nitrogen in $Cu(H_{-1}GGG)GGG^-$ and $Cu(H_{-1}GG)GG^-$. The pK_a of $Cu(H_{-1}GGG)GGG^-$ (8.7) is, in fact, 2.4 log units lower than that of $Cu(H_{-1}GG)GG^-$ (11.1)[26] thus confirming the prediction.

Conclusions

(1) An equilibrium exists in solution between mono- and bis(triglycinato)cuprate(II) and the interconversion is catalyzed by hydroxide ion. (2) Protonated peptide nitrogen groups are very poor nucleophiles and hydroxide ion catalyzes the interconversion by deprotonating the incoming peptide nitrogen, thereby enhancing its nucleophilic character. (3) The bis(triglycinato)cuprate(II) complex involves both triglycine molecules bonding in a square-planar cis bidentate configuration. (4) The preference for the cis rather than the trans configuration for the copper complexes appears to be due to the strong σ-donor ability of deprotonated peptide nitrogen atoms. This effect may be important in the bonding of copper(II) in polypeptide complexes of biological significance.

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Effect of Potentially Coordinating Amino Acid Side Chains on the Cobalt(III) Promoted Hydrolysis of Peptides and Esters Containing Trifunctional Amino Acids

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Abstract: The cobalt(III) promoted hydrolysis of the diethyl ester of L-aspartic acid and dipeptides containing aspartic acid, glutamic acid, and methionine is reported. $Co(trien)(OH)(H_2O)^{2+}$ promotes the hydrolysis of glycyl-L-aspartic acid and glycyl-L-glutamic acid to form the free amino acids and $Co(trien)(gly)^{2+}$, but, contrary to previous studies of the simpler dipeptides N-terminal in glycine, an intermediate of considerably greater stability is formed. From deuterium isotopic exchange of the glycine methylene protons, infrared spectra in D_2O, and circular dichroism spectra, it is postulated that the dipeptides are chelated through the N-terminal amine and amide carbonyl. Interaction of the complex with the potentially hydrogen-bonding side chain on the penultimate trifunctional amino acid is invoked as a factor in the stabilization of the intermediate. This is substantiated by the relative ease of hydrolysis of glycyl-DL-methionine which has a side chain of comparable bulk but with little tendency to hydrogen bond. If the trifunctional amino acid is N-terminal as in α-L-aspartylglycine and L-aspartic acid diethyl ester, essentially no hydrolysis is observed when the "two-site" complex $Co(trien)X_2$ is used. However, the three-site complex $Co(dien)X_3$ promotes hydrolysis with the formation of $Co(dien)(L\text{-}Asp)^+$ where the amino acid is coordinated as a tridentate.

It has been known for some time that certain metal ion complexes promote hydrolysis of simple esters and peptides.[1-3] Of particular interest have been the studies with cobalt(III) initiated by Buckingham and Collman.[2] Tetraaminecobalt(III) complexes with two cis reactive coordination sites, such as *cis*-$[Co(en)_2(OH)(H_2O)]^{2+}$,[4] *cis*-$\beta$-$[Co(trien)(OH)(H_2O)]^{2+}$, and *cis*-$[Co(tren)(OH)(H_2O)]^{2+}$,[4] have been found to effect the hydrolysis of bidentate amino acid residues from amino acid esters, amides, and simple peptides. In aqueous solution it was found that the cationic chelates promote hydrolysis by chelation of the N-terminal amino acid forming $[Co(N_4)(\text{amino acid})]^{2+}$ chelates, where N_4 represents the tetradentate amine ligand. The N-terminal specificity of the reaction was shown by the characterization of the amino acid complexes and residual amino acid or peptide formed. In the case of some simple peptides it was shown that the N-terminal bidentate amino acid can be cleaved in a sequential manner.[2]

Thus far only simple di- and tripeptides and, in a few cases, tetrapeptides of the general formula $NH_2CHRCONHR'$, where R' is the remainder of the peptide and R the side chain, have been studied, but in all cases investigated R was a noncoordinating group. For these peptides, tetradentate cobalt(III) amine chelates were used to cleave the N-terminal bidentate amino acid since they can provide two cis coordinating sites for the interaction of the metal ion with the N-terminal, bidentate amino acid.

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(4) The following abbreviations are used throughout this paper: en = ethylenediamine, dien = diethylenetriamine, trien = triethylenetetramine, tren = 4-(2-aminoethyl)diethylenetriamine.

Of interest to us, however, was whether the hydrolysis would be affected by the presence of a potentially ligating side chain either on the N-terminal amino acid or the penultimate amino acid. The most obvious interference would come from coordination of this side chain which would result in the chelation of the peptide rather than hydrolysis as illustrated for a peptide N-terminal in aspartic acid in Figure 1, path B. A selective isotopic deuterium exchange study of the chelation of aspartic acid and glutamic acid in the model system Co(en)$_2$(L-Asp or L-Glu)$^+$, where only two sites are available for coordination, revealed that both amino acids coordinate exclusively through the five-membered glycinate rings.[5] This suggested that a complex with two available sites such as *β-cis*-Co(trien)(OH)(H$_2$O)$^{2+}$ could be used to achieve hydrolysis of peptides N-terminal in aspartic and glutamic acids, path A in Figure 1.

One of the goals of these studies is to investigate the general applicability of cationic chelates of cobalt(III) for sequential degradation of polypeptides. If this were to become a simple and useful technique, dipeptides which contain amino acids with a potentially coordinating side chain, R, would need to be studied. Because of this and our general interest in cobalt(III) complexes of trifunctional amino acids, we have been investigating the scope of metal ion promoted hydrolysis of dipeptides containing both N-terminal and C-terminal trifunctional amino acids using both the "two-site" chelate Co(trien)(OH)(H$_2$O)$^{2+}$ and the "three-site" chelate Co(dien)Cl$_3$. The latter was selected because of the availability of three sites for a trifunctional amino acid and because the expected products of hydrolysis of peptides N-terminal in aspartic acid have been characterized.[6] It was expected that Co(dien)Cl$_3$ would be suitable if it were found that the potentially ligating side chains of the trifunctional amino acids interfered with the hydrolysis when the two-site tetraamine was employed. This report deals with studies of the hydrolysis of dipeptides containing aspartic acid, glutamic acid, and methionine as well as the hydrolysis of the diethyl ester of aspartic acid.

Experimental Section

Materials. The dipeptides were purchased "chromatographically pure." L-α-Aspartylglycine hydrate was obtained from Cyclo Chemical Corp. (reported analysis for [C$_6$H$_{10}$C$_5$N$_2$·H$_2$O]: N, 13.45 (calcd); N, 13.98 (found)). Glycyl-L-aspartic acid, glycyl-L-glutamic acid, glycyl-DL-methionine, and L-aspartic acid diethyl ester hydrochloride were obtained from Mann Research Laboratories, Inc.

Trichloro(diethylenetriamine)cobalt(III) was prepared in 80% yield as previously reported[7] by heating Co(dien)(NO$_3$)$_3$ in concentrated HCl. The hydrolyzed form of Co(dien)Cl$_3$[8] was produced *in situ* by dissolving Co(dien)Cl$_3$ in water at 50° and then precipitating the chloride ion using 3 equiv of silver nitrate.[9]

The starting material, β-[Co(trien)(CO$_3$)]Cl, was prepared in a similar manner to that described by Sargeson and Searle,[10] and β-[Co(trien)(OH)(H$_2$O)]$^{2+}$ was produced *in situ* by treatment of β-[Co(trien)CO$_3$]Cl with 2 equiv of HCl followed in 30 min by the addition of freshly prepared LiOH solution to the desired pH.

Dowex 50W-X4 cation-exchange resin (200–400 mesh, hydrogen form) was obtained from Bio-Rad Laboratories; Sephadex G-15 for gel filtration (particle size 40–120 μ) and SE-Sephadex C-25 cation-exchange cellulose (particle size 40–120 μ) were obtained from Pharmacia Fine Chemicals Inc. Eastman Chromatogram sheet silica gel (Type K301R2) was used for thin layer chromatography.

Physical Measurement:, Visible absorption and circular dichroism spectra were recorded for aqueous solutions (*ca.* 10^{-3} *M*) on a Cary Model 14 spectrophotometer and a JASCO Model ORD/UV-5 with CD attachment, respectively. Infrared solution spectra in D$_2$O were obtained on a Perkin-Elmer 700 infrared spectrophotometer.

Proton magnetic resonance spectra were recorded on a Varian A-60 and/or T-60 spectrometer. The samples were dissolved in deuterium oxide, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (TMS*) was used as an external reference. Isotopic exchange studies were obtained by the addition of a granule of anhydrous Na$_2$CO$_3$ to the D$_2$O solution of the compound to be studied.

Peptide and Ester Hydrolyses. A. Reaction of Hydroxoaquotriethylenetetraminecobalt(III) Ion with the Dipeptides. Hydrolysis reactions were carried out using previously reported methods.[1,2,4] Hydrochloric acid (2 ml, 1 *N*) was added to a 5-ml solution of 0.336 g (1.0 mmol) of *β-cis*-[Co(trien)CO$_3$]Cl·2H$_2$O, carbon dioxide evolved, and the resulting solution had a pH <1. The solution was allowed to stand for 30 min; the pH was then adjusted to 7.2–7.6 using freshly prepared LiOH solution. The calculated quantity of peptide (1.0 mmol) was added, and the volume and pH were adjusted to 25 ml and 7.2–7.6, respectively. The solution (0.04 *M* in each component) was then heated in a constant-temperature bath at 70° for the desired length of time. At the end, the pH was rechecked and the hydrolysis products were separated on either a Dowex 50W-X4 or a Sephadex cation-exchange column, isolated, and identified. The presence of amino acids and/or peptides was checked using tlc on silica gel. The dipeptides studied were glycyl-L-aspartic acid, glycyl-L-glutamic acid, and glycyl-DL-methionine. Chromatography and isolation of the products are described in the last portion of this section.

B. Reaction of Hydroxodiaquo(diethylenetriamine)cobalt(III) Ion with α-L-Aspartylglycine.[8] A 20-ml solution of Co(dien)Cl$_3$ (0.271 g, 1.0 mmol) was heated at 50° with stirring for *ca.* 10 min until the Co(dien)Cl$_3$ was completely dissolved. To the purple solution was added AgNO$_3$ (0.516 g, 3.0 mmol). After heating at 50° with stirring for an additional 10 min, the precipitated silver chloride was filtered and 0.11 g of charcoal (carbon blood charcoal, acid washed) was added. The mixture was heated again at 45–50° for 10 min. α-L-Aspartylglycine hydrate (0.213 g, 1.0 mmol) in 20 ml of water, whose pH was adjusted to 8 using freshly prepared LiOH solution, was then added and the volume brought to 100 ml (pH 5.0–5.5). The mixture was heated for 2 hr in a constant-temperature bath at 70° and was then allowed to cool. The charcoal was removed by filtration; the pH did not change. Hydrolysis products were then separated on a Dowex 50W-X4 cation-exchange column, isolated, and identified by comparison of their nmr and/or CD spectra to those of known model complexes.[6]

To check the effect of carbon, the hydrolysis reaction was carried out in exactly the same manner as before except carbon was omitted. Thin layer chromatography of the reaction mixture after heating for 2 hr revealed the presence of glycine; also some dipeptide was

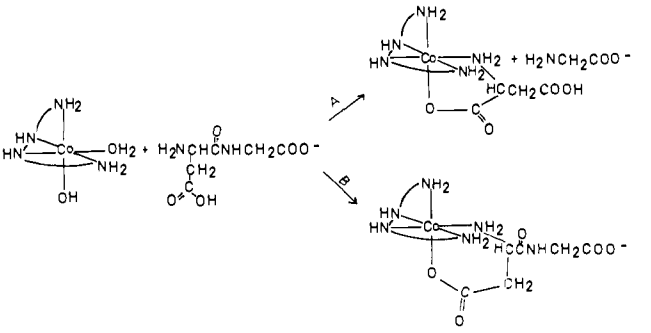


Figure 1. Possible reactions of Co(trien)(OH)(H$_2$O)$^{2+}$ with α-L-aspartylglycine.

(5) J. I. Legg and J. Steele, *Inorg. Chem.*, **10**, 2177 (1971).
(6) J. I. Legg and D. W. Cooke, *J. Amer. Chem. Soc.*, **89**, 6854 (1967).
(7) P. H. Clayton, *Inorg. Syn.*, **7**, 207 (1963).
(8) The identity of the species obtained from the hydrolysis of Co(dien)Cl$_3$ is not known with certainty but is probably Co(dien)(OH)(H$_2$O)$_2^{2+}$ at pH 5–6.
(9) Gravimetric determination of the AgCl produced indicated that over 90% of the Cl$^-$ had been removed.
(10) A. M. Sargeson and G. H. Searle, *Inorg. Chem.*, **6**, 787 (1967).

detected. The hydrolysis products were then separated on a cation-exchange SE-Sephadex column. Three cobalt species were detected, two minor purple bands, one of which was highly charged and stuck to the column top, and a major orange band. Addition of carbon and heating for 1 hr at 70° produced the expected Co(dien)(L-Asp)$^+$ isomers.

C. Reaction of Hydroxodiaquo(diethylenetriamine)cobalt(III) Ion[8] with L-Aspartic Acid Diethyl Ester. The same procecure as that used in the hydrolysis of α-L-aspartylglycine was followed with only one modification; 4 mmol of $AgNO_3$ was used in order to remove all the chloride since the ester was obtained as the hydrochloride salt.

Chromatography and Isolation of Products. A. Ion-Exchange Chromatography. 1. Dowex Resin. Hydrolysis products from the reaction of Co(dien)Cl_3 with α-L-aspartylglyine, L-aspartic acid diethyl ester, and those from the reaction of β-[Co(trien)(OH)$(H_2O)]^{2+}$ with glycyl-L-aspartic acid were separated on an ion-exchange column in the manner described by Legg and Cooke.[6] The reaction mixtures were added to an ion-exchange column (2.2 cm o.d., height of resin = 25.5 cm) containing 130 mequiv of Dowex 50W-X4, 200–400 mesh resin which had been converted to the sodium form. The column had been uniformly packed by backwashing. The compounds formed a narrow, 1-cm band. Dipeptides and amino acids formed in the hydrolyses reactions are neutral or negatively charged under these conditions and were eluted with water; to ensure their complete recovery, elution with water was continued until *ca.* 500 ml of eluent was collected. The solution was then concentrated and the presence of amino acids was checked by thin layer chromatography.

α-L-Aspartylglycine and L-Aspartic Acid Diethyl Ester. Elution of the cobalt complexes with 0.35 *M* $NaClO_4$ (1 drop/10 sec) was complete in 7 days. The band separated into the three expected Co(dien)(L-Asp)$^+$ isomers.[6] These three bands were collected in fractions, and CD spectra showed that each consisted of only one isomer. Slower moving bands were also observed, but their isolation was not possible owing to the small quantities present. In the case of L-aspartic acid diethyl ester some brown decomposition product stuck to the top of the column. The perchlorate isomers were isolated by evaporation of the eluent with simultaneous removal of $NaClO_4$. The remainder of the $NaClO_4$ was removed by chromatography on a G-15 Sephadex column (2.2 cm o.d., height of gel = 34 cm, particle size 40–120 μ). The pure isomer moved faster than $NaClO_4$. The yield of each isomer was then determined spectrophotometrically. The first, *s-cis*-Co(dien)(L-Asp)$^+$, isomer was recrystallized by dissolving it in a minimum amount of water and setting the solution in a desiccator containing concentrated sulfuric acid which permitted slow crystallization to take place.

Glycyl-L-aspartic Acid. The cobalt complexes obtained from the reaction of β-[Co(trien)(OH)$(H_2O)]^{2+}$ and glycyl-L-aspartic acid formed an even 1-cm band at the top of the column. Upon elution with 0.35 *M* $NaClO_4$, three bands separated, two of which (a pink and an orange) eluted well before another orange band (+2 species). After the collection of the two bands, 0.7 *M* $NaClO_4$ was used for the elution of the last orange band. A brown decomposition product stuck to the top of the column. The three eluted bands were collected in fractions, and circular dichroism spectra showed that the second eluted orange band consisted of more than one isomer (diastereoisomers) and the first and third eluted bands were not optically active. The products were purified, isolated, and identified spectroscopically. Elemental analyses of the optically active, unipositive species is consistent with [Co(trien)(Gly-L-Asp)]$ClO_4 \cdot 5H_2O$. *Anal.* Calcd for $[Co(C_{12}N_6O_5H_{26})]ClO_4 \cdot 5H_2O$: C, 24.72; H, 6.18; N, 14.42. Found: C, 25.18; H, 5.98; N, 13.97. Found (for a different preparation): C, 24.45; H, 5.09; N, 14.23.

2. SE-Sephadex. Products from the hydrolysis reaction of β-[Co(trien)(OH)$(H_2O)]^{2+}$ with glycyl-L-aspartic acid, glycyl-L-glutamic acid, and glycyl-DL-methionine were separated on a SE-Sephadex C-25 cation-exchange cellulose column (2.2 cm o.d., height of cellulose = 36 cm) in the sodium form (columns were packed with Sephadex which was allowed to swell by letting the Sephadex stand in water overnight). The compounds formed a narrow band when loaded on the column. Dipeptides and amino acids produced in the hydrolysis reaction were recovered in the same manner as when Dowex ion-exchange resin was used as the chromatographic medium. The elution rate was 1 drop/10 sec. Results for the various reactions are given below. In all cases, a brown decomposition product stuck to the top of the column.

Glycyl-L-aspartic Acid. Elution of the cobalt complexes obtained from the hydrolysis of glycyl-L-aspartic acid with 0.2 *M* $NaClO_4$ gave the same results as those observed when Dowex was used; however, elution time, which was complete in 2–3 days, was much shorter than in the former case.

Glycyl-L-glutamic Acid. Elution of the cobalt complexes obtained from the hydrolysis of glycyl-L-glutamic acid with 0.2 *M* $NaClO_4$ gave three bands which behaved analogously to those obtained from the glycyl-L-aspartic acid hydrolysis. The first eluted pink-orange band and the third eluted highly charged orange band were optically inactive. The second eluted orange band was optically active, and circular dichroism spectra of the fractions showed that it consisted of more than one isomer (diastereoisomers). Elution was complete in 2–3 days. Elemental analyses of the optically active, unipositive species are consistent with [Co(trien)(Gly-L-Glu)]$ClO_4 \cdot 6H_2O$. *Anal.* Calcd for $[Co(C_{13}H_6O_5H_{28}]ClO_4 \cdot 6H_2O$: C, 25.94; H, 6.62; N, 13.89. Found: C, 25.82, 25.74; H, 5.42, 5.53; N, 13.61, 13.70.

Glycyl-DL-methionine. Elution of the cobalt complexes obtained from the hydrolysis of glycyl-DL-methionine with 0.2 *M* $NaClO_4$ gave two well-separated bands, a pink band that eluted first and a highly charged orange band.

B. Thin Layer Chromatography. The water fractions eluted from the ion-exchange columns were checked for the presence of amino acids and/or dipeptides using tlc on silica gel. The solutions were spotted on strips of silica gel with capillaries and air-dried. The plates were eluted in the ascending manner using 1-propanol–water (70:30, v/v) as eluent. Generally, amino acids or dipeptides moved faster than the cobalt complexes. After elution, the spots were detected by spraying the air-dried plate with freshly prepared ninhydrin solution (0.25 g in 100 ml acetone) and heating at 100°. Amino acids appeared after heating for 1–2 min. Identification was accomplished by comparison to known marker amino acids.

Base Hydrolysis of the Intermediate Dipeptide Complexes. Base hydrolysis of the intermediate dipeptide complexes was carried out by dissolving *ca.* 0.03 g of Co[(trien)(Gly-L-Asp)]$^+$ or [Co(trien)-(Gly-L-Glu)]$^+$ in 5 ml of water; the solution was made basic (*p*H 10–10.5) using LiOH and heated for 1 hr at 55°. The presence of aspartic acid and glutamic acid, respectively, was established by tlc. Very small traces of dipeptides were also detected.

Results and Discussion

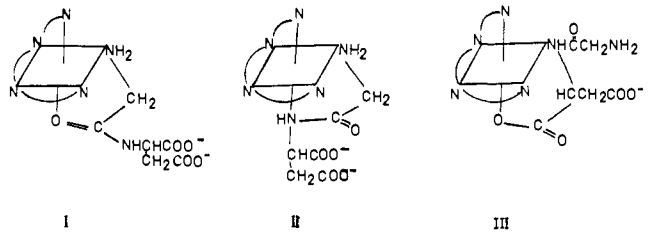
Reactions of Hydroxoaquotriethylenetetraminecobalt(III) Ion with Dipeptides Containing C-Terminal Trifunctional Amino Acids. Three dipeptides were selected: glycyl-L-aspartic acid, glycyl-L-glutamic acid, and glycyl-DL-methionine. All three possess potentially coordinating side chains of comparable bulk on the C-terminal amino acid. However, the Gly-Asp and Gly-Glu peptides differ from Gly-Met in that hydrogen bonding involving the side chain is possible for the former two but not for the latter. This difference was found to have a significant effect on the hydrolysis reactions.

The final products formed upon treatment of the peptides with β-[Co(trien)(OH)$(H_2O)]^{2+}$ at pH 7.2–7.6 and 70° are given in Table I. In each case, the products

Table I. Products Obtained from the Reaction of Co(trien)(OH)$(H_2O)^{2+}$ with Dipeptides Containing C-Terminal Trifunctional Amino Acids

Substrate[a]	Products	Yield, %[b]
Glycyl-L-aspartic acid	Co(trien)(Gly)$^{2+}$	18
	Co(trien)(Gly-L-Asp)$^{1+}$	55
	Co(trien)$(OH)_2^+$	6
	L-Aspartic acid	
Glycyl-L-glutamic acid	Co(trien)(Gly)$^{2+}$	10
	Co(trien)(Gly-L-Glu)$^+$	54
	Co(trien)$(OH)_2^+$	8
	L-Glutamic acid	
Glycyl-DL-methionine	Co(trien)(Gly)$^{2+}$	60
	Co(trien)$(OH)_2^+$	10
	DL-Methionine	

[a] Reaction conditions: [reactants] = 0.04 *M*, *t* = 70°, pH 7.2–7.6. [b] Reaction time 2 hr.



I II III

Figure 2. Possible modes of chelation of glycyl-L-aspartic acid.

were separated by cation-exchange column chromatography (Sephadex and/or Dowex), isolated as their perchlorates, and characterized. Amino acids formed were identified by tlc on silica gel. In all cases studied $[Co(trien)(Gly)]^{2+}$ was one of the products. The formation of this complex as opposed to the formation of the trifunctional amino acid complex demonstrates the N-terminal specificity of the hydrolysis reactions.

(1) Reaction with Glycyl-L-aspartic acid and Glycyl-L-glutamic Acid. Other investigators have found that hydrolysis of dipeptides containing bifunctional amino acids was essentially quantitative and the N-terminal amino acid was selectively removed as $[Co(trien)(amino\ acid)]^{2+}$.[2,4] Thus, if no interference from the side chain of the trifunctional C-terminal amino acid were to occur, it was anticipated that the hydrolysis of glycyl-L-aspartic acid and glycyl-L-glutamic acid would parallel those reactions carried out previously. If such were the case, the expected hydrolysis products would be $[Co(trien)(Gly)]^{2+}$ and aspartic acid or glutamic acid, respectively. However, it was found that treatment of either of these two dipeptides with $[Co(trien)(OH)(H_2O)]^{2+}$ resulted in the formation of aspartic acid or glutamic acid and three cobalt containing complexes: a pink +1 species, a dominant orange +1 species, and an orange +2 species.

Following the hydrolysis reaction of glycyl-L-aspartic acid as a function of time at pH 7.2–7.6 using ion-exchange column chromatography revealed that the orange +2 cobalt complex increased at the expense of the orange +1 species; this suggested that the +1 species is an intermediate in the hydrolysis reaction. However, the +1 species was observed even after 25 hr of heating at 70°. Various additional products were obtained when the reaction was carried out at a higher pH (8.5) as had been observed for the simpler dipeptides.[11]

The orange +2 species was identified as β-$[Co(trien)(Gly)]^{2+}$ on the basis of chromatographic behavior, visible spectra (maxima at 348 nm (ε 135) and 480 nm (ε 127)); the pmr spectra (absorptions at 3.5 and 3.0 (broad 2.6–3.3) ppm downfield from TMS* integrating 1:6). These spectral results are consistent with those obtained previously for $[Co(trien)(Gly)]^{2+}$.[12]

Based on elemental analyses, chromatographic behavior, and spectral data (visible, CD, ir, and pmr), it is proposed that the unipositive, orange species are the dipeptide complexes, $[Co(trien)(Gly\text{-}L\text{-}Asp)]^{+}$ and $[Co(trien)(Gly\text{-}L\text{-}Glu)]^{+}$. Support for this conclusion also comes from a crystallographic study of a similar complex, $[Co(trien)(Gly\text{-}Gly\text{-}OEt)]^{3+}$.[13]

(11) D. A. Buckingham, C. E. Davis, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **92**, 5571 (1970).
(12) J. P. Collman and E. Kimura, *ibid.*, **89**, 6096 (1967).
(13) D. A. Buckingham, P. A. Marzilli, I. E. Maxwell, and A. M. Sargeson, *Chem. Commun.*, 488 (1968).

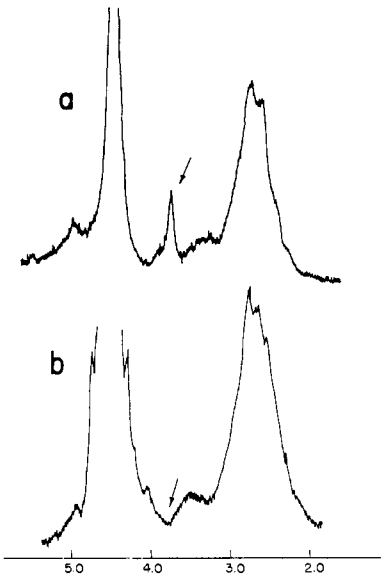


Figure 3. (a) Nmr spectra of $Co(trien)(Gly\text{-}L\text{-}Asp)^{+}$ before and (b) after H–D exchange.

Chromatography revealed that each complex consisted of two isomers, and the CD spectra suggested that the isomers were diastereomeric. From the chromatographic behavior and the elemental analyses it could be concluded that the dipeptide was coordinated to the complex. Since these complexes behaved as intermediates in the hydrolysis and since amide carbonyl coordinated peptides had previously been obtained in nonaqueous solvents,[11,12] it was of particular interest to identify the nature of these dipeptide complexes formed in aqueous solution.

Figure 2 shows the different possible modes of coordination of the dipeptide, *e.g.*, glycyl-L-aspartic acid. Structure I is the five-membered ring isomer formed by dipeptide coordination through the terminal amine group and the amide carbonyl oxygen, structure II is formed by coordination through the terminal amine group and the amide carbonyl nitrogen, and structure III is the five-membered ring isomer formed by coordination of the trifunctional amino acid through the α-carboxyl group and the amide group. Structure III was not expected, for it was shown under the conditions used in these studies[3,4] that hydrolysis of dipeptides proceeds *via* the chelation of N-terminal amino acids. A fourth structure, involving coordination through the β-carboxyl group to form a six-(Asp) or seven- (Glu) membered ring was not expected on the basis of previous studies.[5] Experimental data used to eliminate structure III would also apply to the elimination of this structure.

Visible spectra of the unipositive species, $[Co(trien)(Gly\text{-}L\text{-}Asp)]^{+}$ (maxima at 478 nm (ε 161) and 345 nm (ε 167)) and $[Co(trien)(Gly\text{-}L\text{-}Glu)]^{+}$ (maxima at 478 nm (ε 138) and 345 nm (ε 157)) are consistent with either $Co(N_4O_2)$ or $Co(N_5O)$ complexes[1,2,4-6,12] and, therefore, are not helpful in distinguishing modes of coordination.

The pmr spectra of $[Co(trien)(Gly\text{-}L\text{-}Asp)]^{+}$ and $[Co(trien)(Gly\text{-}L\text{-}Glu)]^{+}$ are summarized in Figures 3 and 4 (spectrum a). The ethylene protons of the trien ligand exhibit a broad resonance centered at 2.7 and 3.1 ppm downfield from TMS*, respectively. The glycine methylene protons exhibit resonances at 3.77

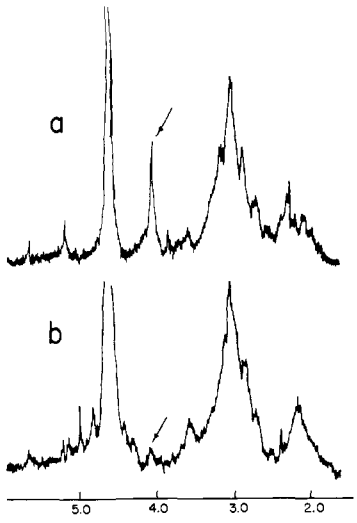


Figure 4. (a) Nmr spectra of Co(trien)(Gly-L-Glu)$^+$ before and (b) after H–D exchange.

and 4.07 ppm, respectively, which are the same as or further downfield from the corresponding values of the free peptides. The methylene chemical shifts are consistent with those found for similar chelates where glycylglycine esters are coordinated through the terminal amino group and the amide carbonyl oxygen.[12] The glutamic acid dipeptide complex shows an absorption centered at 2.2 ppm due to the methylene protons on the side arm.[5]

Several investigators[5,14] have found the α-methylene protons of glycine or glycine-like chelates to be labile by demonstrating the disappearance of the resonance signal upon isotopic substitution of D for H in basic (or acidic) solution. The addition of Na_2CO_3 to the dipeptide complexes resulted in the disappearance of the glycine–methylene peak, Figures 3 and 4 (spectrum b). The exchange of the α-methylene protons eliminates structure III and supports structure I. Based on work done by Gillard and Phipps,[15] no such exchange is expected for structure II. They have shown that for [Co(dien)(dipeptide)]$^{2+}$ (dipeptide = Gly-Gly, Gly-L-Ala, and L-Ala-Gly), where the dipeptide is acting as a tridentate, methylene proton exchange occurs only for the C-terminal residue of the peptides. Coordination of the dipeptides in these systems is probably similar to the analogous bis(dipeptide) complexes and bis(glycylglycinato)- and bis(alanylglycinato)cobalt(III).[16] An X-ray crystal structure[17] determination of $NH_4[Co(Gly\text{-}Gly)_2]\cdot 2H_2O$ showed that the glycylglycine is coordinated through the amino group, amide nitrogen, and the carboxyl group. It is reasonable to assume that dipeptides in complexes of the type [Co(dien)(dipeptide)]$^{2+}$ are coordinated in the same fashion, and their studies suggest that no isotopic exchange will occur if coordination of the amino acid is through the terminal amino group and the amide carbonyl nitrogen.

(14) D. H. Busch and D. H. Williams, *J. Amer. Chem. Soc.*, 87, 4644 (1965); J. L. Sudmeier and G. Occupati, *Inorg. Chem.*, 7, 2524 (1968); J. B. Terrill and C. N. Reilley, *ibid.*, 5, 1988 (1966); P. F. Coleman, J. I. Legg, and J. Steele, *ibid.*, 9, 937 (1970).

(15) R. D. Gillard and D. A. Phipps, *Chem. Commun.*, 800 (1970).

(16) R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Coord. Chem. Rev.*, 1, 163 (1966); R. D. Gillard, P. M. Morrison, and E. D. McKenzie, *J. Chem. Soc. A*, 618 (1967).

(17) R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Nature (London)*, 209, 1347 (1966).

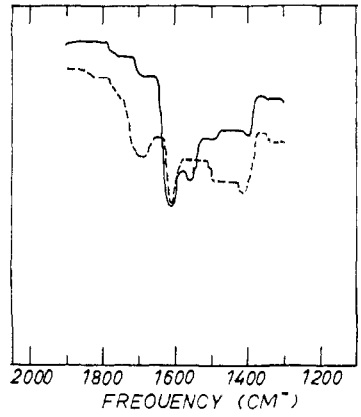


Figure 5. Ir spectra of Co(trien)(Gly-L-Glu)$^+$ at pH 5.5 (——) and pH 1 (– – –).

Since H–D exchange was observed for the dipeptide complexes reported here, structure I, Figure 2 is favored. It should also be noted that Buckingham, *et al.*, did not report H–D exchange for N,N-chelated glycine amides in alkaline solution.[11]

Further support for this assignment comes from the solution infrared spectra in D_2O of the glycyl-L-aspartic acid and glycyl-L-glutamic acid intermediates in the region 1300–1900 cm^{-1}. Representative spectra obtained in D_2O are shown in Figure 5 for [Co(trien)(Gly-L-Glu)]$^+$. An intense band at 1620 cm^{-1} observed for both complexes is assigned to the coordinated amide carbonyl normal stretching mode. This frequency in free glycyl-L-aspartic acid and glycyl-L-glutamic acid is found between 1670 and 1680 cm^{-1}. The spectral shift upon coordination is similar to that found for the cobalt(III) triethylenetetramineglycylglycine methyl ester complexes.[12] It is also in accord with coordination through the amide carbonyl oxygen. A study of the infrared spectra of both N and O bound urea complexes had demonstrated that N-coordination causes the carbonyl frequency to increase whereas O-coordination has the opposite effect.[18]

Bands at 1700 (Asp), 1690 (Glu) cm^{-1} and 1580 (Asp), 1560 (Glu) cm^{-1} are attributed to the normal carbonyl stretching modes of free carboxylic acid (COOH) and to the free carboxylate (COO$^-$), respectively.[19] These values indicate little or no interaction with the metal ion because of their proximity to the intense bands 1700 (Asp), 1700 (Glu) cm^{-1} and 1580 (Asp), 1560 (Glu) cm^{-1} of the free peptides. A pD study further confirmed the presence of an uncoordinated carboxyl group; a decrease in pD increased the intensity of the higher frequency band in each complex while decreasing the intensity of the lower frequency bands as illustrated for [Co(trien)(Gly-L-Glu)]$^{n+}$ in Figure 5. As expected the coordinated amide carbonyl stretching frequency at 1620 cm^{-1} was unaffected (Figure 5).

Alkaline aqueous hydrolysis of [Co(trien)(Gly-L-Asp)]$^+$ and [Co(trien)(Gly-L-Glu)]$^+$ resulted in the formation of aspartic acid and glutamic acid, respectively, which further supports coordination of the amide through the carbonyl oxygen as shown in structure I,

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(19) R. E. Sievers and J. C. Bailar, Jr., *Inorg Chem.*, 1, 174 (1962), and references therein.

Figure 2. If the unipositive species possessed structure III, glycine would be expected to be the residual amino acid and had coordination of the glycine been through the carbonyl nitrogen, hydrolysis of these complexes would not have been anticipated. It has been previously reported[20] that essentially no hydrolysis is observed when the amide group is coordinated through the nitrogen in a variety of complexes.

These observations are consistent, then, with the conclusion that these peptide complexes are intermediates in the N-terminal hydrolysis of the peptides by β-$[Co(trien)(OH)(H_2O)]^{2+}$. One possible explanation for the stability of these intermediates arises from a consideration of the interaction of the penultimate amino acid side chain with the complex. Examination of molecular models reveals that the ω-carboxyl group is within hydrogen bonding distance of the amine hydrogens as shown in Figure 6. It is proposed that the stability of these intermediate dipeptide complexes is due in part to this interaction.

(2) Reaction with Glycyl-DL-methionine. Treatment of $[Co(trien)(OH)(H_2O)]^{2+}$ with glycyl-DL-methionine resulted in complete hydrolysis of the dipeptide and the formation of the expected products $[Co(trien)(Gly)]^{2+}$ and methionine. This result supports the proposal that the unipositive species obtained in the previous cases are stabilized due to hydrogen bonding. Methionine, although possessing a potentially ligating side chain of comparable bulk to those of aspartic and glutamic acid, is not expected to hydrogen bond to the amine hydrogens, and hence the hydrolysis is expected to parallel previously studied systems.[1,2,4]

Reactions of Hydroxydiaquo(diethylenetriamine)cobalt(III)[8] with α-L-Aspartylglycine and Aspartic Acid Diethyl Ester. Treatment of α-L-aspartylglycine with $Co(trien)(OH)(H_2O)^{2+}$ produced some glycine but also a series of difficult to identify cobalt(III) complexes which suggested that the aspartic acid side chain was interfering with the hydrolysis. This was not surprising since even when the trifunctional amino acid was penultimate, interference had been encountered (*vide supra*).

It was found that by rendering the metal ion more accessible to the incoming substrate and simultaneously making available three sites to the N-terminal trifunctional amino acid by changing from a tetradentate chelate ($Co(trien)X_2$) to a tridentate chelate ($Co(dien)X_3$), hydrolysis was obtained. The final products formed upon treatment of α-L-aspartylglycine and L-aspartic acid diethyl ester with $Co(dien)(OH)(H_2O)_2^{2+}$ at a pH 5.5 and 70° were the three isomers of Co(dien)(L-Asp)$^+$ and glycine (former case). The isomers were separated chromatographically and were identified through their CD spectra.[6] In addition, the perchlorate salt of the first eluted isomer (s-cis) was isolated and identified by CD and pmr spectroscopy in both cases. The CD spectrum of the isomer obtained from the ester hydrolysis was identical with that of the known complex;[6] however, that obtained from the dipeptide hydrolysis showed minor but consistent discrepancies although pmr spectra were identical with the isomer prepared directly from L-aspartic acid. The glycine formed was detected by tlc. The N-terminal specificity of the reaction with α-L-aspartylglycine is demonstrated by the formation of the complex Co(dien)(L-Asp)$^+$ and glycine.

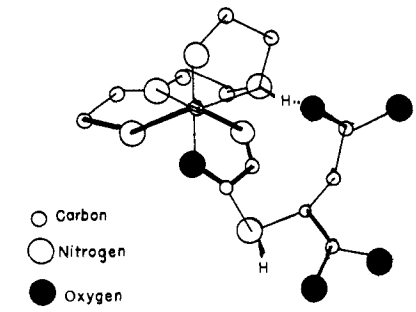

Figure 6. Proposed structure of Co(trien)(Gly-L-Asp)$^+$ intermediate.

Although it cannot be concluded that charcoal is not the active promoter in the hydrolysis (*e.g.*, directly or indirectly through the production of Co(II)), evidence thus far obtained suggests that charcoal is only required to obtain fully coordinated aspartic acid. When the reaction was carried out in the absence of charcoal, glycine was obtained indicating that some hydrolysis had taken place, but the complex isolated chromatographically from the reaction mixture had to be further subjected to charcoal to obtain Co(dien)(L-Asp)$^+$ where aspartic acid is fully coordinated as a tridentate. In line with these observations direct reaction of $Co(dien)(H_2O)^{3+}$ with L-aspartic acid produces two intermediates, both of which yield Co(dien)(L-Asp)$^+$ on treatment with charcoal.[21] The relationship between these intermediates and that formed in the hydrolysis of L-aspartylglycine remains to be determined.

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