en)$_2$(L-glu)]$ClO_4 \cdot H_2O$	24.48	5.71	15.85	24.63	5.61	15.90
Δ-[Co(en)$_2$(L-glu)]$ClO_4 \cdot 2.5H_2O$	23.07	5.82	14.94	23.23	5.34	14.80
Λ-[Co(en)$_2$(L-Hasp)]$(ClO_4)_2 \cdot 0.5H_2O$	18.52	4.47	13.48	18.43	4.60	13.31
Δ-[Co(en)$_2$(L-Hasp)]$(ClO_4)_2$	18.84	4.38	13.73	18.86	4.55	13.62
Λ-[Co(en)$_2$(L-Hglu)]$(ClO_4)_2 \cdot 1.5H_2O$	19.62	4.98	12.71	19.87	4.45	12.52
[Co$(NH_3)_4$(L-Hasp)]$(ClO_4)_2$	10.49	3.96	15.29	10.53	4.08	15.31

amount of cobalt used. Each fraction was dissolved in a minimum amount of warm water and ethanol was added until the solution became cloudly. A few drops of water were added to remove the cloudiness and the solution was allowed to stand for several days. The complex precipitated as red-orange crystals which were filtered, washed with ethanol, and air-dried. From circular dichroism data all the fractions of each band proved to be identical. Analytical results are recorded in Table I.

Synthesis and Separation of the Isomers of Glutamatobis(ethylenediamine)cobalt(III) Perchlorate.—The same procedure was followed as for the aspartic acid complex, except that 1.48 g of L-glutamic acid was used. The yield of unrecrystallized products was 87%. Circular dichroism data demonstrated that the two bands obtained consisted each of one isomer. Analyses of the recrystallized products are recorded in Table I.

Preparation of the Protonated Amino Acid Complexes.—The previously prepared isomers were dissolved in a minimum amount of water and 70% perchloric acid was added to a pH of 1.0. Then excess ethanol was added to cause precipitation of the isomer which was filtered, washed with a minimum amount of ethanol, and air-dried. Analytical results are shown in Table I. The Δ-Co(en)$_2$(L-Hglu)$^{2+}$ isomer could not be isolated due to its apparent high solubility in ethanol. The protonated form of this isomer was generated in solution from the basic form when needed.

Synthesis of Aspartatotetraamminecobalt(III) Perchlorate.—The complex was prepared from the aquopentaamminecobalt(III) complex by modification of the method described by Shimura and coworkers.[3] Five grams of the aquo complex was dissolved in 50 ml of water at 50°, and then 1.40 g of aspartic acid was added with 2.0 g of activated charcoal. After stirring at 70° for 20 min, the mixture was cooled, the charcoal was filtered off, and 6 *N* HNO_3 was added to adjust the pH to 1.0. The solution was diluted to 500 ml with water and loaded on a Dowex 50W-X4 cation-exchange resin column (100–200 mesh, 1150-mequiv capacity). The complex was eluted with 0.2 *N* $NaClO_4$. Several bands were obtained, but only the red-orange band which showed optical activity was completely characterized. The desired complex was isolated and purified as was described for the other complexes. The yield was less than 50 mg due to extensive hydrolysis and disproportionation of the starting materials. Analytical results are shown in Table I.

Physical Measurements.—The visible absorption spectra were recorded on a Cary Model 14 spectrophotometer. The circular dichroism spectra were obtained on a JASCO Model ORD/UV-5 with a CD attachment using (1.1–9.3) × 10^{-3} *M* solutions. Proton magnetic resonance spectra were run on a Varian A-60 nmr spectrometer. Deuterium oxide was used as solvent with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (TMS*) as an internal reference. Isotopic exchange studies were achieved by the addition of a granule of anhydrous Na_2CO_3 to the D_2O solution of the isomer to be studied. Microanalytical analyses were performed by Galbraith Laboratories.

Results and Discussion

Synthesis and Separation of the Isomers.—These complexes may be prepared by three different methods. Two of the syntheses involve the use of *trans*-dichlorobis(ethylenediamine)cobalt(III) in a reaction with an equimolar quantity of the sodium salt of the amino acid in the presence of base[1] or a reaction based on a modification of a method developed by Legg and Cooke,[10] using the silver salt of the amino acid. In both methods hydrolysis of the starting material resulted in low yields of the amino acid complex. The method which gave the highest yield was the reaction of carbonatobis(ethylenediamine)cobalt(III) with an equimolar amount of the amino acid in the presence of activated charcoal.

Ion-exchange chromatography suggested the presence of the diastereoisomers of only one isomer of the respective amino acid complexes. The purity of the isomers was checked by collecting the bands in fractions and running quantitative circular dichroism spectra. Initially using a nonbuffered eluent of sodium perchlorate, there appeared to be a large amount of trailing for each band on the ion-exchange resin; also, analytical data indicated an approximate 50:50 mixture of the protonated and nonprotonated species. The use of a Na_2CO_3-buffered eluent caused the nonprotonated complex to be eluted from the column as a 1+ species. In acid solution (pH 1.0), the protonated 2+ species is formed and the analytical data for both sets of isomers are shown in Table I. The Λ isomer (see later discussion for assignment of isomers) is the predominant diastereoisomer and is eluted first, whether the amino acid is protonated or nonprotonated.

Stereochemistry of the System.—In the system Co(en)$_2$(L-aa)$^+$ there are three geometrical isomers which result from different modes of coordination of the amino acid, each capable of existing in diastereomeric pairs as shown in Figure 1. Isomer I involves the five-membered chelate ring formed by the amino and α-carboxylate groups, leaving the non-α-carboxylate uncoordinated. In isomer II the ring is formed by the amino group and the non-α-carboxylate, leaving the α-carboxylate free. Aspartic acid and glutamic acid, then, form six- and seven-membered rings, respectively, in isomer II. In isomer III the ring is formed by both carboxylate groups, leaving the amino group free. In this case aspartic and glutamic acids form seven- and eight-membered rings, respectively. We have previ-

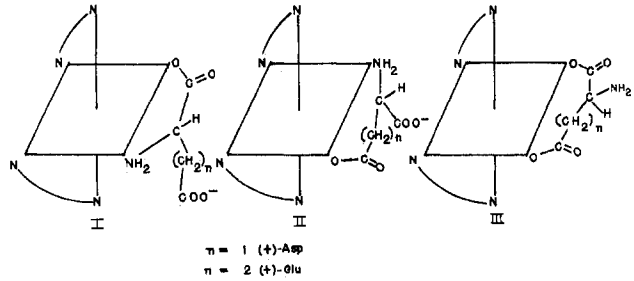

Figure 1.—The geometrical isomers of Co(en)$_2$(L-aa)$^+$ where aa is aspartic acid or glutamic acid.

(10) J. I. Legg and D. W. Cooke, *J. Amer. Chem. Soc.*, **89**, 6854 (1967).

ously demonstrated that all three rings can form when aspartic acid functions as a tridentate ligand.[10] However, it was expected in this case that isomer III would not occur because of the excessively large chelate rings and because of the incorporation of the two carboxylates at the expense of the amine (and the smaller more stable rings).

The only isomers with a dangling carboxylate group which are capable of existing in the protonated or nonprotonated forms are isomers I and II, Figure 1. The third isomer with the dangling amino group should be protonated in the pH range used, and the complex formed should be a 2+ species. As was anticipated, no evidence for this isomer was obtained. In the preparation of $Co(NH_3)_4$(L-Hasp)$^{2+}$ only one isomer was expected on the basis of what was formed for the bis-ethylenediamine analog and only one was found. Isomer I was the only isomer found for glutamic acid by earlier workers, and, furthermore, the presence of the other diastereoisomer was not established with any certainty.[7]

Electronic Absorption Spectra.—Geometrical isomers I and II (Figure 1) should be of the type $Co^{III}N_5O$ and the electronic absorption spectra are not expected to be particularly helpful in distinguishing the isomers. The third isomer is, however, *cis*-CoN_4O_2 and could be readily distinguished from the other two. All of the complexes have visible absorption maxima (490 nm with extinction coefficients of about 100) in the range of previously prepared (L-amino acidato)bis(ethylenediamine)cobalt(III) complexes.[1-5] There is no spectral evidence for the *cis*-CoN_4O_2 isomer.

Characterization of the Isomers by Their Proton Magnetic Resonance Spectra.—The pmr study of these complexes in deuterium oxide gave strong evidence for the existence of the five-membered ring isomers. The $Co(NH_3)_4$(L-asp)$^{2+}$ has no ethylenediamine protons, and only the ABX portion for the aspartic acid was detected. The pmr spectrum shows a triplet at 3.85 ppm (one proton from integration) with a splitting between the two outer peaks of 3 cps and a doublet at 2.95 ppm (two protons). It follows then that the triplet belongs to the methine proton (X of ABX) of the α carbon, and the doublet, to the methylene protons (AB of ABX) of the β carbon. The chemical shifts are consistent with this assignment. $Co(NH_3)_4$(L-asp)$^{2+}$ served as a model for assigning the more complex spectra found for the bis-ethylenediamine analogs.

The only definitive method for deciding if the complex were isomer I or II is based on the anticipated deuterium exchange of the methine proton of the glycinate ring if isomer I had formed. Such exchange was first observed by Busch and Williams[11] with $Co(en)_2gly^{2+}$ in basic media and has been very useful in the study of the stereochemistry of glycinate type complexes.[12] These five-membered ring systems of amino acids appear to be the only ones which readily exchange with the solvent. Kinetic studies support a mechanism which involves the chelated glycine unit;[12a,b] and the stereospecificity of substitution, with respect to *both* the chelate rings involved and the individual protons on a ring, strongly suggests that the glycine portion of a ligand must be coordinated for exchange to take place. This does not rule out the possibility that exchange may occur when the ligand is not chelated, *i.e.*, when it is either just N bonded or O bonded. However, that such is the case for these complexes is highly unlikely. If the aspartate and glutamate were only N bonded, then the two forms (nonprotonated and protonated) would give a 1+ and 3+ salt instead of the 1+ and 2+ salts found. The crystal structure of the glutamate complex showed that it was N and O bonded.[7b] It is unlikely that a cobalt(III) complex involving a chelated amine-containing ligand would break the N bond simply on dissolution at room temperature. It should also be noted that the spectral evidence supported an N_5O ligand environment. This then makes it highly improbable that the ligands are bonded only through one carboxylate.

Figure 2 shows the $Co(en)_2$(L-Hasp)$^{2+}$ pmr spectrum.

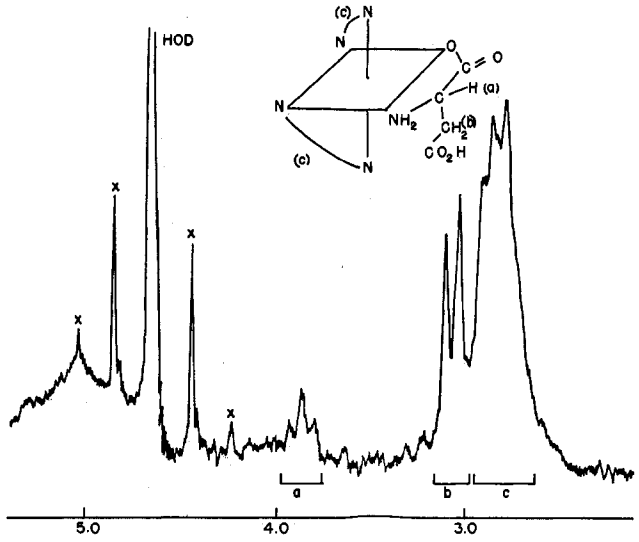

Figure 2.—The pmr spectrum of $Co(en)_2$(L-Hasp)$^{2+}$.

There are eight protons on the ethylenediamine backbone with resonances between 2.5 and 3.0 ppm (labeled "c" in Figure 2). The X proton (proton a) is assigned on the basis of its relative intensity and its chemical shift (3.90 ppm) in comparison with the $Co(NH_3)_4$(L-asp)$^{2+}$ model complex. The AB portion (b protons) is centered at 3.18 ppm. Figure 3 shows the nonprotonated aspartic acid complex. The triplet occurs in the same region as before; however, the AB portion has shifted under the broad peak arising from the ethylenediamine. Both the Λ and Δ isomers of the aspartic acid complex exhibited this same type of behavior. The fact that deprotonation changes the chemical shift of the methylene protons and not of the methine proton suggests that it is the γ-carboxylate which is free.

The glutamic acid complex shows a more complex pmr spectrum since it is a five-spin system, as can be seen in Figure 4. As before, the ethylenediamine proton resonances (d) occur between 2.5 and 3.0 ppm. The methine (a) proton resonances occur at 3.70 ppm as a poorly defined triplet. The methylene protons α to the methine proton (b) are attached to the carbon which lies between two other carbons and are expected to be the most shielded; these are assigned to the reso-

(11) D. H. Busch and D. H. Williams, *J. Amer. Chem. Soc.*, **87**, 4644 (1965).

(12) (a) J. B. Terrill and C. N. Reilley, *Inorg. Chem.*, **5**, 1988 (1966); (b) J. L. Sudmeier and G. Occupati, *ibid.*, **7**, 2524 (1968); (c) P. F. Coleman, J. I. Legg, and J. Steele, *ibid.*, **9**, 937 (1970).

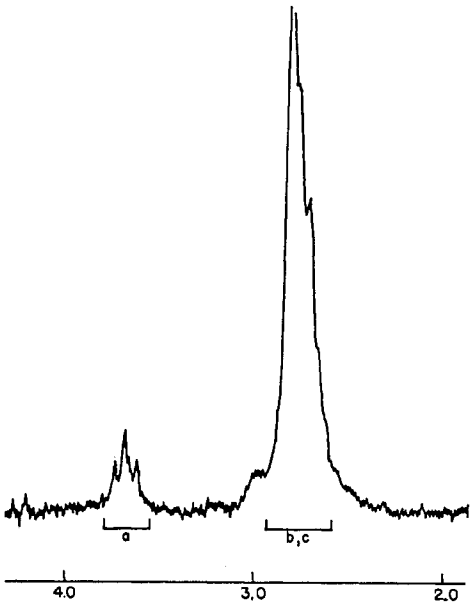

Figure 3.—The pmr spectrum of $Co(en)_2(L\text{-asp})^+$.

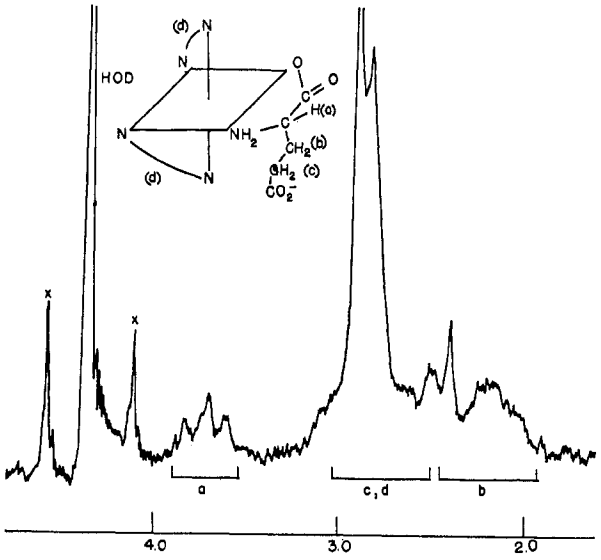

Figure 4.—The pmr spectrum of $Co(en)_2(L\text{-glu})^+$.

nances furthest upfield. The resonances of the β-methylene protons (c) located next to the γ-carboxylate lie beneath the ethylenediamine protons as indicated by integration. The similarity between the chemical shifts of the methine protons in the glutamic and aspartic acid may result from the protons being in the same chemical environment in each complex as would be expected if both were part of a five-membered ring.

The addition of Na_2CO_3 to the nonprotonated isomers results in the disappearance of the triplet in 72 hr for both optical isomers of the aspartate and glutamate as illustrated in Figures 5 and 6. The exchange of the methine proton for deuterium strongly supports chelation of aspartic acid and glutamic acid through the five-membered glycinate ring. Thus, the two bands eluted for each amino acid complex are in fact diastereoisomers of isomer I separated by ion-exchange chromatography.

Circular Dichroism Spectra and Absolute Configuration.—Figure 7 shows the CD curves obtained for the diastereoisomers of $Co(en)_2(L\text{-Hasp})^{2+}$ and $Co(NH_3)_4$-

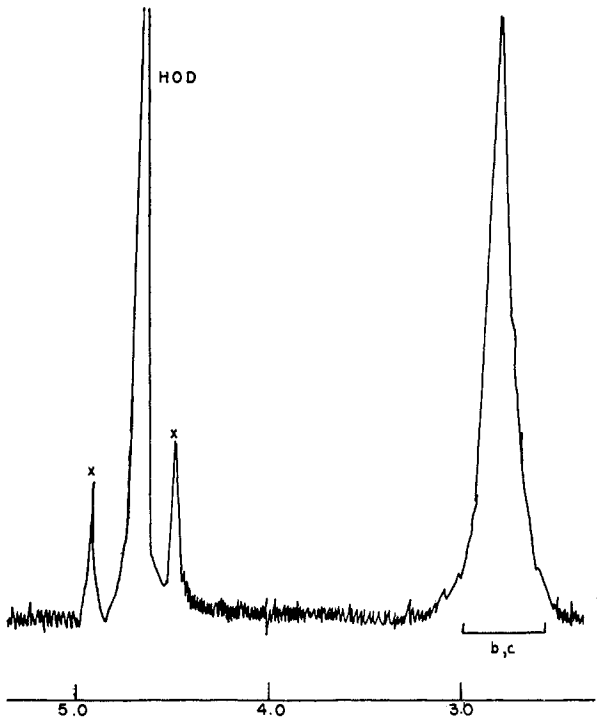

Figure 5.—The pmr spectrum of $Co(en)_2(L\text{-asp})^+$ after addition of Na_2CO_3.

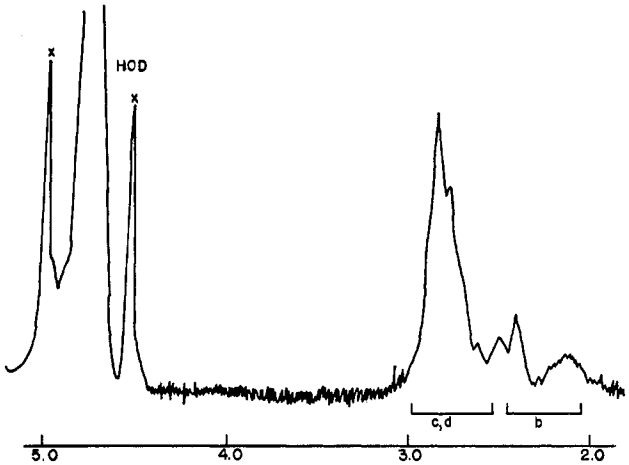

Figure 6.—The pmr spectrum of $Co(en)_2(L\text{-glu})^+$ after addition of Na_2CO_3.

$(L\text{-Hasp})^{2+}$. These are exemplary of the spectra obtained for the other isomers for which data are summarized in Table II. As would be anticipated these spectra are very similar to those obtained for other $Co(en)_2aa^+$ type complexes.[1,3-5] The salient features are the dominant band with a maximum just below 500 nm and two or three very weak bands at higher energy. Deprotonating the free carboxylate results in a decrease in the intensity of the strong band for the glutamate and an increase for the aspartate isomers.

The absolute configuration of $(+)_{589}$-$Co(en)_2(L\text{-glu})^+$ has been deduced as Λ[13] from its crystal structure and the knowledge of the absolute configuration of L-glutamic acid.[7b] The dominant CD band (Figure 7) is

(13) Λ and Δ refer to a left- and right-handed helix, respectively, as defined by any pair of chelate rings. This nomenclature has been suggested by IUPAC in *Inorg. Chem.*, **9**, 1 (1970). Λ corresponds to D as used by Gillard and coworkers.[7]

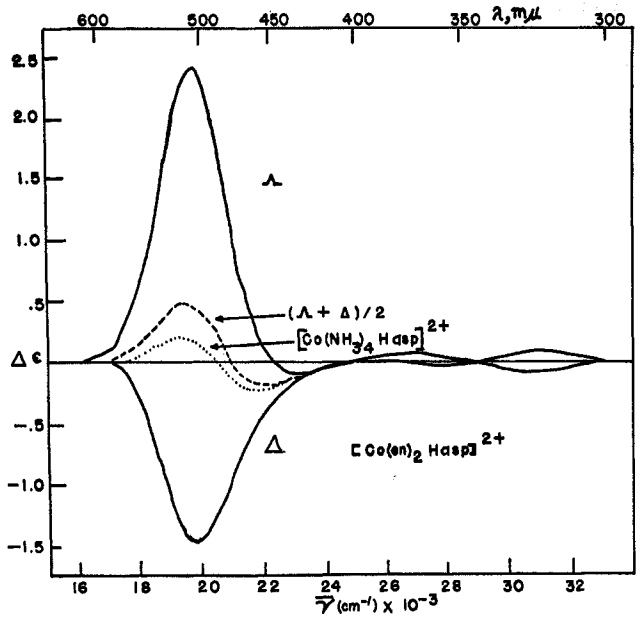

Figure 7.—The CD spectrum of Λ- and Δ-Co(en)$_2$(L-Hasp)$^{2+}$ and Co(NH$_3$)$_4$(L-Hasp)$^{2+}$.

TABLE II
CIRCULAR DICHROISM DATA OBTAINED FOR THE COBALT(III)–AMINO ACID COMPLEXES INVESTIGATED

	$10^{-4}\nu$, cm^{-1}	$\Delta\epsilon^a$	$\Delta\epsilon^b$
Λ-Co(en)$_2$(L-asp)$^{n+}$	1.97	2.43	2.55
	2.35	−0.07	...
	2.73	0.07	0.05
	3.10	0.09	0.07
Δ-Co(en)$_2$(L-asp)$^{n+}$	1.98	−1.47	−1.53
	2.76	−0.04	−0.05
	3.13	−0.11	−0.07
Λ-Co(en)$_2$(L-glu)$^{n+}$	1.97	2.53	2.23
	2.30	−0.14	−0.08
	2.72	0.04	−0.06
	3.12	0.05	−0.08
Δ-Co(en)$_2$(L-glu)$^{n+}$	1.96	−1.65	−1.55
	2.73	−0.09	−0.07
	3.14	−0.10	−0.08
Co(NH$_3$)$_4$(L-Hasp)$^{2+}$	1.94	0.18	
	2.18	−0.21	
	2.89	−0.07	

[a] Protonated. [b] Nonprotonated.

positive. The Λ configuration is that shown in Figure 1. The assignment of absolute configuration to the remaining isomers follows logically from a comparison of their CD spectra. It is interesting to note that there is a direct correspondence between the sign of the dominant CD band for the Co(en)$_2$aa^{n+} complexes reported here and that of the configurationally related Co(en)$_3^{3+}$ with the same absolute configuration which is positive for Λ-(+)$_{589}$-Co(en)$_3^{3+}$.[14] Substituting an amine–carboxylate chelate ring for ethylenediamine in Co(en)$_3^{3+}$ would not be expected to alter the environment about the cobalt(III) to any great extent as has been observed previously.[15]

Since the optical isomers are diastereomeric, the CD curves are not mirror images. If the curves obtained for the diastereoisomers of Co(en)$_2$(L-Hasp)$^{2+}$ are added together, it might be expected that the configurational contribution (resulting from the dissymmetric distribution of the chelate rings) to the optical activity would cancel leaving a contribution primarily derived from the presence of the asymmetric amino acid. This has been termed the vicinal contribution.[16] The results of this addition are shown in Figure 7. The curve has been divided by 2 to obtain the contribution from one aspartate. This curve is similar to that obtained for Co(NH$_3$)$_4$HAsp^{2+} in both the sign of the bands and band positions. In the case of the tetraammine only a vicinal contribution is expected. This interesting relationship between vicinal and configurational effects has been observed for aspartic acid functioning as tridentate[10] and in other examples (see additional references in ref 10). The fact that the curve obtained by adding together the spectra of the diastereoisomers is more intense than that of Co(NH$_3$)$_4$Hasp^{2+} suggests that there is a conformational difference between the two diastereoisomers. The ramifications of this observation are further discussed in the next section.

The Question of Stereospecificity.—The study of the bis(ethylenediamine)(trifunctional amino acid)cobalt(III) complexes shows that aspartic acid and glutamic acid coordinate exclusively through the glycinate portion of the amino acid to form five-membered rings. There is little reason to doubt that when only two sites are available for coordination, these two amino acids will preferentially form the five-membered ring chelates. However, other amino acids may exhibit more than one mode of coordination. Cysteine, for example, could coordinate through the sulfide and the amine, as well as through the amine and carboxylate of the glycinate portion due to the strong ligating properties of the sulfide. It has already been demonstrated that cysteine will preferentially chelate through the sulfide and amine in Co(en)$_2$cys$^+$ where a five-membered ring is formed.[6] An understanding of these interactions is essential to our studies of the stereochemistry of metal ion hydrolysis of peptides containing these multifunctional amino acids.

As has been previously noted[7] there is stereospecificity (suggested to be kinetic) with respect to the diastereoisomers formed when glutamic acid coordinates as a bidentate. Hydrogen bonding of the free carboxylate to an ethylenediamine proton in the Λ isomer (D isomer in the report) was used to explain the stability of this diastereoisomer. The change in the CD spectrum of this isomer with change in pH was rationalized in terms of an equilibrium between a protonated and nonprotonated form. The protonated form would not be hydrogen bonded, and thus a difference in chelate ring conformations might be anticipated between the two isomers which should be reflected in the CD spectrum.

We have examined space-filling and framework molecular models and find no particular reason to favor hydrogen bonding in the Λ isomer over the Δ isomer for the glutamic acid complex. The isomer distribution which we obtained supports these observations. Contrary to what had previously been observed, we found a substantial quantity of the Δ isomer although the Λ isomer was still the predominant species. In fact, the 70:30 distribution between the Λ and Δ isomers leads to

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a free energy difference of only -0.5 kcal mol^{-1} (assuming this represents the equilibrium distribution). With such a small difference it is difficult to pinpoint any particular steric factor as being dominant. Denning and Piper carried out a very exacting analysis of the Co(aa)$_3$ (aa = L-alanine, L-leucine, L-proline) isomer system.[17] They found that among the important steric factors was the relative pseudoaxial and pseudoequatorial positioning of the R group in the Δ and Λ isomers, respectively, the axial position being less favored. Such an argument might be invoked to rationalize the difference in stability between the Δ and Λ isomers of Co(en)$_2$(L-aa)$^{n+}$. However, consideration of molecular models suggests that the steric interactions in these isomers are small relative to those found in the Co(aa)$_3$ isomers and that hydrogen bonding might even be favored when R is in an axial position. Furthermore molecular models suggest that the conformation of the ethylenediamine ring involved in hydrogen bonding makes little difference in the stability of the molecule. It should be noted, however, that the CD spectra obtained for the protonated and nonprotonated species of the Δ isomer exhibit a relatively smaller change. This observation supports the hydrogen-bond postulate, since this isomer was predicted not to be hydrogen bonded.

The aspartate isomers exhibit a *reversal* in CD behavior in going from the protonated to the nonprotonated form for both the Λ and Δ isomers as compared to the glutamate analogs. Molecular models suggest that hydrogen bonding is possible for both isomers. In fact the removal of one methylene group in changing from glutamate to aspartate would appear to eliminate some steric crowding arising from the puckered nature of the $-CH_2CH_2CO_2-$ group in glutamate. For the aspartate isomers even less stereospecificity was observed (a 60:40 distribution between the Λ and Δ isomers, respectively).

The foregoing observations lead to several important conclusions. First, in accounting for the distribution of isomers it is essential, if at all possible, to perform a careful chromatographic analysis of the contents of the total reaction solution. Such an analysis has cast a different light on the Co(en)$_2$aa^{n+} system reported in this paper as it did for Co(EDDA)(L)$^-$ (L = ox and mal) where the presence and predominance of a hitherto undetected isomer were established.[12c] Second, when differences in stability involve less than 1 kcal/mol, any number of subtle variations in structure may be invoked to account for the difference. Third, although crystallographic analysis can be very helpful in suggesting structural behavior in solution, conclusions about stereochemical *details* in solution are tenuous at best.

Finally, we have previously demonstrated that when *three sites* are available for coordination as when a diethylenetriamine–cobalt(III) system is employed, aspartic acid functioning as tridentate shows absolute stereospecificity on coordination. This observation has been misinterpreted in a recent study where a comparison of stereospecificity in *bidentate* amino acid chelates was made[18] (see, for example, Table IV and associated discussion in the paper). The complexes that should be included in that report are those discussed in this paper and not Co(dien)asp$^+$.

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μ-Carbonato-bis(pentaamminecobalt(III)) Salts

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Potentiometric titration, conductometric charge determination, visible, ultraviolet, and infrared spectra, behavior of resin ion exchange, counterion substitution, tga, and dta have been considered as evidence for binuclear carbonate-bridged structure, composition, and purity of $[(NH_3)_5CoCO_3Co(NH_3)_5](SO_4)_2 \cdot 4H_2O$. The nitrate, perchlorate, and chloride of the binuclear cation are presented and characterized, using the same properties.

Several carbonato-bridged complexes of cobalt(III) have been described,[1,2] but so far they have not been studied in as much detail as mononuclear carbonatocobalt(III) complexes.

It was called to our attention that comparisons including the behavior of carbonato-bridged complexes are missing in the whole subject of physical chemistry researches on carbonatoammines of cobalt(III) such as aquation, base hydrolysis, exchange kinetics, and corresponding mechanisms. The only comparative study including carbonato-bridged compound we have noted refers to infrared spectra.[3]

Considering particularly the μ-carbonato-bis(pentaamminecobalt(III)) sulfate tetrahydrate, the older and better known bridged complex,[4,5] we believe that the reason

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