Requirements for the Multiple Formation of Peptide Bonds within the Coordination Spheres of Metal Ions. A Three-Site System Based on Cobalt(III)

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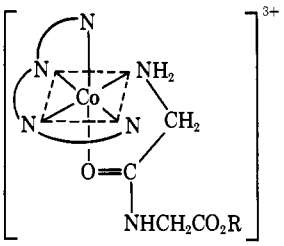
Abstract: The formation of peptides using the three-site moiety $Co(dien)^{3+}$ has been exemplified in the reaction of $Co(dien)X_3$ (X = Cl, NO_2) with glycine esters and glycylglycine esters to produce $[Co(dien)(GlyGlyOR)X]^{2+}$ and $[Co(dien)(GlyGlyGlyGlyOEt)]^{2+}$. Pertinent reactions of these species have been investigated and a number of other interesting and related complexes have been prepared, including $[Co(dien)(GlyO)X]^{+}$, $[Co(dien)(GlyNHR')X]^{2+}$ (R' = H or CH_3), $[Co(dien)(GlyGlyOH)X]^{2+}$, and $[Co(dien)(GlyGlyO)]^{+}$. The application of infrared and nmr spectroscopy to the characterization of such complicated adducts is well illustrated by these studies. Formation of the dipeptide proceeds, from either GlyOR or GlyGlyOR, as expected on the basis of earlier reports on two-site systems. An interesting complication is associated with formation of the tetrapeptide complex. At low and neutral pH bidentate chelated dipeptides bind to Co(III) through their terminal amines and the oxygen atoms of their adjacent carboxamide functions. In such a configuration the oxygen atom of the second amino acid residue cannot reach the metal to produce tridentate chelation and activation of the second carboxyl function. In order for the second peptide linkage to form, the binding of the first such linkage must isomerize from O-metal bound to N-metal bound. The conditions for such reactions are indicated.

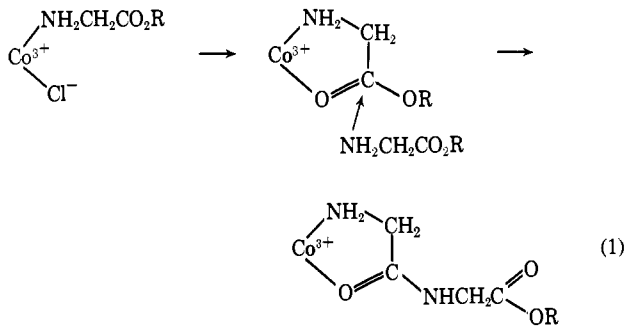
To investigate the nature of cobalt ion promotion of the hydrolysis of α-aminocarboxylic acid esters, Alexander and Busch and Wu and Busch[1] prepared the series of complexes *cis*-$[Co(en)_2(GlyOR)Cl]^{2+}$, where R is CH_3, C_2H_5, C_3H_7, *t*-C_4H_9, by the reactions of glycine esters with *trans*-$[Co(en)_2Cl_2]Cl$ in water.[2] When the reaction of the cobalt complex with a glycine ester is performed in dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), rather than in water, the products are the dipeptide complexes $[Co(en)_2(GlyGlyOR)]^{3+}$ (R = CH_3, C_2H_5, C_3H_7).[3] Corresponding results were obtained[4,5] upon reaction of glycine esters with *cis*-$[Co(trien)Cl_2]^{+}$ in water to yield *cis*-$[Co(trien)(GlyO)]^{2+}$ and in DMF or DMSO medium to give *cis*-$[Co(trien)(GlyGlyOR)]^{3+}$. In the latter, the GlyGlyOR acts as a chelate ligand bonded to cobalt through the terminal amino group and amide carbonyl oxygen (structure I).



I

The structures of these complexes have been established by the X-ray method for the case of $[Co(trien)(GlyGlyOC_2H_5)](ClO_4)_3 \cdot H_2O$.[6]

It has been concluded[1,3-5] that peptide formation takes place through activation of the glycine ester group by coordination of the carbonyl oxygen by cobalt, followed by nucleophilic attack at the carbonyl carbon by the amino group of a second amino acid or peptide reagent (eq 1).



(1)

In each case, the hydrolysis of coordinated amino acid esters or of peptides leaves chelated amino acid anions bound to the metal.[1,5,7] However, Nakahara, *et al.*,[8] reported that almost no hydrolysis occurs when an amide group is coordinated through the amide nitrogen (pH 8.5).

Most of the recent work concerned with the promotion of peptide formation or hydrolysis of amino acid derivatives by metal ions has involved tetramine complexes of cobalt(III) containing only two adjacent coordination sites available to accommodate the reaction; *e.g.*, the residual units $Co(en)_2^{3+}$ and $Co(trien)^{3+}$ were designed to persist throughout the reactions. The en and trien serve to block four of the six coordina-

(1) M. D. Alexander and D. G. Busch, *Inorg. Chem.*, **5**, 602 (1966); *J. Amer. Chem. Soc.*, 88, 1130 (1966); Y. Wu and D. H. Busch, *ibid.*, **92**, 3326 (1970).

(2) The following abbreviations are used throughout this paper: en = ethylenediamine; dien = diethylenetriamine; GlyO = glycinate; GlyOR = glycine ester; GlyGlyO = glycylglycinate; GlyGlyOR = glycylglycine ester; GlyGlyGlyGlyOR = glycylglycylglycylglycine ester; GlyN = glycine amide; IDA = iminodiacetate; MIDA = methyliminodiacetate; trien = triethylenetetramine.

(3) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Amer. Chem. Soc.*, 89, 4538 (1967).

(4) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *ibid.*, 89, 2772 (1967).

(5) J. P. Collman and E. Kimura, *ibid.*, 89, 6096 (1967).

(6) D. A. Buckingham, P. A. Marzilli, I. E. Maxwell, and A. M. Sargeson, *Chem. Commun.*, 488 (1968).

(7) D. A. Buckingham, J. P. Collman, D. A. R. Happer, and L. G. Marzilli, *J. Amer. Chem. Soc.*, 89, 1082 (1967).

(8) A. Nakahara, K. Hamada, Y. Nakao, and T. Higashiyama, *Coord. Chem. Rev.*, 3, 207 (1968).

tion sites on the Co(III), making it a bifunctional or two-site reagent.

We have been interested in investigating the formation of peptides by using a metal complex ion with three coordination sites available for the condensation process. This requires that three sites on Co(III) be blocked and the $Co(dien)^{3+}$ unit is well suited to this use (dien is diethylenetriamine). Such studies provide the opportunity to elucidate the requirements for the stepwise formation of peptides. The materials developed in this work also permit elaboration on the selective hydrolysis of peptide bonds in metal complexes.

Experimental Section

Materials. Glycine methyl and ethyl ester hydrochloride and glycine amide hydrochloride were obtained from Aldrich Chemical Co. Glycylglycine was obtained from Nutritional Biochemicals Co. Glycylglycine ethyl ester was obtained from Cyclo Chemical Co. All of these reagents were used without further purification.

$[Co(dien)Cl_3]$ and $[Co(dien)(NO_2)_3]$ were prepared by the method of Crayton and Mattern.[9] *Anal.* Calcd for $[Co(C_4H_{13}N_3)Cl_3]$: C, 17.9; N, 15.7; H, 4.9. Found: C, 18.2; N, 16.1; H, 4.8. Calcd for $[Co(C_4H_{13}N_3)(NO_2)_3]$: C, 16.0; N, 28.0; H, 4.4. Found: C, 16.0; N, 28.2; H, 4.2.

Glycine *N*-Methylamide Hydrochloride. This compound was prepared according to the method of Collman and Kimura.[5] *Anal.* Calcd for $C_3H_8N_2O \cdot HCl$: C, 28.9; H, 7.3; N, 22.5. Found: C, 29.2; H, 7.8; N, 23.1.

N,N-Dimethylformamide was purified by distillation under reduced pressure and dried over type 4A or 5A molecular sieves ($^1/_{16}$ in. pellets, Fisher Scientific Co.). Dimethyl sulfoxide was purified by adding a small amount of CaH_2 and distillation under reduced pressure followed by drying over type 4A or 5A molecular sieves. Absolute ethanol was dried over type 3A molecular sieves.

$[Co(dien)(NH_2CH_2CO_2)NO_2]ClO_4$. Trinitro(diethylenetriamine)cobalt(III) (1.50 g, 0.005 mol) is gently heated with 1.2 ml of concentrated perchloric acid (60%) until nitrogen(IV) oxide evolution ceases. The resulting solution is diluted to 10 ml with water. The excess acid in the solution is neutralized with diethylamine (Beckman Model G pH meter was used as indicator). Glycine (380 mg, 5 mmol) is added to the solution, followed by diethylamine (0.5 ml). A golden crystalline precipitate begins to form almost immediately. The precipitate is filtered and washed with methanol, ethanol, and ether. The crude product is dissolved in a minimum amount of hot water (50–60°) and recrystallized on cooling, after the addition of a few drops of saturated sodium perchlorate solution. The product is dried *in vacuo* overnight over $Mg(ClO_4)_2$ at 50°. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_2H_4NO_2)(NO_2)]ClO_4$: C, 18.88; H, 4.49; N, 18.35. Found: C, 18.95; H, 4.75; N, 18.10.

$[Co(dien)(NH_2CH_2CONH_2)(NO_2)](ClO_4)_2$. The procedure described above is used except that the glycine is replaced with glycine amide hydrochloride (yield 40%). The perchlorate salt of the glycine amide complex is more soluble in water than the perchlorate salts of the glycine complex. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_2H_6N_2O)(NO_2)](ClO_4)_2$: C, 15.0; H, 4.0; N, 17.5. Found: C, 15.1; H, 4.0; N, 17.7.

$[Co(dien)(NH_2CH_2CO_2)Cl]ClO_4$. $Co(dien)Cl_3$ (1.34 g, 0.005 mol) and 0.4 g (0.005 mol) of glycine are made into a paste with 5 ml of water using a mortar and pestle. Diethylamine (0.5 ml, 0.005 mol) is added to the mixture which is ground continuously. After this mixture has been ground intermittantly over a period of 30 min, water-insoluble materials are removed by filtration using a glass filter and washed with 12 ml of water. The residue on the glass filter is $[Co(dien)Cl_3]$ (10%). The wash solution is combined with the filtrate, to which a few drops of concentrated perchloric acid (60%) are added. The product separates as the solution is concentrated under a stream of air. The crude product is washed with methanol, ethanol, and ether, and air-dried. It is recrystallized from water and dried over $Mg(ClO_4)_2$ *in vacuo* at 50°. Yield 50%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_2H_4NO_2)Cl]ClO_4$: C, 19.4; H, 4.62; N, 15.1. Found: C, 19.6; H, 4.62; N, 15.0.

$[Co(dien)(NH_2CH_2CONHCH_2CO_2R)(NO_2)](ClO_4)_2$. The methyl and ethyl derivatives are prepared as follows. Trinitro(diethylenetriamine)cobalt(III) (0.90 g, 3 mmol) is gently heated with 1.2 ml of concentrated perchloric acid (60%) until nitrogen(IV) oxide evolution ceases. Dimethylformamide (20 ml) is added to the resulting solution and the solution is neutralized with diethylamine. Glycine ester hydrochloride (6 mmol) is dissolved in the solution. Diethylamine (0.6 ml, 6 mmol) is added slowly into the solution with stirring, and stirring is continued at 50° for 1 hr. Acetone is added to the resulting solution in order to precipitate a yellow solid. The crude product is converted into the perchlorate salt by dissolution in 1–2 ml of concentrated sodium perchlorate solution. After standing overnight, golden crystals separate from the solution. These are recrystallized from water and dried over $Mg(ClO_4)_2$ *in vacuo* at 50°. Yield 15%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_5H_{10}N_2O_3)(NO_2)](ClO_4)_2$: C, 19.5; H, 4.2; N, 15.2. Found: C, 19.5; H, 4.5; N, 15.2. Calcd for $[Co(C_4H_{13}N_3)(C_6H_{12}N_2O_3)(NO_2)](ClO_4)_2$: C, 21.2; H, 4.4; N, 14.8. Found: C, 21.0; H, 4.6; N, 14.6.

$[Co(dien)(GlyGlyOR)Cl](ClO_4)_2$. This preparation is similar to that used by Collman and Kimura[5] to prepare *cis*-β-$[Co(trien)(GlyGlyOR)]^{3+}$. The methyl and ethyl derivatives are prepared as follows. A mixture of $[Co(dien)Cl_3]$ (0.793 g, 0.003 mol) and silver nitrate (1.04 g, 0.0061 mol) is made into a paste with 15 ml of DMF or DMSO using a mortar and pestle. The mixture is ground continually. After 30 min, the insoluble silver chloride is removed by filtering and washed with DMF or DMSO until the combined filtrates and washings totaled about 30 ml. Glycine ester hydrochloride (0.006 mol) is dissolved in this solution and 0.6 ml (0.006 mol) of diethylamine is added slowly with stirring. The use of a pH meter (glass electrode) is recommended in order to monitor pH during the addition of dimethylamine (although the absolute value of the indicated pH is not significant in this DMF solution). The apparent pH of the solution must not rise above 7.7. It takes approximately 15 min to complete the addition of dimethylamine in this way. Finally, the solution is stirred at 50° for 1 hr. Acetone is added to precipitate a grey hygroscopic solid which is washed with acetone and ether and then converted into a purple-black crystalline perchlorate salt following the above procedure. Yield 39–45%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_5H_{10}N_2O_3)Cl](ClO_4)_2$: C, 19.92; H, 4.27; N, 12.91; Cl, 19.60. Found: C, 20.25; H, 4.41; N, 12.84; Cl, 19.57. Calcd for $[Co(C_4H_{13}N_3)(C_6H_{12}N_2O_3)Cl](ClO_4)_2$: C, 21.57; H, 4.53; N, 12.58; Cl, 19.11. Found: C, 21.63; H, 4.57; N, 12.58; Cl, 19.09.

Preparation of $[Co(dien)(GlyGlyOC_2H_5)Cl](ClO_4)_2$ from $[Co(dien)Cl_3]$ and Glycylglycine Ethyl Ester Hydrochloride. The method employed is analogous to that used to prepare the chloroglycine complex. Yield 60%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_6H_{12}N_2O_3)Cl](ClO_4)_2$: C, 21.6; H, 4.53; N, 12.6. Found: C, 21.8; H, 4.78; N, 12.9. The infrared and visible spectrum are identical with those of the previously described product.

Preparation of $[Co(dien)(GlyGlyOC_2H_5)NO_2](ClO_4)_2$ from $[Co(dien)(NO_2)_3]$ and Glycylglycine Ethyl Ester Hydrochloride. The method employed is analogous to that used to prepare the nitroglycine complex. Yield 75%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_6H_{12}N_2O_3)(NO_2)](ClO_4)_2$: C, 21.2; H, 4.44; N, 14.8. Found: C, 21.3; H, 4.61; N, 15.0. The infrared and visible spectrum are identical with those of the previously described product.

Preparation of $[Co(dien)(GlyGlyO)]ClO_4 \cdot H_2O$ from the Reaction of $[Co(dien)Cl_3]$ with Glycylglycine. $[Co(dien)Cl_3]$ (1.34 g, 5 mmol) is heated with 0.66 g (5 mmol) of glycylglycine in 5 ml of H_2O at 50°. Triethylamine (2.1 ml, 15 mmol) is added to the mixture with stirring and stirring is continued at 50° for 1 hr. To the resulting solution is added 1–2 ml of sodium perchlorate solution to precipitate a red solid which is recrystallized from water and washed with methanol, ethanol, and ether, and dried over $Mg(ClO_4)_2$ *in vacuo* overnight at 50°. Yield 55%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_4H_6N_2O_3)]ClO_4 \cdot H_2O$: C, 23.5; H, 5.16; N, 17.1. Found: C, 23.5; H, 5.43; N, 17.2.

Preparation of $[Co(dien)(GlyGlyO)]ClO_4 \cdot H_2O$ from $[Co(dien)(GlyGlyOC_2H_5)Cl]$ in Basic Solution. $Co(dien)][(GlyGlyOC_2H_5)Cl(ClO_4)_2$ (1.11 g, 2 mmol) is dissolved in 15 ml of DMF. Sodium ethoxide (2.2 mmol) in 15 ml of absolute ethanol is added slowly into the solution with stirring. The solution is stirred at 55° for 2 hr. The red crystalline precipitate is recrystallized from a sodium perchlorate solution by dissolution in 1 ml of the sodium perchlorate solution. After standing overnight, the fine red crystals that separate are washed with methanol, ethanol, and ether, and dried over $Mg(ClO_4)_2$ *in vacuo* overnight at 50°. The infrared and visible spectra are identical with those of the previously described product.

(9) P. H. Crayton and J. A. Mattern, *J. Inorg. Nucl. Chem.*, 13, 248 (1960).

$[Co(dien)(GlyGlyGlyGlyOC_2H_5)]Cl_2$. The purple-black complex $[Co(dien)(GlyGlyOC_2H_5)Cl](ClO_4)_2$ (0.56 g, 1 mmol) is dissolved in 5 ml of DMF at 50°. Sodium ethoxide (1.1 mmol) in 5 ml of absolute ethanol is added to the solution with stirring. After 15 min, 0.10 ml (1 mmol) of diethylamine is added, immediately followed by the addition of 0.20 g (1 mmol) of glycylglycine ethyl ester hydrochloride. The resulting solution is heated at 55° for 1 hr. The trace amount of precipitate in the solution is observed. The solution is allowed to stand overnight at room temperature. The fine orange-red crystals that separate are collected, washed with ethanol and ether, and air-dried. The fine crystals are dissolved in a minimum volume of DMSO and recrystallized on slow addition of ethanol. The fine crystals of purified product are filtered, washed with ethanol and ether, and dried *in vacuo* overnight over $Mg(ClO_4)_2$ at 50° Yield 40%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_{10}H_{17}N_4O_5)]Cl_2$: C, 33.23; H, 5.98; N, 19.38; Cl, 13.85. Found: C, 33.05; H, 5.97; N, 19.52; Cl, 13.87.

Preparation of $[Co(dien)(GlyGlyGlyGlyOC_2H_5)]Cl$ from $[Co(dien)Cl_3]$ and Glycylglycine Ethyl Ester Hydrochloride. Procedure 1. A mixture of 1.34 g (5 mmol) of $[Co(dien)Cl_3]$, 1 g (5 mmol) of glycylglycine ethyl ester hydrochloride, and 15 ml of DMF is heated at 50° with stirring. Diethylamine (1.5 ml, 15 mmol) is added slowly into the mixture. During the course of reaction the mixture gradually becomes a deep purple solution (~15 min). An additional 1.0 g of glycylglycine ethyl ester hydrochloride (5 mmol) is added to the solution. The resulting solution is heated at 50° for 1 hr. The fine orange-red crystals are filtered and washed successively with ethanol and ether. The crude product was recrystallized from DMSO and ethanol as described previously. Yield 60%. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_{10}H_{17}N_4O_5)]Cl_2$: C, 33.23; H, 5.98; N, 19.38; Cl, 13.85. Found: C, 33.31; H, 5.71; N, 19.67; Cl, 13.77.

Procedure 2. A mixture of 0.54 g (2 mmol) of $[Co(dien)Cl_3]$, 0.40 g (2 mmol) of glycylglycine ethyl ester hydrochloride, and 10 ml of DMSO is heated at 50° with stirring. Diethylamine (0.6 ml, 6 mmol) is added slowly into the mixture. After the mixture converts to a solution (~15 min), another 0.40 g (2 mmol of glycylglycine ethyl ester hydrochloride is added. The solution is heated at 50° for 1 hr. Ethanol, followed by ether, is added to the solution in order to precipitate an orange-red solid. The crude product is recrystallized from DMSO and ethanol as described previously. This product is identical with that obtained by the alternate procedures as shown by comparing infrared and visible spectra.

$[Co(dien)(GlyGlyOH)X]Cl_2$, X = NO_2, Cl. These complexes are prepared by an adaptation of the method of Alexander and Busch.[10] Glycylglycine ethyl ester complex (6 g), $[Co(dien)(GlyGlyOC_2H_5)X]$, where X is NO_2 or Cl, is shaken with 200 ml of 3 *N* hydrochloric acid for 24 hr. Acetone (1500 ml) is added to this mixture, and after cooling in ice for several hours, the product is removed by filtration. The product is recrystallized from the minimum amount of water and washed successively with acetone and ether and dried *in vacuo* overnight over $Mg(ClO_4)_2$ at 50°.

$[Co(dien)(NH_2CH_2CONHR)Cl](ClO_4)_2$ where R is H or CH_3. The method employed is analogous to that used to prepare $[Co(dien)(GlyO)Cl]ClO_4$ complex. *Anal.* Calcd for $[Co(C_4H_{13}N_3)(C_2H_6N_2O)Cl](ClO_4)_2$: C, 15.32; H, 4.07; N, 14.89; Cl, 22.60. Found: C, 15.45; H, 4.05; N, 14.72; Cl, 22.63. Calcd for $[Co(C_4H_{13}N_3)(C_3H_8N_2O)Cl](ClO_4)_2$: C, 17.35; H, 4.37; N, 14.46; Cl, 21.95. Found: C, 17.48; H, 4.67; N, 14.41; Cl, 21.71.

Physical Measurements. Visible and ultraviolet spectra were obtained using a Cary Model 14 recording spectrophotometer equipped with matched 1-cm quartz cells or 1-cm glass cells. Measurements were made on aqueous solutions of each complex within the concentration range between 6.000 ± 0.002 m*M* and 1.000 ± 0.002 m*M*. Infrared spectra were obtained using a Perkin-Elmer Model 337 grating infrared spectrophotometer. Nujol mulls and potassium bromide disks were used.

Elemental analyses were obtained from Galbraith Analytical Laboratories. Most of the nitrogen, hydrogen, and carbon analyses were run in this department on an F & M Model 185 carbon–hydrogen–nitrogen analyzer.

The conductances of complexes in water were obtained using an Industrial Instruments Model RC 16B conductivity bridge and a conductance cell with a constant of 2.116. Conductances were measured at 25° and 1000 cps on samples at concentrations of about 10^{-3} *M*. Conductivity water was used as solvent.

Nmr spectra were recorded on an HR A-60A spectrometer (60 Mc/sec) at about 35° (the internal temperature of the probe). Deuterium oxide solutions were prepared by dissolving as much of the finely powdered sample as necessary to obtain a good spectrum (minimum sample size 0.5 ml). The addition of a fraction of a drop of concentrated HCl was found to shift the HDO peak downfield about 0.3–0.5 ppm facilitating the integration of adjacent sample peaks. A granule of NaTMS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) was added to serve as an internal reference. The chloride salts of the complexes were sufficiently soluble to obtain good spectra.

Determination of the Acid Dissociation Constants (pK_a) of the Peptide–Co(III) Complexes and Related Compounds. The dissociation constants were measured by potentiometric titration at 25.0 ± 0.1° in a constant temperature bath. The Beckman Model G pH meter was used. The titration was carried out quickly to avoid possible amide or ester saponification. The procedure consisted of dissolving a weighed amount of complex in 500 ml of conductivity water which had been boiled for 20 min and saturated with nitrogen. A 100-ml aliquot of the solution at 25° was pipetted into a three-necked flask which was immersed in 25.0 ± 0.1° constant temperature bath. A 100-ml aliquot of specially diluted sodium perchlorate solution was added to bring the ionic strength up to 0.1. After equilibrating for 25 min the temperature of the solution was assumed to be constant and the complex was titrated with 0.1163 *N* NaOH while being stirred under nitrogen. The calculations of pK_a utilized a correction for the concentration of hydroxide ion in the alkaline region.[11]

Paper Chromatography. Paper chromatograms of the tetrapeptide complex and glycylglycinato complex were obtained using a 1-butanol–water–acetic acid (70:20:10) or 1-butanol–pyridine–water–acetic acid (40:30:20:10) eluent. These solvent mixtures have been used to separate the α and β isomers of $[Co(trien)(NO_2)_2]^+$.[12] In addition, an acetone–water–HCl solvent mixture (70:20:10) also has been used. For each complex a single spot chromatogram was obtained. The spot moved fastest in the case of the tetrapeptide complex and slowest in the glycylglycinato complex case. The single spot chromatograms may suggest that in the synthesis of the compounds only a single isomer is isolated.

Deuteration of Selected Complexes. Approximately 50 mg of a given complex was dissolved in a minimum amount of hot deuterium oxide (50–60°). Recrystallization upon cooling resulted in isolation of the product containing deuterated N–H groups as shown by its infrared spectrum.[13]

Results and Discussion

Syntheses. The preparation of the chloro and nitro dipeptide complexes, $[Co(dien)(GlyGlyOR)X]^{2+}$ (X = Cl, NO_2) by the condensation of 2 mol of glycine ester within the coordination sphere of Co(III) in DMF or DMSO is a strict application of the procedure used by Collman and Kimura[5] to prepare *cis*-β-$[Co(trien)(GlyGlyOR)]^{3+}$. The chloro and nitro dipeptide complexes have also been prepared from the corresponding glycylglycine esters in water. The related chloro- and nitroglycinato complexes, $[Co(dien)(GlyO)X]^+$, and the chloro- and nitroglycine amide complexes, $[Co(dien)(GlyNHR)X]^{2+}$ (R = H, CH_3), were prepared *via* the reaction of $[Co(dien)X_3]$ with glycine or glycine amide in aqueous solution.

Perchlorate salts of the nitro dipeptide complexes, the nitroamide complex and the nitroglycine complex are sufficiently stable to be recrystallized from water at 50°. The chloro complexes readily undergo aquation.

Treatment of the purple-black $[Co(dien)(GlyGlyOC_2H_5)Cl](ClO_4)_2$ with glycylglycine ethyl ester in a basic (EtO^-) solution of water-free DMF–EtOH results in the condensation of the two glycylglycine ester residues and the formation of the orange-red tetrapep-

(10) M. D. Alexander and D. H. Busch, *Inorg Chem.*, **5**, 1590 (1966).

(11) A. Albert and E. P. Sargeant, "Ionization of Acids and Bases," Wiley, New York, N. Y., 1962.

(12) A. M. Sargeson and G. H. Searle, *Inorg. Chem.*, **6**, 787 (1967).

(13) M. L. Morris and D. H. Busch, *J. Amer. Chem. Soc.*, **82**, 1521 (1960).

tide cobalt(III) complex, $[Co(dien)(GlyGlyGlyGlyOC_2H_5)]Cl_2$ (eq 2). The identical product is formed

$$[Co(dien)(GlyGlyOC_2H_5)Cl]^{2+} + GlyGlyOC_2H_5 \xrightarrow[NaOC_2H_5]{DMF + EtOH} [Co(dien)(GlyGlyGlyGlyOC_2H_5)]^{2+} \quad (2)$$

when $[Co(dien)Cl_3]$ is treated with glycylglycine ethyl ester in a basic, water-free DMF or DMSO solution (eq 3). The related glyclyglycinatocobalt(III) complex

$$Co(dien)Cl_3 + GlyGlyOC_2H_5 \xrightarrow[HN(C_2H_5)_2]{DMSO \text{ or } DMSO} [Co(dien)(GlyGlyGlyGlyOC_2H_5)]^{2+} + 3Cl^- \quad (3)$$

$[Co(dien)(GlyGlyO)]Cl_2 \cdot H_2O$ has been prepared by the reaction of $Co(dien)Cl_3$ with glycylglycine in basic aqueous solution.

Structures. The characterization and structural assignments for the cobalt(III) complexes are based on their modes of synthesis, chemical reactions, molar conductives, and infrared, visible, and proton magnetic resonance spectra.

The molar conductivities of the chloro and nitro dipeptide complexes, the tetrapeptide complex, and the glycylglycinato complex are presented in Table I.

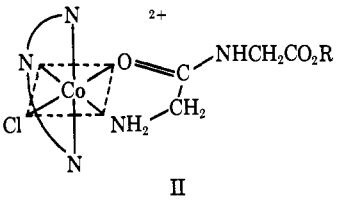
Table I. Molar Conductances for Cobalt(III) Complexes

Complex	Molar conductance[a] (ohm^{-1} at 25°)	
$[Co(dien)(GlyGlyOMe)Cl](ClO_4)_2$	230	364[b]
$[Co(dien)(GlyGlyOEt)Cl](ClO_4)_2$	226	335[b]
$[Co(dien)(GlyGlyOMe)NO_2](ClO_4)_2$	205	
$[Co(dien)(GlyGlyOEt)NO_2](ClO_4)_2$	218	
$[Co(dien)(GlyNH_2)Cl](ClO_4)_2$	246	386[b]
$[Co(dien)(GlyNHCH_3)Cl](ClO_4)_2$	228	358[b]
$[Co(dien)(GlyO)Cl](ClO_4)$	100	217[b]
$[Co(dien)(GlyNH_2)NO_2](ClO_4)_2$	230	
$[Co(dien)(GlyO)NO_2](ClO_4)$	97	
$[Co(dien)(GlyGlyGlyGlyOC_2H_5)]Cl_2$	209	
$[Co(dien)(GlyGlyO)]ClO_4$	92	
$[Co(trien)(GlyGlyOMe)](ClO_4)_3$[c]	340	
α-$[Co(trien)(NO_2)_2](ClO_4)_2$[c]	98	
cis-$[Co(en)_2(GlyOR)Cl]Cl_2$[c]	220	

[a] Measured at 10^{-3} *M* concentration in H_2O. [b] Data pertain to the solutions after the conclusion of the aquation reaction of the complex. [c] Reference 5.

The data for a number of other representative cobalt(III) complexes are listed for comparison. The conductance data support formulation of the nitro dipeptide, nitroamide, and tetrapeptide complexes as uni-divalent electrolytes and the nitroglycine and glycylglycine complexes as uni-univalent electrolytes. The rates of aquation of the chloro complexes were observed and the molar conductivities reported for these complexes were obtained by extrapolation to zero time.[14] These data show the chloro dipeptide complexes and chloroamide complexes to be uni-divalent electrolytes,[15] and the chloroglycine complex to be a uni-univalent electrolyte. Further, data of Table I confirm the presumption that the coordinated chloride is replaced by water at long times.

The only infrared spectral bands showing significant differences between the nitro and chloro dipeptide complexes occur at 1435 and 1333 cm^{-1}. These are assigned to the asymmetric and symmetric stretching modes of NO_2.[16] The intense bands at 1739 and 1240 cm^{-1} are assigned to the normal CO_2 stretching modes of the uncoordinated ester group.[17] An intense band occurring at 1631 cm^{-1} is assigned to the oxygen coordinated amide group[5] (structure II). The carbonyl

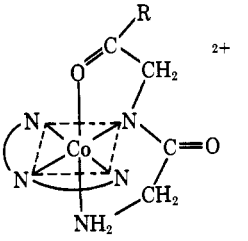


II

frequency of the free amide group in uncoordinated glycylglycine ethyl ester hydrochloride falls at 1686 cm^{-1}. The infrared spectra of the glycine amide complexes show similar features.

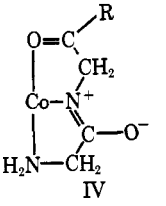
The hydrogens of the NH_2 groups are exchanged when the dipeptide compounds are recrystallized from hot D_2O and the NH stretching modes at 3100–3200 cm^{-1} are replaced by N–D stretching modes in the 2470 2270-cm^{-1} region.[18] The band in the 1590–1560-cm^{-1} region is replaced by a new band at 1510 cm^{-1}. The magnitude of this isotopic shift (NH/ND of 1.05) is due to the mixing of the C–N stretching and N–H deformation vibrations.[19,20]

The infrared spectrum of the tetrapeptide complex (structure III) shows a sharp and intense band at 1745



III, R = $NHCH_2CONHCH_2CO_2C_2H_5$

cm^{-1}, which is attributed to the stretching mode of the free carbonyl group and a band at 1678 cm^{-1} that is assigned to the uncoordinated secondary amide. The intense band at 1633 cm^{-1} is assigned to a carbonyl stretching mode of the coordinated secondary amide. This band also appears in the infrared spectra of the $[Co(dien)(GlyGlyOR)X]^{2+}$ complexes. The intense band at 1610 cm^{-1}, which also appears in the spectrum of the glycylglycinato complex, is assigned to the carbonyl stretching mode of the N-bonded coordinated



IV

(14) A. A. Frost and R. G. Pearson, "Kinetics and Mechanisms," 2nd ed, Wiley, New York, N. Y., 1965, p 36.
(15) M. M. Jones, "Elementary Coordination Chemistry," Prentice-Hall, Englewood Cliffs, N. J., 1964, p 254.
(16) K. Nakanishi, "Practical Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962.
(17) M. P. Springer and C. Curran, *Inorg. Chem.*, **2**, 270 (1963).
(18) M. E. Baldwin, *J. Chem. Soc.*, 4369 (1960).
(19) T. Miyazawa, T. Shimanouchi, and S. J. Mizushima, *J. Chem. Phys.*, **24**, 408 (1956).
(20) L. Bellamy, "The Infrared Spectra of Complex Molecules," Wiley, New York, N. Y., 1958.

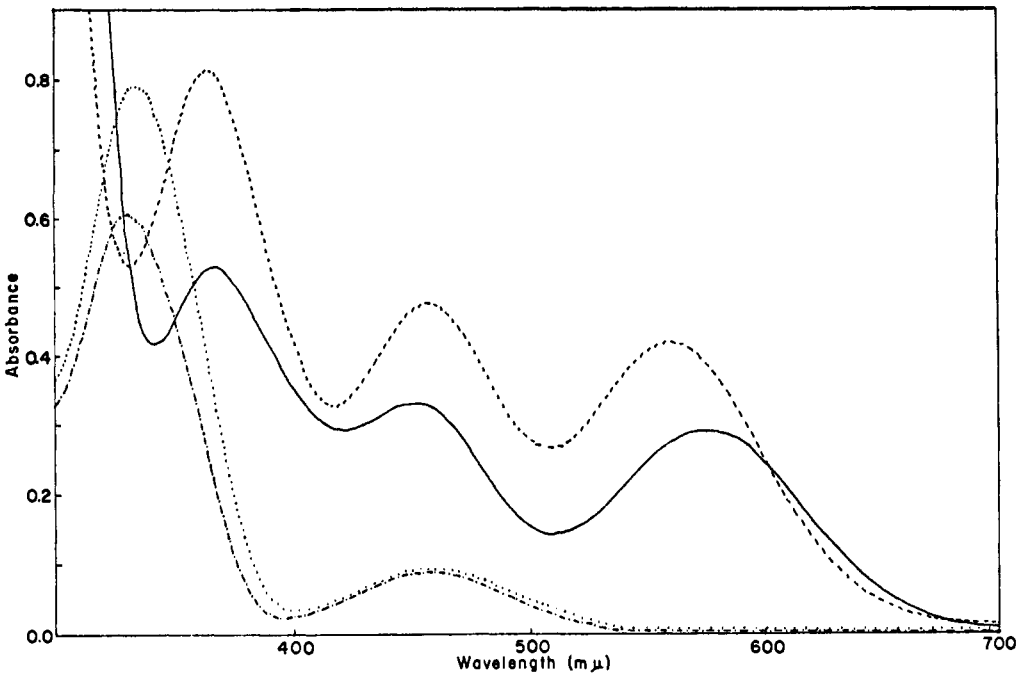


Figure 1. The visible spectra of $[Co(dien)(GlyGlyOC_2H_5)Cl]^{2+}$ (—), $[Co(dien)(GlyO)Cl]^{+}$ (---), $[Co(dien)(GlyGlyOC_2H_5)NO_2]^{2+}$ (· · ·), and $[Co(dien)(GlyO)NO_2]^{+}$ (-·-·-).

secondary amide, for the proton ionization that accompanies formation of the cobalt–nitrogen bond implies an increased contribution from the resonance form given in structure IV.[21]

signed to the transition from $^1A_{1g}$ to the ^1E_g and $^1A_{2g}$ components derived by descent in symmetry from the $^1T_{1g}$ state (O_h symmetry). The absorption band at shorter wavelengths (373 or 364 mμ) may be assigned to the

Table II. Visible Spectral Data for Dipeptide, Tetrapeptide, and Related Complexes

Complex	λ_{max}, mμ ± 2 (ϵ_{max} ± 1)		
$[Co(dien)(GlyGlyOMe)Cl](ClO_4)_2$[a]	573 (68)	454 (75)	373 (130)
$[Co(dien)(GlyGlyOEt)Cl](ClO_4)_2$[a]	573 (67)	454 (76)	373 (131)
$[Co(dien)(GlyGlyOMe)NO_2](ClO_4)_2$[b]	458 (182)		333 (1532)
$[Co(dien)(GlyGlyOEt)NO_2](ClO_4)_2$[b]	458 (182)		333 (1530)
$[Co(dien)(GlyNH_2)Cl](ClO_4)_2$[a]	573 (68)	455 (78)	373 (136)
$[Co(dien)(GlyNHCH_3)Cl](ClO_4)_2$[a]	573 (68)	455 (76)	373 (138)
$[Co(dien)(GlyO)Cl]ClO_4$[a]	562 (67)	457 (76)	364 (129)
$[Co(dien)(GlyNH_2)NO_2](ClO_4)_2$[b]	458 (180)		333 (1690)
$[Co(dien)(GlyO)NO_2](ClO_4)$[b]	457 (161)		331 (1370)
$[Co(dien)(GlyGlyOH)NO_2]Cl_2$[b]	459 (162)		333 (1390)
$[Co(dien)(GlyGlyOH)Cl]Cl_2$[a]	573 (64)	454 (76)	359 (143)
$[Co(dien)(GlyGlyGlyGlyOC_2H_5)]Cl_2$[b]	488 (311)		333 (91)
$[Co(dien)(GlyGlyO)]ClO_4 \cdot H_2O$[b]	483 (262)		337 (69)
trans-$[Co(en)_2Cl_2]$	616	455	375
cis-$[Co(en)_2Cl_2]$	596	530	383
trans-[Co(dien)(IDA)]$^+$ [c]	510 (174)	445 (sh)	361 (189)
u-cis-[Co(dien)(IDA)]$^+$ [c]	512 (105)		358 (124)
s-cis-[Co(dien)(IDA)]$^+$ [c]	548 (sh)	486 (72)	350 (85)

[a] Measured in CH_3OH. [b] Measured in H_2O. [c] Reference 23.

Visible spectral data for the dipeptide, tetrapeptide, and related complexes are given in Table III. The spectra of other representative cobalt(III) complexes are listed for comparison. The complete spectra of several of these complexes are presented in Figure 1. Three bands are observed in the visible spectra of the chloro complexes. The bands at 573 and 454 mμ of the chloro dipeptide and chloroamide complexes and the bands at 562, 457 mμ for the chloroglycine complex can be as-

unresolved transitions from the $^1A_{1g}$ ground state to the ^1E_g and $^1B_{2g}$ states coming from the $^1T_{2g}$ state (for octahedral symmetry).[22] This large splitting[23] in the $^1T_{1g}$ state strongly suggests the trans configuration for each of the chloro complexes (structure V) for only in such a structure are the two weaker field donor atoms trans to each other. The alternative isomeric forms are shown in structures VI and VII. (The designation cis or trans is used to define the positions of the coordi-

(21) (a) A. S. Brill, R. B. Martin, and R. J. P. Williams, "Electronic Aspects of Biochemistry," B. Pullman, Ed., Academic Press, New York, N. Y., 1964, p 519; (b) H. C. Freeman, *Advan. Protein Chem.*, 256 (1966).

(22) C. J. Ballhausen and W. J. Moffitt, *J. Inorg. Nucl. Chem.*, 3, 178 (1956).

(23) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, 5, 594 (1966).

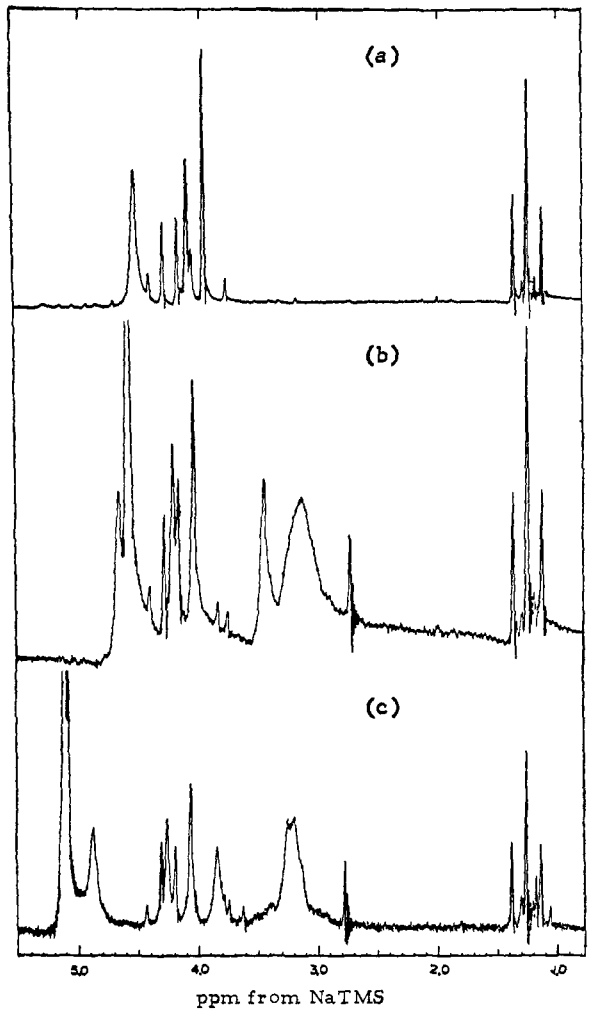
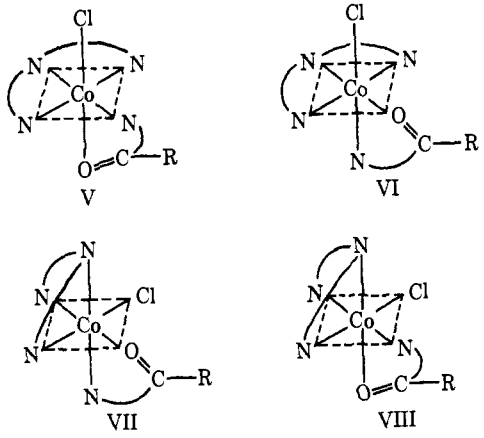


Figure 2. The pmr spectra of tetraglycine derivatives: (a) $Cl^-H_3{}^+NCH_2CONHCH_2COC_2H_5$, (b) $[Co(dien)(GlyGlyGlyGlyOC_2H_5)]^{2+}$, and (c) $[Co(dien)(GlyGlyGlyGlyOC_2H_5)]^{2+}$ (trace of HCl present).

nated oxygen and chlorine atoms within the cobalt(III) octahedron.) Molecular models show less steric repulsion for the trans configuration than the cis.

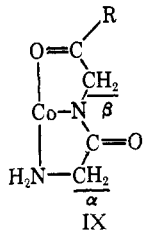


Two bands (λ_{max} 458, 333 mμ) corresponding to the two spin-allowed d–d transitions for octahedral cobalt(III) are observed in the visible spectra of the nitro complexes. These are shown in Table II and Figure 1. The trans configuration for each of the nitro complexes is indicated, for the ligand field of NO_2^- is stronger than that of an amine nitrogen while that of the carbonyl oxygen is weaker so that their average ligand field is similar to that of two amines.

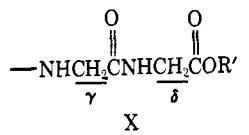
Two bands are observed in the visible spectrum for the tetrapeptide and the glycylglycinato complexes (Table II). The general nature of the visible spectra of these complexes is similar to that of the bis(glycylglycinato)cobalt(III) ion.[24]

Although diethylenetriamine could coordinate in either facial (structures VII and VIII) or meridional configuration (structures V and VI), models show that the tetrapeptide and glyclyglycinate are very strained in the facial configuration. Paper chromatographic studies suggest that the products are single isomers.

Further evidence supporting the meridional structure (structure III) for these complexes is found in their pmr spectra. The data for the tetrapeptide and glycylglycinato complexes are summarized in Table III and several of the spectra are shown in Figure 2. The two methylene groups on the coordinated glycylglycine ring are designated α and β, and those on the dangling or free glycylglycine group are designated γ and δ as illustrated in structures IX and X.



IX



X

Legg and Cooke[2,23] reported that the methylene protons of dien in *trans*-$[Co(dien)(IDA)]^+$ and *trans*-$[Co(dien)MIDA)]^+$ appear at 2.8 ppm for the cis isomers and at approximately 3.1 for the trans-meridional isomers. Further, the dien spectrum of the trans isomer is more symmetrical and simpler than that of the cis isomer. From Figure 2 it can be seen that the characteristic resonance of dien occurs at 3.1 ppm and is fairly symmetrical in the spectra of the complexes in question. The dien resonance bands for the tetrapeptide and glycylglycinato complexes also do not exhibit large splittings such as might be caused by the crowding of the methylene or ethylene protons into the cis configuration. These evidences suggest that the tetrapeptide and glycylglycinate chelate in the trans (meridional) configuration (structure III).

The pmr spectra of the tetrapeptide and glycylglycinato complexes deserve further comment. (1) The β methylene on the tetrapeptide and glycylglycinato complexes exhibit chemical shifts at lower fields (4.66 and 4.10 ppm, respectively) than the corresponding values (4.10 and 3.83 ppm) for the hydrochloride salts of the free ligands. Simultaneously, the α-methylene groups of the complexes show chemical shift toward higher fields (3.45 and 3.40 ppm, respectively). This is consistent with ionization of the amide hydrogen (structure IV). (2) The α and β methylenes of the complexes are shifted downfield when the solution is made acidic. This is associated with the protonation of the complexes (see Reactions). (3) The β-methylene hydrogens on the tetrapeptide complex (4.66 ppm) are

(24) R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Coord. Chem. Rev.*, 1, 263 (1966).

Table III. Chemical Shifts and Intergration Values for the Diethylenetriamine and Peptide Protons of the Tetrapeptide and Glycylglycinato Complexes[a,b]

Assignment	GlyGlyOH δ, ppm	No. of H's	$GlyGlyOC_2H_5 \cdot HCl$ δ, ppm	No. of H's	$[Co(dien)GlyGlyO]^+$ δ, ppm	No. of H's	$[Co(dien)GlyGlyGlyGlyOC_2H_5]^{2+}$ δ, ppm	No. of H's
$>NCH_2CN$ γ	3.89	2.0	3.96	2.1			4.03 *4.04*	
$>NCH_2COR$ δ	3.83	1.0[c]	4.10	1.9			*4.16* *4.16*	6.0
$-OC_2H_5$			4.24 1.26	2.1 3.0			4.24 *4.25* 1.25 *1.24*	3.0
Co–N–C=O –N–CH_2 α					3.40 *3.83*	2.1	3.45 *3.81*	2.1
O–C(R) Co–N–CH_2 β					4.10 *4.28*	2.0	4.66 *4.85*	*1.9*
$>NCH_2CH_2N<$ (dien)					3.12 *3.13*	7.9	3.13 *3.20*	8.0

[a] The data in italics were taken in acidic solution. [b] All frequencies on low-field side of Na TMS (Na TMS = 0). [c] A proton on methylene has been deuterated.

more deshielded than are those on the β-methylene of the glycylglycinato complex (4.10 ppm). (4) The bands associated with chelate ring members (α and β methylenes) are broader than those of the methylene on the dangling groups.

Reactions. The nonlability of coordinated NO_2^- makes $[Co(dien)(GlyGlyOR)NO_2]^{2+}$ useful in the study of the hydrolysis of the bidentate chelated dipeptide. Alkaline aqueous hydrolysis of the nitro dipeptide complexes at pH ~10 affords the glycinato complex (eq 4). This product is identical with that prepared

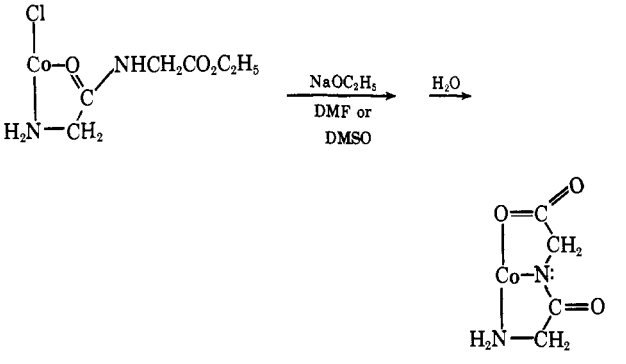
$$[Co(dien)(NH_2CH_2CONHCH_2CO_2R)NO_2]^{2+} \xrightarrow{\sim pH\ 10} [Co(dien)(NH_2CH_2CO_2)NO_2]^{+} \quad (4)$$

from the reaction of $[Co(dien)(NO_2)_3]$ with glycine. At high pH, a further substitution reaction occurs. This behavior is consistent with the conclusion that the amide group of the amino terminal peptide unit is coordinated to the metal through the carbonyl oxygen atom (structure II).

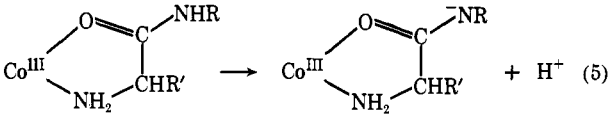
Acid hydrolysis (3 *N*) of the dipeptide complexes in aqueous solution at room temperature affords complexes of the formulation $[Co(dien)(GlyGlyOH)X]Cl_2$, where X is NO_2 or Cl. This indicates that the hydrolytically most reactive site is the dangling ester function and not the peptide linkage.

Alkaline aqueous hydrolysis of the tetrapeptide complex produces the same glycylglycinato complex as is prepared from the reaction of $Co(dien)Cl_3$ with glycylglycine. All previous examples of such hydrolyses of cobalt(III) peptide complexes have yielded glycinato complexes.

The formation of the tetrapeptide complex from the reaction of the chloro dipeptide complex with glycylglycine ester appears to require isomerization of the cobalt–amide linkage in the dipeptide complex from the O-bonded to the N-bonded form. This isomerization is also demonstrated by the reaction of the chloro dipeptide complex with NaOEt in dry DMSO or DMF, followed by recrystallization from water. The reaction did not afford the glycinato complex. Instead the reaction yields a tridentate glycylglycinato complex within which the peptide is coordinated to cobalt(III) through the nitrogen atom of the amide group instead of the oxygen atom.



Carbonyl oxygen-bonded amide complexes of cobalt(III) exhibit considerable acidity[5] (eq 5). The pK_a



values for the complexes reported here are $[Co(dien)(GlyGlyOR)NO_2](ClO_4)_2$, 9.89; $[Co(dien)(GlyONH_2)NO_2](ClO_4)_2$, 10.58; and $[Co(dien)(GlyGlyOH)(NO_2)](ClO_4)_2$, 11.1. The pK_a of the carboxylic acid function of $[Co(dien)(GlyGlyOH)NO_2]^{2+}$ was found to be 2.86. This value is high when compared to that of $[Co(en)_2(GlyOH)Cl]^{2+}$ (pK_a = 2.1)[10] and glycine hydrochloride

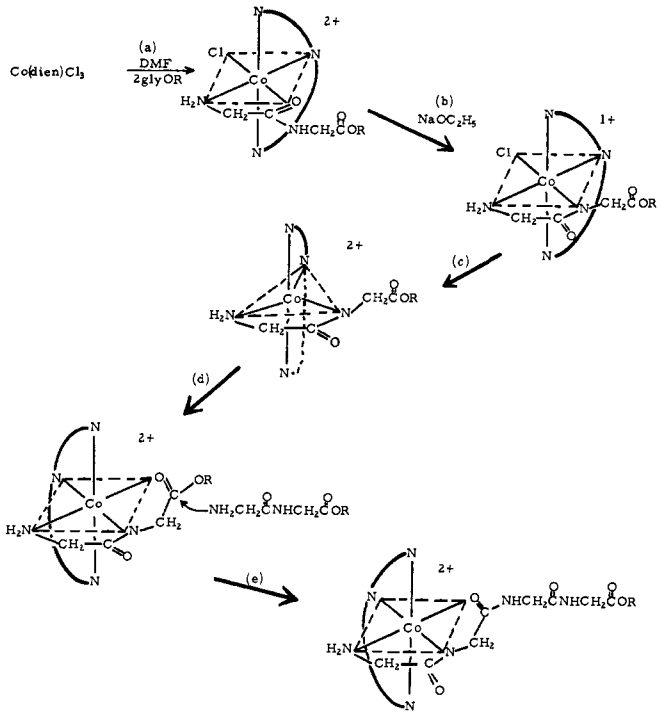
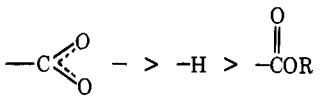


Figure 3. Reaction sequence for tetrapeptide formation from a dipeptide under the influence of $Co(dien)^{2+}$.

(pK_a = 2.34),[25] but quite low compared to the value for alkylcarboxylic acids. The acidity of the amide hydrogen is consistent with the order of electron-donating inductive effects



The complexes containing a tridentate peptide moiety contain an ionized amide nitrogen bound to the metal ion. The complexes undergo significant protonation reactions. Gillard, *et al.*, detected the protonation of the bis(glycylglycinato)cobalt(III) complex in concentrated mineral acids (0.1–6 *M*).[24] When a mineral acid is added to an aqueous solution of either the tetrapeptide or glycylglycinato complex, the spectral bands positioned at 483 mμ for glycylglycinato complex and at 488 mμ for the tetrapeptide derivatives are shifted to 475 and 478 mμ, respectively.

(25) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold, New York, N. Y., 1943.

Conclusions

In the studies summarized here, it has been found that the cleavage of the peptide linkage in dipeptide complexes does not occur in the presence of ethoxide in nonaqueous media. Instead, the system gives a product in which the peptide is coordinated around the metal as a tridentate ligand bound by the amine nitrogen, the ionized amide nitrogen, and the terminal carbonyl, as illustrated in Figure 3. If the reaction solution contains glycylglycine ethyl ester, the tetrapeptide complex is obtained (Figure 3). This is an example of the stepwise condensation of a polypeptide within the coordination sphere of a metal ion. The reaction sequence of Figure 3 is presented to explain the observations made on the reactions in DMF and EtOH.

The amide proton of the dipeptide complexes, which is made more acidic by coordination of the oxygen of the amide carbonyl group, is abstracted by the ethoxide ion leaving the negative, electron-rich anion of the amide. The dissociation of the proton from the amide nitrogen appears to be necessary in order to cause isomerization of the cobalt–amide linkage from O-bonded to the N-bonded form (step b in Figure 3). Recently, Nakahara[8] reported that the coordination of the amide group through the nitrogen is dominant at high pH, while O coordination dominates at low pH.

It has been illustrated previously that the coordinated chloride of the chloro dipeptide complexes is labile. Upon loss of the coordinated chloride, the complex can presumably form a trigonal-bipyramidal intermediate (Figure 3, step c) (as is usually assumed in substitution at Co(III)), followed in step d by the coordination of the ester carbonyl.[1,4] The carbonyl oxygen of the ester group could not reach the metal ion if the peptide group were O bonded instead of N bonded.

The final step (Figure 3, e) involves the formation of the new peptide linkage. This takes place because of activation of the ester carbonyl due to coordination of the carbonyl oxygen of the glycylglycine ester. The activated carbonyl carbon suffers nucleophilic attack by the amino group of the peptide reagent.

The several steps exemplified in Figure 3 represent the set of elemental processes that must be used to form a polypeptide in a multisite system based on metal ion activation. Clearly more than three sites might be involved within the coordination sphere of one metal ion. It is also obvious that a greater number of sites might participate in such a process if the promoter were a bridged complex containing two or more metal ions suitably arrayed.

Acknowledgment. The support of the National Science Foundation is gratefully acknowledged.