The Formation of Peptide Bonds in the Coordination Sphere of Cobalt(III)[1,2]

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Abstract: Peptide formation in the coordination sphere of cobalt(III) is observed in the reaction of *cis*-[Co(trien)Cl_2]Cl with glycine esters to form *cis*-β-[Co(trien)(glyglyOR)]$^{3+}$. Similar peptide and glycinamide complexes were prepared from *cis*-[Co(trien)Cl_2]Cl and glycylglycine esters or glycinamides. Other peptide complexes, [Co(trien)(glyglyNH$_2$)]$^{3+}$, [Co(trien)(glyglyglyOR)]$^{3+}$, and [Co(trien)(glyalaOR)]$^{3+}$, were prepared from the intermediate, *cis*-β-[Co(trien)(glyOC_2H_5)Cl]$^{2+}$. The peptide complexes are shown to be intermediates in the previously described[4] peptide hydrolysis using [Co(trien)(OH)(OH_2)]$^{2+}$.

Tetramine–cobalt(III) complexes with two reactive coordination sites react with peptides in aqueous solution in such a way that the N-terminal amino acid residue is hydrolytically cleaved from the peptide chain to form a chelate ring with the complex ion.[5] We have been studying this reaction because of its relevance to mechanisms of reactions of coordinated ligands and its potential as a selective method of peptide modification.

Complexes such as *cis*-[Co$(en)_2$(OH)(OH_2)]$^{2+}$,[6] *cis*-β-[Co(trien)(OH)(OH_2)]$^{2+}$,[4] *cis*-[Co(tren)(OH)(OH_2)]$^{2+}$,[7] and [Co(cyclen)(OH)(OH_2)]$^{2+}$ [8] have been found to be effective in this stoichiometric peptide hydrolysis.[9] We now report related reactions whereby metal ions promote the *formation* of peptide bonds through coordination of ester carbonyl groups.

This discovery emanated from our attempt to prepare *cis*-chlorotriethylenetetramine–glycine ester complexes, *cis*-[Co(trien)(glyOR)Cl]$^{2+}$. Alexander and Busch[10] had previously reported the reaction of aliphatic amines and glycine esters with *trans*-[Co$(en)_2Cl_2$]$^+$ to yield *cis*-[Co$(en)_2$(RNH_2)Cl]$^{2+}$ and *cis*-[Co$(en)_2$(glyOR)Cl]$^{2+}$. We found the tren analog, [Co(tren)Cl_2]$^+$ (I), also forms [Co(tren)(glyOR)Cl]$^{2+}$ (II) upon treatment with methyl and ethyl glycinates (Figure 1). In the trien series Pearson, Boston, and Basolo[11] had prepared *cis*-[Co(trien)(NH_3)Cl]$^{2+}$ from a similar reaction with ammonia. We have found that *n*-butylamine forms *cis*-[Co(trien)(*n*-$C_4H_9NH_2$)Cl]$^{2+}$ (IV) (Figure 1). In each of these examples a single chloride ion in the coordination sphere is replaced by an amino group.

Reaction of *cis*-[Co(trien)Cl_2]$^+$ (III) with a series of glycine esters in DMF or DMSO did not afford the expected *cis*-[Co(trien)(glyOR)Cl]$^{2+}$ but instead gave complexes *cis*-[Co(trien)(glyglyOR]$^{3+}$ (V) in which the glycylglycine ester acts as a chelate bonded to cobalt through the terminal amino group and the amide carbonyl oxygen (Figure 2). During the course of our investigation certain of these peptide complexes were independently prepared by Buckingham, *et al.*[12,13] Herein are described the synthesis and characterization of these novel complexes as well as implications of these results to the mechanism of the peptide hydrolysis reactions.

Discussion

When an aqueous suspension of the sparingly soluble violet *cis*-β-[Co(trien)$^+Cl_2$] (III) was treated with methyl or ethyl glycinate at room temperature, the violet crystals dissolved to form an orange solution. Addition of acetone resulted in the precipitation of a methanol soluble, hygroscopic orange solid which was then converted into a crystalline perchlorate salt of V. The same dipeptide complexes were obtained in higher yields using DMF or DMSO as the reaction medium. In DMSO the reaction is almost instantaneous at 50°. Perchlorate salts of these dipeptide complexes are sufficiently stable to be recrystallized from hot water. The structures which are assigned to the dipeptide ester complexes (Va–c) are based on their syntheses, chemical reactions, molar conductivities, and infrared, visible, and proton magnetic resonance spectra, and have gained support from an X-ray diffraction study.[14]

The dipeptide complexes V were prepared independently from the corresponding glycylglycine esters under similar conditions. That the glycine esters are not converted into glycylglycine esters or diketopiper-

(1) This work was supported by the National Institutes of Health under Grant GM08350. Portions of this work were presented at the 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967, Abstract L 139.

(2) Abstracted from the Ph.D. dissertation of E. Kimura, University of North Carolina, 1967.

(3) Department of Chemistry, Stanford University, Stanford, Calif.

(4) J. P. Collman and D. A. Buckingham, *J. Am. Chem. Soc.*, **85**, 3039 (1963).

(5) D. A. Buckingham, J. P. Collman, A. Happer, and L. G. Marzilli, *ibid.*, **89**, 1082 (1967).

(6) D. A. Buckingham and J. P. Collman, *Inorg. Chem.*, in press.

(7) S. L. Young, Ph.D. dissertation, University of North Carolina, 1967.

(8) J. P. Collman and P. W. Schneider, *Inorg. Chem.*, **5**, 1380 (1966).

(9) The following abbreviations are used throughout this paper: en for ethylenediamine, trien for triethylenetetramine, tren for 4-(2-aminoethyl)diethylenetriamine, cyclen for 1,4,7,10-tetraazacyclododecane, gly for glycine, ala for alanine, glyglyOR for glycylglycine esters, glyNR$_2$ for glycinamides, and glyOR for glycine esters.

(10) (a) M. D. Alexander and D. A. Busch, *J. Am. Chem. Soc.*, **88**, 1130 (1966); (b) *Inorg. Chem.*, **5**, 602 (1966).

(11) R. G. Pearson, C. R. Boston, and F. Basolo, *J. Phys. Chem.*, **59**, 305 (1955).

(12) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.*, **89**, 2772 (1967).

(13) We are indebted to D. A. Buckingham for making his results available to us prior to publication.

(14) Unpublished results of M. Fehlmann, H. Freeman, D. A. Buckingham, and A. M. Sargeson communicated to us through D. A. Buckingham. The β structure of V and related complexes is thus correct as shown in Figure 2. The structure of the glycinato complex follows from this and is the one dipicted in Figure 2. The same structure was suggested in our earlier paper.[5] The structure of the tren complex II is uncertain. One of the two possible isomers is depicted in Figure 1.

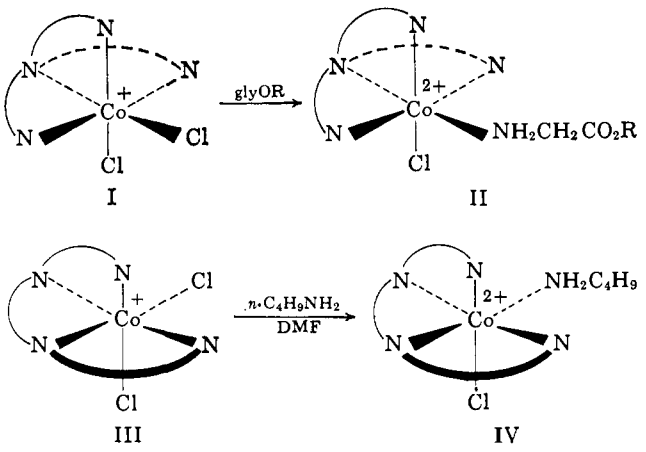


Figure 1.

azine in the absence of the metal complex but under otherwise identical reaction conditions was demonstrated by thin layer chromatographic analyses. Thus the complex metal ion is promoting the formation of a peptide bond.

Alkaline aqueous hydrolysis of the dipeptide complexes V (a, b, or c) at 50° affords the glycinato complex VI, which is identical with the complex prepared from treating *cis*-β-[Co(trien)(OH)(OH_2)]$^{2+}$ (VII) with glycine, glycylglycine, or glycylalanine, the latter being examples of the peptide cleavage reaction (Figure 2).[5]

Related glycinamide complexes VIII have been prepared by the reaction of the dichlorotrien complex III with a series of glycinamides in DMF. The characterization of these bidentate amide complexes VIII is also discussed below. Alkaline hydrolysis of the coordinated glycinamide bonds in VIII yielded the glycinato complex VI; however, hydrolysis of the coordinated N,N-dialkylamides VIII was markedly slower than hydrolysis of the glycylglycine complexes V. It is not clear whether this kinetic difference is due to electronic or steric effects.

Molar conductivities of the glycylglycine and glycinamide complexes V and VIII are presented in Table I along with data for other representative examples for comparison. The conductance data support the repre-

Table I. Conductivity Values for Dipeptide and Related Complexes

Complex	Molar conductance,[a] $ohms^{-1}$
[Co(trien)(glyglyOMe)](ClO_4)$_3$ (Va)	340
[Co(trien)(glyglyOEt)](ClO_4)$_3$ (Vb)	335
[Co(trien)(glyglyO-*i*-Pr)](ClO_4)$_3$ (Vc)	343
[Co(trien)(glyNHMe)]Cl(ClO_4)$_2$ (VIIIa)	395 (28°)
[Co(trien)(glyNH-*i*-Pr)]Cl(ClO_4)$_2$ (VIIIb)	375 (28°)
[Co(trien)(glyNMe$_2$)](ClO_4)$_3$ (VIIIc)	390 (27°)
[Co(trien)(glyNEt$_2$)](ClO_4)$_3$ (VIIId)	398 (27°)
[Co(trien)(gly)](ClO_4)$_2$ (VI)	205
β-[Co(trien)(*n*-BuNH$_2$)Cl](ClO_4)$_2$ (IV)	232
α-[Co(trien)(NO_2)$_2$](ClO_4)$_2$[b]	98
cis-[Co(en)$_2$(glyOR)Cl]Cl_2[c]	220

[a] Measured in 10^{-3} *M* concentrations in H_2O at 25° otherwise noted. [b] Prepared according to the method of A. M. Sargeson and G. H. Searle, *Inorg. Chem.*, **6**, 787 (1967). [c] Prepared according to the method of Alexander.[10b]

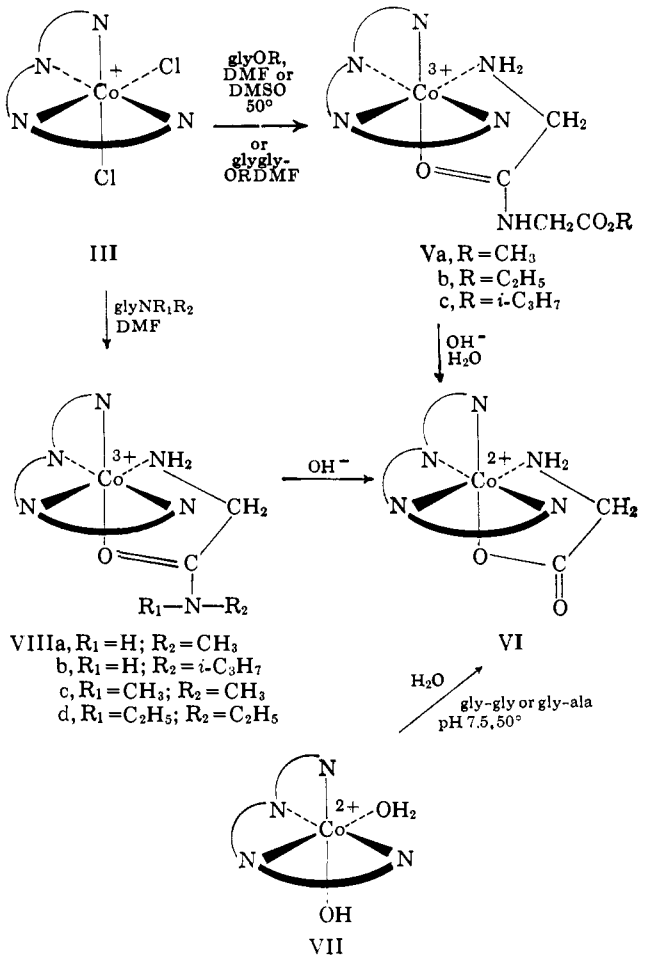


Figure 2.

sentation of these complex salts as unitrivalent electrolytes.[15] Certain of the glycinamide complexes VIII were isolated as mixed chloride perchlorate salts. Qualitative experiments demonstrated the presence of ionic chloride. Elemental analyses of the perchlorate derivatives V show the absence of ionic chloride.

Visible spectra of the dipeptide and glycinamide complexes are summarized in Table II. The spectra of other relevant cobalt(III) complexes are listed for comparison. The detailed spectra of four of these complexes are presented in Figure 3. Two bands corresponding to the two spin-allowed d–d transitions for octahedral cobalt(III) are observed in the visible spectrum of each complex.[16] The similarity between the spectra of glycylglycine, glycinamide, and glycinato complexes V, VIII, and VI is particularly evident. This suggests analogous ligand fields about the central metal due to one oxygen and five nitrogen atoms. Alexander and Busch[10] report that chelated amino acid esters, *cis*-[Co(en)$_2$(glyOR)]$^{3+}$ exhibit spectra (λ 487 mμ (ε 80) and 344 mμ (ε 92)) very similar to that of the corresponding glycinato complex, *cis*-[Co(en)$_2$(gly)]$^{2+}$ (λ 487 mμ (ε 98) and 346 mμ (ε 107)). The contrast with the spectra of complexes containing chloropentamine ligands is illustrated in Figure 3 and Table II. The near congruence (Figure 3)

(15) M. M. Jones, "Elementary Coordination Chemistry," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1964, p 254.
(16) C. J. Ballhausen, "Introduction to Ligand Field Theory," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 259.

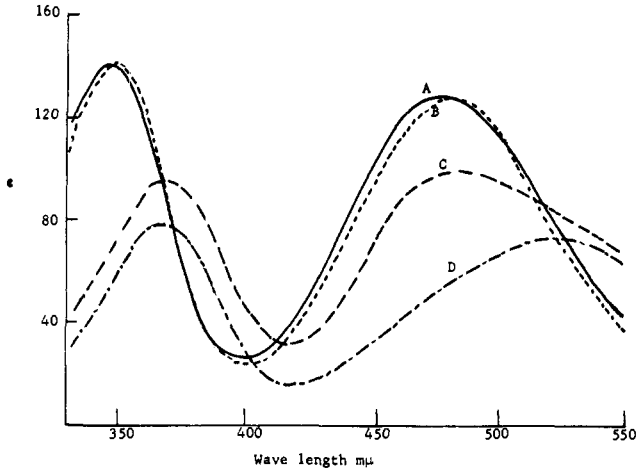


Figure 3. Visible absorption spectra of (A) [Co(trien)(glyglyOR)]$^{3+}$, (B) [Co(trien)(gly)]$^{2+}$, (C) *cis*-β-[Co(trien)(BuNH$_2$)Cl]$^{2+}$, (D) *cis*-[Co(en)$_2$(BuNH$_2$)Cl]$^{2+}$.

Table II. Visible Spectral Data for Dipeptide and Related Complexes in H_2O Solution

Complex	λ_{max}, mμ (ϵ_{max})	
[Co(trien)(glyglyOMe)](ClO$_4$)$_3$ (Va)	347 (137)	479 (128)
[Co(trien)(glyglyOEt)](ClO$_4$)$_3$ (Vb)	356 (140)	478 (129)
[Co(trien)(glyglyO-*i*-Pr)](ClO$_4$)$_3$ (Vc)	346 (137)	478 (126)
[Co(trien)(glyNHMe)]Cl(ClO$_4$)$_2$ (VIIIa)	346 (153)	478 (132)
[Co(trien)(glyNH-*i*-Pr)]Cl(ClO$_4$)$_2$ (VIIIb)	347 (164)	479 (138)
[Co(trien)(glyglyNH$_2$)]Cl(ClO$_4$)$_2$ (XI)	346 (138)	478 (127)
[Co(trien)(glyNMe$_2$)](ClO$_4$)$_3$ (VIIIc)	346 (154)	479 (132)
[Co(trien)(glyNEt$_2$)](ClO$_4$)$_3$ (VIIId)	346 (185)	480 (167)
[Co(trien)(glyglyglyOEt)]Cl$_3$ (XIII)	346 (133)	480 (125)
[Co(trien)(glyalaOEt)]Cl$_3$ (XII)	346 (166)	480 (152)
[Co(trien)(gly)](ClO$_4$)$_2$ (VI)	348 (135)	480 (127)
β-[Co(trien)(*n*-BuNH$_2$)Cl](ClO$_4$)$_2$ (IV)	368 (96)	483 (99)
β-[Co(trien)(glyOEt)Cl]Cl$_2$ (X)	372 (103)	487 (99)
α-[Co(trien)(glyOEt)Cl](ClO$_4$)$_2$ (XV)	369 (96)	510 (107)
cis-[Co(en)$_2$(glyOEt)Cl]Cl$_2$[a]	367 (82)	525 (77)

[a] Reference 10.

of the spectra of the dipeptide complexes and the glycinato complex resulted in our failure to detect such peptide complexes as intermediates in our earlier spectral study of the kinetics of the peptide hydrolysis reaction.[5] This point is elaborated below.

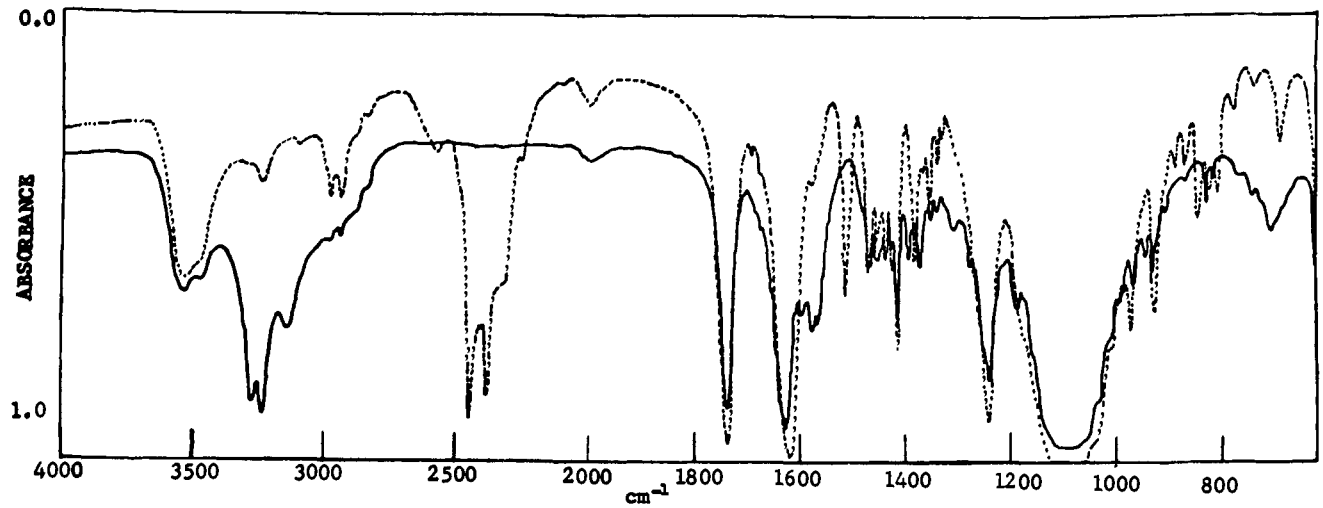


Figure 4. Infrared spectrum of [Co(trien)(glyglyOCH$_3$)](ClO$_4$)$_3$ (broken line indicates partially deuterated sample).

The infrared spectrum of the glycylglycine methyl ester complex Va is presented in Figure 4 along with the spectrum of the partially deuterated complex. The intense band at 1740 cm^{-1} and another at 1240 cm^{-1} are assigned to the normal stretching modes of the uncoordinated ester group,[17] indicating little or no interaction with the metal ion. The strong band at 1625 cm^{-1} is assigned to coordinated amide. The carbonyl frequency of the amide group in uncoordinated glycylglycine methyl ester hydrochloride is at 1675 cm^{-1}. The infrared spectra of the glycinamide complexes show similar features.

This spectral shift upon coordination is similar to that of the chromium(III) nicotinamide complex[18] whose spectrum in D_2O shows a band at a frequency (1618 cm^{-1}) lower than that of the free ligand (1665 cm^{-1}). An earlier study of the infrared spectra of both N and O bound urea complexes had demonstrated that coordination of N causes the carbonyl frequency to increase whereas O coordination has the opposite effect.[19]

Recrystallization of the dipeptide complexes V from hot D_2O resulted in the deuteration of all N–H groups as shown by nmr spectra. The infrared spectra of the deuterated complexes show N–D stretching bands ~2400 cm^{-1}. The perturbed amide I band at 1625 cm^{-1} is not affected by deuteration, but the absorption at 1575 cm^{-1} is replaced by a new band at 1510 cm^{-1} as shown in Figure 4. If the band at 1575 cm^{-1} were due to an N–H asymmetric deformation, deuteration would be expected to shift it to ~1150 cm^{-1}.[20] The small isotopic shift (NH/ND of 1.04) is consistent[21] with an amide II band which usually occurs in this region as a mixed vibration of C–N stretching and N–H deformation.[22]

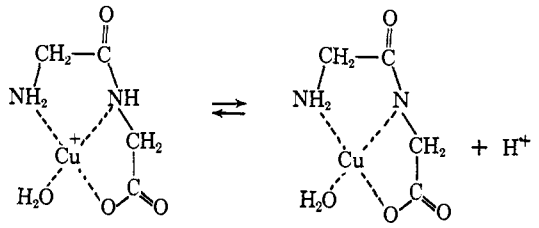
(17) M. P. Springer and C. Curran, *Inorg. Chem.*, 2, 270 (1963).
(18) F. R. Nordmeyer and H. Taube, *J. Am. Chem. Soc.*, 88, 4295 (1966).
(19) R. B. Penland, S. Mizushima, C. Curran, and J. V. Quagliano, *ibid.*, 79, 1575 (1957).
(20) A. Rosenberg, *Acta Chem. Scand.*, 11, 1390 (1957).
(21) T. Miyazawa, T. Shimanouchi, and S. Mizushima, *J. Chem. Phys.*, 24, 408 (1956).

Table III. Proton Magnetic Resonance Spectra of Peptide and Related Complexes

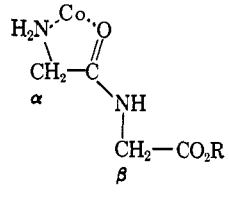
Compound	Band positions[a] α-CH_2	β-CH_2	Ester or amide alkyl group[b]
$[Co(trien)(glyglyOMe)]^{3+}$	4.31[c]	4.48[c]	3.92 S (CH_3)[c]
	4.30	4.35	3.83 S (CH_3)
$^+NH_3CH_2CONHCH_2CO_2MeCl^-$	4.07	4.10	3.98 S (CH_2)
$[Co(trien)(glyglyO\text{-}i\text{-}Pr)]^{3+}$	4.20	4.30	5.12 Qi (CH), 1.27 D (*gem*-CH_3)
$[Co(trien)(gly)]^{2+}$	3.70		
cis-$[Co(en)_2(glyOMe)Cl]^{2+}$[d]	3.47		3.75 S (CH_3)
$^+NH_3CH_2CO_2MeCl^-$	4.0		3.85 S (CH_3)
cis-$[Co(en)_2(glyO\text{-}i\text{-}Pr)Cl]^{2+}$[d]	3.46		5.13 Qi (CH), 1.28 D (*gem*-CH_3)
$^+NH_3CH_3CO_2\text{-}i\text{-}PrCl^-$	3.92		5.16 Qi (CH), 1.30 D (*gem*-CH_3)
$[Co(en)_2(gly)]^{2+}$[e]	3.65		
$[Co(trien)(glyNHMe)]^{3+}$	4.02		2.94 S (CH_3)
$^+NH_3CH_2CONHMeCl^-$	3.83		2.80 S (CH_3)
$[Co(trien)(glyNH\text{-}i\text{-}Pr)]^{3+}$	4.00		~4.1 (CH), 1.20 D (*gem*-CH_3)
$^+NH_3CH_2CONH\text{-}i\text{-}Pr\text{-}Cl^-$	3.78		~4.0 (CH), 1.15 D (*gem*-CH_3)
$[Co(trien)(glyNMe_2)]^{3+}$	4.15		3.13 S ($2CH_3$)
$^+NH_3CH_2CONMe_2Cl^-$	4.01		2.95 S (CH_3), 3.0 S (CH_3)
$[Co(trien)(glyNEt_2)]^{3+}$	4.15		3.43 Q ($2CH_2$), 1.17 T (CH_3), 1.24 T (CH_3)
$^+NH_3CH_2CONEt_2Cl^-$	4.01		1.11 T (CH_3), 1.18 T (CH_3)

[a] Sodium 3-(trimethylsilyl)-1-propanesulfonate was used as an internal standard in D_2O solutions. Values (ppm) are downfield from the standard. [b] Line splittings: S = singlet, D = doublet, T = triplet, Q = quartet, Qi = quintet. [c] Measured in acidic D_2O. [d] Prepared according to the method of Alexander.[10] [e] Prepared according to the method of Buckingham and Collman.[6]

One proton in the glycylglycine complex V is acidic (pK_a = 9.4). This is undoubtedly the amide hydrogen which is made more acidic by coordination of the amide carbonyl group. A nitrogen-coordinated amido hydrogen would be expected to be an even stronger acid. For example, the pK_a of the glycylglycine complex of copper(II) is 4.4.[23]



The nmr data for the peptide and amide complexes V and VIII are summarized in Table III. Related compounds are included for comparison. The ethylene protons of the trien ligand exhibit a broad resonance centered at 3.2 ppm downfield from TMS* (sodium 3-(trimethylsilyl)-1-propanesulfonate). The amine protons undergo deuterium exchange with D_2O unless this solvent has been previously acidified. The two methylene groups on the glycylglycine ester ligand are designated α and β as illustrated in structure IX.



IX

The α-methylene in V exhibits a triplet in acidified D_2O due to coupling with the adjacent amino group. The β-methylene shows a singlet at lower field inasmuch as the amide NH is rapidly exchanging with the solvent even in acidic medium. The α- and β-methylenes in these complexes (Va–c) each exhibit chemical shift values at lower magnetic fields (4.31 and 4.48 ppm, respectively) than the corresponding values (4.07 and 4.10) in the hydrochloride salt of the free ligands. The relative positions of the α- and β-methylenes do not change upon chelation. For the uncoordinated ligands (glycylglycine esters) the α- and β-methylene groups are distinguishable since the chemical shift of the β-methylene protons is sensitive to the pH of the solution, whereas the β-methylene position is nearly pH independent.[24]

Williams and Busch[25] illustrated the lability and acidity of the α-methylene hydrogens in $[Co(en)_2gly]^{2+}$ by demonstrating the disappearance of this peak upon making the D_2O solution alkaline. Both methylenes in V are exchanged with deuterium when the D_2O solutions are made alkaline. This could act as a mechanism to reduce the stereochemical integrity of asymmetric centers in cases of coordinated optically active amino acid residues.

The magnetic environment of the ester alkyl groups is influenced by chelation. Comparison of the integrals of these peaks with those of the trien CH_2 groups in the complex ions serves as a method of internal hydrogen analysis, the results of which are consistent with the structure proposed.

The nmr spectra of the glycinamide complexes VIII are very similar and require no further comment.

Trituration of *cis*-β-$[Co(trien)Cl_2]^+$ (III) with a limited amount of water in the presence of ethylglycinate gives a mixture of *cis*-β-$[Co(trien)(glyOC_2H_5)Cl]^{2+}$ (X) and the glycylglycine ethyl ester complex Vb (Figure 5). These are easily separated since in ethanol the chloro complex X is insoluble whereas the peptide complex V is quite soluble. The chloro complex X can be converted into a series of peptide complexes by allowing it to react with amino acid esters, glycinamide, and glycylglycine esters in DMSO (Figure 5).

(22) L. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958.
(23) M. K. Kim and A. E. Martell, *Biochemistry*, 3, 1169 (1964).
(24) A similar result has been reported for di- and tripeptides: M. Sheinblatt, *J. Am. Chem. Soc.*, 88, 2845 (1966).
(25) D. H. Williams and D. H. Busch, *ibid.*, 87, 4644 (1965).

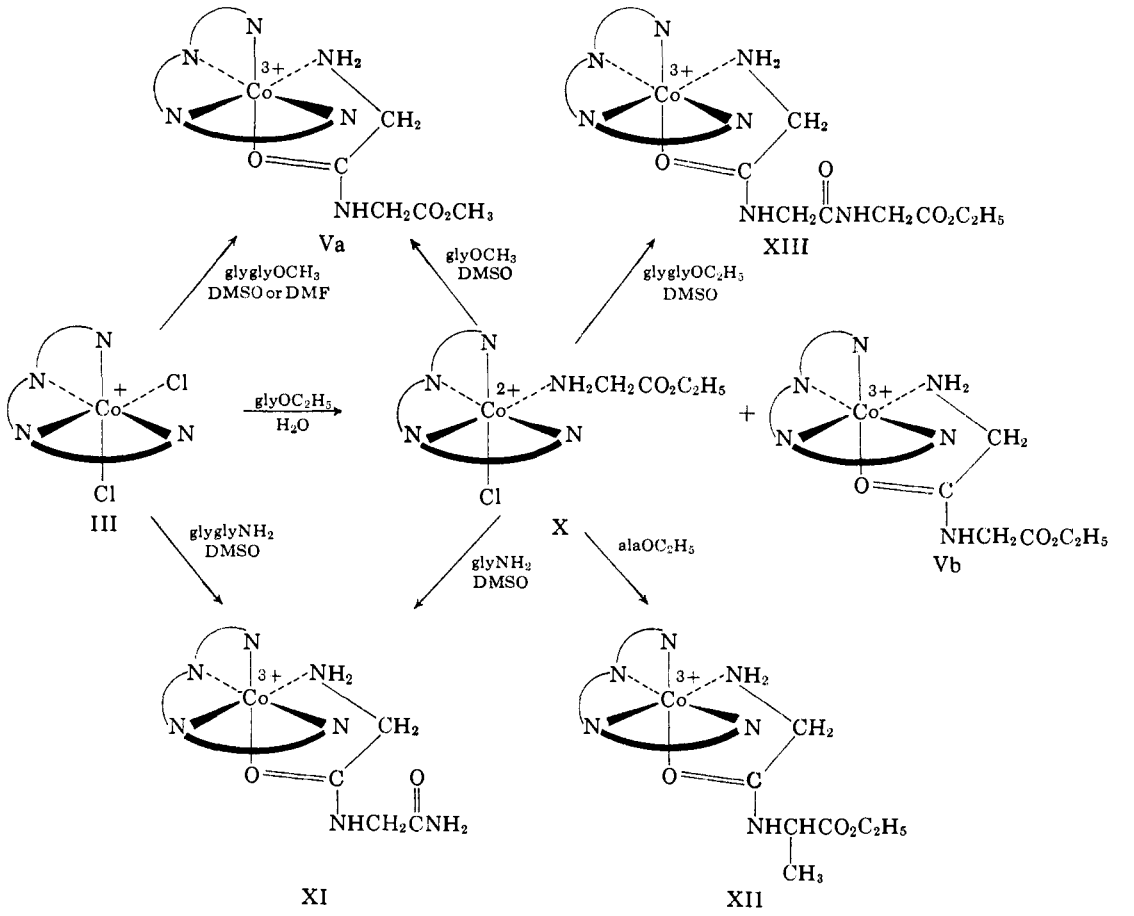


Figure 5.

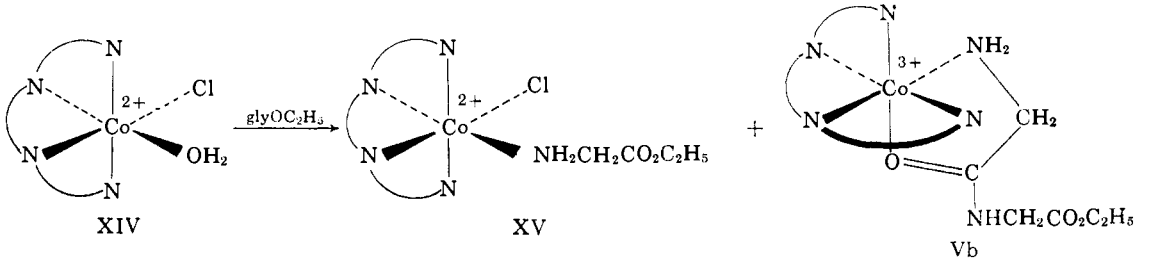


Figure 6.

In each instance peptide formation takes place through activation of the glycine ester group by coordination with cobalt followed by nucleophilic attack by the amino group on the other amino acid or peptide reagent. In these examples the distinctive role of the two amino acid units forming the coordinated peptide bond is clear. Solvolytic replacement of the remaining coordinated chloride in X probably precedes coordination of the ester carbonyl. The chloro complex X is quite stable in the solid state. Busch[10] has provided evidence for a similar coordinated ester intermediate in the metal-promoted hydrolysis of amino acid esters. More recently Buckingham[13] has isolated such a chelated ester intermediate in the bisethylenediamine series and demonstrated its role in forming coordinated peptide bonds.

The characterization of complexes XI–XIII follows along the lines developed earlier. Pertinent spectral data in Tables II and III are consistent with the structures depicted. The infrared spectrum of the tripeptide complex XIII deserves further comment. Strong carbonyl peaks at 1740, 1675, and 1625 cm^{-1} are assigned to the uncoordinated ester, the uncoordinated amide, and the coordinated amide, respectively.

The α isomer of *cis*-$[Co(trien)Cl_2]^+$ was also found to react with glycine esters in DMF or DMSO to form dipeptide complexes V of the β configuration. When partially resolved D-α-$[Co(trien)Cl_2]^+$ was used, the dipeptide complex V was found to be totally racemic. Treatment of *cis*-α-$[Co(trien)(Cl)(H_2O)]^{2+}$ (XIV) with glycine ethyl ester in a small amount of water afforded the α isomer, *cis*-$[Co(trien)(glyOC_2H_5)Cl]^{2+}$ (XV) and the β peptide complex, *cis*-$[Co(trien)glyglyOC_2H_5)]^{3+}$ (Vb) (Figure 6). The ratio of Vb to XV increased when higher concentrations of glycine ethyl ester were used. The assignment of an α configuration to the chloro ester complex XV is based on a comparison of its infrared spectrum with that of the β isomer X using the criterion of Buckingham.[26]

(26) D. A. Buckingham and D. Jones, *Inorg. Chem.*, 4, 1387 (1965).

It is now clear that peptide complexes such as V are intermediates in the stoichiometric N-terminal hydrolysis of peptides by *cis*-β-[Co(trien)(OH)(OH_2)]$^{2+}$ (VII). Our previous failure to detect these intermediates[5] must be attributed to the remarkable similarity of their visible spectra to those of the amino acid chelates such as VI. Hydrolysis of the intermediate peptide complex occurred during our preparation of analytical samples. Paper and thin layer chromatograms of the peptide complex V and the amino acid chelate VI are difficult to distinguish with the eluent systems originally employed. Having both complexes in hand, a thin layer chromatographic procedure was developed to separate V from VI.

The reaction of the hydroxoaquo complex VII with glycylglycine ethyl ester was followed by chromatography and pH. The initial pH (7.5) of the solution increased to a maximum (8.5) and then gradually decreased to the starting value. Chromatography revealed the intermediate formation of the peptide complex V, followed by formation of the glycinato chelate VI. The formation of V occurred during the pH rise, whereas the formation of VI accompanied the pH decline. The stoichiometric reactions (eq 1) predict this behavior. Very similar results were obtained by following the reaction of the hydroxoaquo complex VII with glycine-N,N-diethylamide. In this case the hydrolysis of the amide complex is much slower (eq 2).

$$\underset{\text{VII}}{cis\text{-}\beta\text{-}[Co(trien)(OH)(OH_2)]^{2+}} + \text{glyglyOR} \longrightarrow \underset{\text{V}}{cis\text{-}\beta\text{-}[Co(trien)(glyglyOR)]^{3+}} + OH^- \longrightarrow cis\text{-}\beta\text{-}\underset{\text{VI}}{[Co(trien)(gly)]^{2+}} + \text{glyOR} \quad (1)$$

$$\underset{\text{VII}}{cis\text{-}\beta\text{-}[Co(trien)(OH)(OH_2)]^{2+}} + \text{glyNR}_2 \longrightarrow \underset{\text{VIII}}{cis\text{-}\beta\text{-}[Co(trien)\text{glyNR}_2]^{3+}} + OH^- \longrightarrow cis\text{-}\beta\text{-}\underset{\text{VI}}{[Co(trien)(gly)]^{2+}} + HNR_2 \quad (2)$$

The elucidation of this mechanism is significant inasmuch as the results can be applied to developing a method of selective modification of peptide chains by removing a single N-terminal residue under mild conditions. We are now turning our attention to this analytical problem.

The formation of peptide bonds by the methods outlined above is significant only in elucidating the mechanisms of coordinated ligand reactions. Even the eloquent, more rapid method of Buckingham and Sargeson[12] will probably not be useful in peptide synthesis. Once a peptide bond is formed on the metal, it would be difficult to remove the peptide without hydrolysis. Furthermore, the labilization of α hydrogens in the chelate ring make the racemization of asymmetric centers probable.

Experimental Section

Infrared spectra (KBr pellets) were recorded on a Perkin-Elmer Model 237B spectrophotometer. Visible spectral measurements were made on a Cary Model 14 recording spectrophotometer. The nmr spectra were determined with a Varian A-60 spectrometer using 99.7% D_2O or previously acidified D_2O with P_2O_5, pH about 1.5, as the solvent and sodium 3-(trimethylsilyl)-1-propanesulfonate as an internal reference. Conductivity measurements were made upon aqueous solutions of the complexes, 0.001 *M*, at room temperature, using an Industrial Instruments conductivity bridge. Measurements of pH were made using a Leeds and Northrup pH meter. Paper chromatograms were obtained using Whatman No. 1 chromatography paper. Eluting solutions used were water–acetic acid–1-butanol in the volume ratio 5:4:10. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Materials. Amino acid and dipeptide esters were obtained from Mann Research Lab, Inc., and Cyclo Chemical Co. Whenever possible these compounds were purchased "chromatographically pure." *cis*-α- and β-[Co(trien)Cl_2]Cl, α-[Co(trien)$(NO_2)_2$]ClO_4, and β-[Co(trien)(CO_3)]ClO_4 were prepared by modification of the methods described by Searle.[27] *cis*-α-[Co(trien)(H_2O)Cl]Cl_2 was prepared according to the method of Gillard and Wilkinson.[28] D-α-[Co(trien)Cl_2]Cl was resolved by the procedure reported by Kyuno, Boucher, and Bailar.[29] Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), reagent grade, were used without further purification.

Preparation of *cis*-β-[Co(trien)(*n*-BuNH_2)Cl]$(ClO_4)_2$ (IV). A mixture of *cis*-β-[Co(trien)Cl_2]Cl (0.93 g, 3 mmoles) and *n*-butylamine (0.22 g, 3 mmoles) in 10 ml of DMF was heated to 50° for 60 min with stirring. The DMF-insoluble pink solid was collected in a glass filter, washed with ethanol, and then dissolved in 5 ml of water, followed by the addition of $NaClO_4$. Pink-violet crystals of IV separated upon allowing the solution to stand overnight. The yield was 0.50 g (30%).

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_4H_{11}N$)Cl]$(ClO_4)_2 \cdot 0.5H_2O$: C, 23.21; H, 5.79; N, 13.43. Found: C, 22.92; H, 5.66; N, 13.75.

Preparation of [Co(tren)(glyOR)Cl]$(ClO_4)_2$ (II) (R = CH_3 or C_2H_5). A mixture of [Co(tren)Cl_2]Cl[7] (1.86 g, 6 mmoles), glycine ester hydrochloride (6 mmoles), and diethylamine (0.44 g, 6 mmoles) in 3 ml of water was allowed to stand at room temperature for 1 hr. The initial violet suspension gradually formed a pink solution. Some insoluble starting material was removed by filtration, and a small excess of $NaClO_4$ was added to precipitate the perchlorate salt. The crude product was recrystallized from water and air-dried, yield ~50%.

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_3H_7NO_2$)Cl]$(ClO_4)_2 \cdot H_2O$: C, 19.77; H, 4.98; N, 12.81. Found: C, 19.91; H, 4.85; N, 12.85. Calcd for [Co($C_6H_{18}N_4$)($C_4H_9NO_2$)Cl]$(ClO_4)_2 \cdot H_2O$: C, 21.42; H, 5.21; N, 12.49. Found: C, 21.29; H, 5.20; N, 12.70.

The infrared spectra of both complexes exhibit ester bands at 1740 and 1240 cm^{-1}. The visible spectra of both complexes show λ_{max} 370 mμ (ε 102) and 514 mμ (ε 99). The molar conductance of freshly prepared aqueous solutions of the methyl and ethyl complexes are 218 and 211 ohm^{-1}, respectively.

Reaction of *cis*-α- or β-[Co(trien)Cl_2]Cl with Glycine Methyl Ester. *cis*- (either α- or β-) [Co(trien)Cl_2]Cl (0.93 g, 3 mmoles) was mixed with glycine methyl ester hydrochloride (0.33 g, 3 mmoles) and made into a paste with 1 ml of water, using a mortar and pestle. Diethylamine (0.22 g, 3 mmoles) was added to the mixture which was ground continuously. The initial violet reaction mixture quickly turned red-brown. After 3 to 5 min for the β complex, but at least 20 min in the case of the α isomer, the mixture was dissolved in 10 ml of methanol and the insoluble starting material was removed by filtration 0.35 g (38% recovered). To the methanol solution was added acetone to precipitate an orange solid, which was collected and dissolved in a small amount of water (3–5 ml), followed by addition of $NaClO_4$. The solution was allowed to stand overnight; the orange-red crystals which separated were collected, washed with water, and dried over P_2O_5 *in vacuo* at room temperature (yield 0.25 g, 13% either from α- or β-[Co(trien)Cl_2]Cl).

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_5H_{10}N_2O_3$)]$(ClO_4)_3 \cdot H_2O$: C, 19.79; H, 4.53; N, 12.59. Found: C, 19.79; H, 4.80; N, 12.67.

Reaction of *cis*-β-[Co(trien)Cl_2]Cl with Glycine Esters in DMF or DMSO. A mixture of β-[Co(trien)Cl_2]Cl (0.93 g, 3 mmoles), glycine ester hydrochloride (6 mmoles), and diethylamine (0.44 g, 6 mmoles) in 10 ml of DMF was heated to 50° with stirring. After 30 to 60 min, DMF-insoluble materials were filtered off on a glass filter and washed well with methanol. The residue on the filter was recovered β-[Co(trien)Cl_2]Cl, ~0.25–0.35 g (~27–38%). The methanol wash was combined with the DMF filtrate, to which acetone was added to precipitate the product. The hygroscopic precipitate was converted into a crystalline perchlorate following

(27) See footnote *b*, Table I.
(28) R. D. Gillard and G. Wilkinson, *J. Chem. Soc.*, 3193 (1963).
(29) E. Kyuno, L. J. Boucher, and J. C. Bailar, Jr., *J. Am. Chem. Soc.*, 87, 4458 (1965).

the above procedure. The yield of the glycylglycine methyl ester complex (Va) was 0.90 g, 45%; that for the ethyl ester complex (Vb) was 0.73 g, 37%; and that for the isopropyl ester complex (Vc) was 0.55 g, 27%. The reactions in DMSO were carried out in the same way. Using DMSO the reactions were very rapid at 50° as demonstrated by the immediate disappearance of the suspended *cis*-β-[Co(trien)Cl_2]Cl. *cis*-α-[Co(trien)Cl_2]Cl reacted in a similar manner to yield the same products but longer reaction times were required.

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_5H_{10}N_2O_3$)](ClO_4)$_3$·H_2O: C, 19.79; H, 4.53; N, 12.59. Found: C, 19.90; H, 4.50; N, 12.65. Calcd for [Co($C_6H_{18}N_4$)($C_6H_{12}N_2O_3$)](ClO_4)$_3$: C, 21.72; H, 4.55; N, 12.66. Found: C, 21.69; H, 4.55; N, 12.63. Calcd for [Co($C_6H_{18}N_4$)($C_7H_{14}N_2O_3$)](ClO_4)$_3$: C, 23.00; H, 4.75; N, 12.39. Found: C, 23.15; H, 4.84; N, 12.20.

Thin Layer Chromatography. An authentic sample mixture of glycine ester, glycylglycine ester, and diketopiperazine was separated on a silica gel thin-layer plate using as eluent a mixture of $CHCl_3$, CH_3OH, and 15% NH_4OH. The glycine esters and glycylglycine esters were developed using ninhydrin solution (Fischer's spray) and warming in an oven at 90° for a few minutes. The diketopiperazine was developed with further application of 1% aqueous $KMnO_4$ solution.

R_f values were 0.89 (glyOCH_3), 0.67 (glyglyOCH_3), and 0.56 (diketopiperazine), using a mixture of $CHCl_3$ (20), CH_3OH (20), and 15% NH_4OH (10), and 0.95 (glyOC_2H_5), 0.88 (glyglyOC_2H_5), 0.75 (diketopiperazine), using a mixture of $CHCl_3$ (20), CH_3OH (20), and 15% NH_4OH (5).

The DMF solution of a mixture of glycine ester (6 mmoles) and diethylamine (440 mg, 6 mmoles) in DMF (10 ml) with or without the presence of *cis*-β-[Co(trien)Cl_2]Cl (930 mg, 3 mmoles) was, after subjection to the same reaction condition giving rise to the dipeptide ester complexes (*i.e.*, at 50° for 60 min), spotted on a silica gel plate and eluted with the above solvents. Neither glycylglycine ester nor diketopiperazine was detected.

Determination of the pK_a of [Co(trien)(glyglyOR)](ClO_4)$_3$ (V). The dissociation constants were measured by potentiometric titration at room temperature (27°). The titration was carried out quickly to avoid possible amide or ester saponification. The general procedure was as follows: the glycylglycine ester complex (V) (*ca.* 5×10^{-4} mole) to be titrated was accurately weighed out and dissolved in 100 ml of ion-free water. Sufficient sodium perchlorate was added to bring the ionic strength up to 0.1. The complex was titrated with 0.1 *N* NaOH under nitrogen, pH readings being taken at 0.2-ml intervals. Since the titration was run mostly in the alkaline region (pH 8 to 11), the calculation of pK_a requires correction for the concentration of hydroxide ion.[30] The pK_a values calculated using the following equation

$$pK_a = pH + \log \frac{[AH]}{[A^-]}$$

where [AH] = [complex] − [NaOH] + [OH^-] and [A^-] = [NaOH] − [OH^-], were 9.45 (±0.09) for the methyl ester complex Va, 9.28 (±0.08) for the ethyl ester complex Vb, and 9.38 (±0.08) for the isopropyl ester complex Vc.

Reaction of β-[Co(trien)Cl_2]Cl with Glycylglycine Methyl and Ethyl Esters. A mixture of β-[Co(trien)Cl_2]Cl (0.93 g, 3 mmoles), glycylglycine methyl (or ethyl) ester hydrochloride (3 mmoles), and diethylamine (0.22 g, 3 mmoles) in 10 ml of DMF was heated to 50–55° with stirring. After 1 hr, the DMF-insoluble material was removed by filtration. The recovered β-[Co(trien)Cl_2]Cl weighed 0.72 g (or 0.61 g). After the work-up described for the reaction of β-[Co(trien)Cl_2]Cl with glycine esters, 0.17 g (12%) of the glycylglycine methyl ester complex Va or 0.27 g (14%) of the ethyl ester complex Vb was obtained. These complexes were identified by comparing their infrared and nmr spectra and paper chromatograms with those samples described earlier.

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_5H_{10}N_2O_3$)](ClO_4)$_3$·H_2O: C, 19.79; H, 4.53; N, 12.59. Found: C, 20.07; H, 4.70; N, 12.92.

Saponification of [Co(trien)(glyglyOMe)](ClO_4)$_3$ (Va). An orange-red solution of 0.60 g (0.9 mmole) of (Va) in 15 ml of warm water was mixed slowly with 1 ml of 1 *N* NaOH solution. Continuous stirring at about 50° for 30 min lowered the pH of the solution from 9.8 to 8.6. The resulting orange-red solution was concentrated to 3 ml on a steam bath and cooled in an ice bath to crystallize the product. The fine orange crystals were washed with ice water and ethanol and dried over P_2O_5 at 100° *in vacuo*. The yield of the glycinato complex VI was 0.30 g (67%).

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_2H_4NO_2$)](ClO_4)$_2$·H_2O: C, 19.36; H, 4.88; N, 14.12. Found: C, 19.67; H, 4.81; N, 14.12.

The infrared and visible spectra, as well as a paper chromatogram of this product, were identical with those of β-[Co(trien)(gly)](ClO_4)$_2$ prepared from β-[Co(trien)(H_2O)$_2$](ClO_4)$_3$ and glycine.

Reaction of β-[Co(trien)(H_2O)$_2$](ClO_4)$_3$ with Glycylglycine and Glycylalanine. To a solution of β-[Co(trien)(CO_3)](ClO_4) (1.10 g, 3 mmoles) in 2 ml of water was added 60% $HClO_4$ dropwise until effervescence ceased. The red solution was allowed to stand at room temperature for 15 min to ensure complete reaction. A freshly prepared dilute LiOH solution was added until pH 7.5, and then 3 mmoles of glycylglycine or glycylalanine was added. The mixture was stirred at 50° for 1 hr, keeping the pH at 7.4–7.6 by adding dilute LiOH solution from time to time. The resulting orange solution was concentrated on a steam bath to *ca.* 4 ml, which was then allowed to stand at room temperature for 2 days to crystallize the glycinato complex VI. The yield was 0.7 g (50%).

Anal. Calcd for [Co($C_6H_{18}N_4$)($C_2H_4NO_2$)](ClO_4)$_2$·H_2O: C, 19.36; H, 4.88; N, 14.12. Found: C, 19.60; H, 4.78; N, 14.17.

The infrared, visible, and nmr spectrum of both products were identical with those of the preceding product.

Preparation of Glycine N-Substituted Amides. Glycine-N-methylamide Hydrochloride. To a solution of N-carbobenzoxyglycine (6.27 g, 0.03 mole) and triethylamine (3.03 g, 0.03 mole) in 50 ml of chloroform in a Dry Ice–acetone bath was added ethyl chloroformate (3.30 g, 0.03 mole) dropwise with magnetic stirring. In 10 min the mixture solidified, 40% aqueous methylamine (2.5 g, 0.03 mole) was added, and the resulting mixture was stirred for 2 hr. At the end of this time the temperature was raised to 25°. The volatile liquid was evaporated to dryness under reduced pressure. A solution of residue in 100 ml of chloroform was washed successively with 5% $NaHCO_3$, 1 *N* HCl, and water and then dried over anhydrous $MgSO_4$. The crude product after the solvent removed was recrystallized from ethyl acetate–petroleum ether to give 2.5 g of pure N-carbobenzoxyglycine-N-methylamide (mp 108–109°).

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.47; H, 6.37; N, 13.05.

Decarbobenzoxylation was carried out by hydrogenolysis in methanol containing 1 *N* HCl using Pd–C. The product which showed no carbobenzoxy absorption in its infrared spectrum was used without further purification for the reaction with β-[Co(trien)-Cl_2]Cl.

Glycine-N-isopropylamide Hydrochloride. A solution of N-carbobenzoxyglycine (6.27 g, 0.03 mole), 1-ethyl-3-(3-dimethylaminisopropyl)carbodiamide hydrochloride (5.40 g, 0.03 mole), and isopropylamine (1.77 g, 0.03 mole) in 50 ml of ethyl acetate was stirred at −40° and later at room temperature for 4 hr. The resulting solution was washed with 5% $NaHCO_3$, 2 *N* HCl, and water, and then dried over anhydrous $MgSO_4$. The residue after evaporation of the solvent was recrystallized from ethyl acetate–petroleum ether to give 4.0 g of N-carbobenzoxyglycine-N-isopropylamide (mp 98°).

Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.46; H, 7.24; N, 11.41.

The carbobenzoxy group was removed by the method described above.

Glycine-N,N-dimethyl- and -diethylamide Hydrochloride. The method used to prepare glycine-N-isopropylamide hydrochloride was used substituting dimethyl- and diethylamine for isopropylamine. The final products were hygroscopic crystals, which form picrates.

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 36.26; H, 3.96; N, 21.11. Found: C, 36.39; H, 4.16; N, 20.85. Calcd for $C_{12}H_{17}N_6O_8$: C, 40.11; H, 4.77. Found: C, 39.99; H, 5.23.

Reaction of β-[Co(trien)Cl_2]Cl with Glycine-N-methyl-, -N-isopropyl-, N,N-dimethyl-, and N,N-diethylamides. A mixture of β-[Co(trien)Cl_2]Cl (990 mg, 3 mmoles), glycine N-substituted amide hydrochloride (3 mmoles), and diethylamine (220 mg, 3 mmoles) in 10 ml of DMF was stirred at 50° for ~1–5 hr. To the resulting orange-red solution, after removal of unreacted β-(Co(trien)Cl_2]Cl (~200–300 mg), was added acetone to precipitate a chloride salt of the product, which was then converted into a crystalline perchlorate in the usual manner. The perchlorate salts of the glycinamide complexes are more soluble in water than the dipeptide ester complexes, yield ~500–700 mg.

(30) A. Albert and E. P. Sergeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962, pp 30–31.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_3H_8N_2O)]Cl(ClO_4)_2 \cdot H_2O$: C, 19.81; H, 5.17; N, 15.40. Found: C, 19.64; H, 5.12; N, 15.24. Calcd for $[Co(C_6H_{18}N_4)(C_5H_{12}N_2O)]Cl(ClO_4)_2 \cdot H_2O$: C, 23.03; H, 5.62; N, 14.65. Found: C, 23.24; H, 5.62; N, 14.30. Calcd for $[Co(C_6H_{18}N_4)(C_4H_{10}N_2O)](ClO_4)_3 \cdot H_2O$: C, 19.26; H, 4.85; N, 13.63. Found: C, 19.38; H, 4.67; N, 13.55. Calcd for $[Co(C_6H_{18}N_4)(C_6H_{14}N_2O)](ClO_4)_3$: C, 22.75; H, 5.09; N, 13.26. Found: C, 23.11; H, 5.20; N, 13.47.

Reaction of β-[Co(trien)Cl$_2$]Cl with Glycine Ethyl Ester. β-[Co(trien)Cl$_2$]Cl (3.13 g, 0.01 mole) was mixed with glycine ethyl ester hydrochloride (2.80 g, 0.02 mole) and made into a paste with 2 ml of water, using a mortar and pestle. While grinding the mixture, diethylamine (1.4 g, 0.02 mole) was added dropwise over a 10-min period. After a few minutes the mixture coagulated and then was allowed to stand at room temperature for 1 hr. The brick-red crude product was washed with ethanol and acetone and then air-dried. The crude product (2.6 g) was taken up in a minimum of hot water and, after the addition of a few drops of concentrated hydrochloric acid, crystallized upon cooling, yield 1.5 g (35%). The molar conductance in freshly prepared aqueous solution is 219 ohm^{-1}. The infrared spectrum of X has ester bands at 1745 and 1250 cm^{-1}.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_4H_9NO_2)Cl]Cl_2 \cdot 0.5H_2O$: C, 28.35; H, 6.66; N, 16.13. Found: C, 28.38; H, 6.49; N, 15.96.

To the above ethanol wash was added acetone to precipitate an orange-red product, which was then converted into a crystalline perchlorate salt in the usual manner, yield 0.3 g (3%). The infrared spectrum and the thin layer chromatograms are identical with those of $[Co(trien)(glyglyOEt)](ClO_4)_3$ (Vb). The product ratio, Vb to X, increased as the amount of water was increased.

Reaction of β-[Co(trien)(glyOEt)Cl]Cl$_2$ (X) with Glycine Methyl and Ethyl Esters. A mixture of β-[Co(trien)(glyOEt)Cl]Cl$_2$ (830 mg, 2 mmoles), glycine ester hydrochloride (4 mmoles), and diethylamine (290 mg, 4 mmoles) in 10 ml of DMSO was stirred at 45° for *ca.* 8 hr. To the resulting orange-red solution, after removal of the insoluble β-[Co(trien)(glyOEt)Cl]Cl$_2$ (*ca.* 100 mg), was added acetone to precipitate the product, which was then converted into a perchlorate salt (V), yield ~700 mg (55%). The same reactions using DMSO-soluble β-[Co(trien)(glyOEt)Cl]Cl(ClO$_4$) instead of the DMSO-insoluble dichloride salt went to completion quite rapidly (~5 min) at 50°.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_5H_{10}N_2O_3)](ClO_4)_3 \cdot H_2O$: C, 19.79; H, 4.53; N, 12.59. Found: C, 19.99; H, 4.67; N, 12.78. Calcd for $[Co(C_6H_{18}N_4)(C_6H_{12}N_2O_3)](ClO_4)_3$: C, 21.72; H, 4.55; N, 12.66. Found: C, 21.58; H, 4.64; N, 12.69.

These complexes are identical with the ones independently prepared from β-[Co(trien)Cl$_2$]Cl and glycylglycine esters.

Reaction of β-[Co(trien)(glyOEt)Cl]Cl$_2$ (X) with Glycinamide. The procedure was identical with the preceding one except using glycinamide hydrochloride instead of glycine ester hydrochlorides. The product was recrystallized from water containing NaClO$_4$ and dried *in vacuo* overnight over P_2O_5 at room temperature, yield 400 mg (32%).

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_4H_9N_3O_2)]Cl(ClO_4)_2$: C, 21.05; H, 4.77; N, 17.18. Found: C, 21.34; H, 4.86; N, 17.18.

Qualitative tests showed the presence of ionic chloride. The infrared spectrum (KBr) exhibits coordinated and uncoordinated amide I bands at 1625 and 1680 cm^{-1}, respectively.

Direct Preparation of [Co(trien)(glygly NH$_2$)]$^{3+}$ (XI) from β-[Co(trien)Cl$_2$]$^+$ and Glycylglycinamide. The method employed is analogous to the one used to prepare glycinamide complexes.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_4H_9N_3O_2)]Cl(ClO_4)_2$: C, 21.05; H, 4.77; N, 17.18. Found: C, 21.65; H, 4.92; N, 17.20.

The infrared spectrum and thin layer chromatogram are identical with those of the preceding product.

Reaction of β-[Co(trien)(glyOEt)Cl]Cl$_2$ (X) with Glycylglycine Ethyl Ester. The procedure described earlier was repeated using glycylglycine ethyl ester hydrochloride in place of glycinamide hydrochloride. The product did not form a crystalline perchlorate salt but was purified as a chloride salt by repeated reprecipitation from a warm methanol–ethanol mixture containing one drop of concentrated HCl followed by addition of acetone. The hygroscopic chloride salt (XIII) was washed with a little acetone and dried *in vacuo* for 2 days over P_2O_5 at room temperature, yield 800 mg (75%).

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_8H_{15}N_3O_4)]Cl_3$: C, 31.82; H, 6.29; N, 18.54; Cl, 20.11. Found: C, 31.61; H, 6.46; N, 18.32; Cl, 20.29.

The infrared spectrum (KBr) shows $\nu_{C=O}$ (ester) at 1740 cm^{-1}, $\nu_{C=O}$ (coordinated amide) at 1625 cm^{-1}, and $\nu_{C=O}$ (uncoordinated amide) at 1675 cm^{-1}.

Reaction of β-[Co(trien)(glyOEt)Cl]Cl$_2$ (X) with DL-Alanine Ethyl Ester. The above procedure was used replacing the glycylglycine ester hydrochloride with DL-alanine ethyl ester hydrochloride. The product was isolated as a hygroscopic chloride salt (XII) after reprecipitation from ethanol–acetone, yield 800 mg (77%). The analytical sample was dried *in vacuo* over P_2O_5 overnight at room temperature.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_6H_{12}N_2O_3)]Cl_3 \cdot 2.5H_2O$: C, 27.89; H, 6.83; N, 16.27. Found: C, 27.63; H, 6.97; N, 16.88.

The infrared spectrum has ester bands at 1740 and 1225 cm^{-1} and a coordinated amide band ($\nu_{C=O}$) at 1625 cm^{-1}.

Reaction of *cis*-[Co(trien)(H$_2$O)Cl]Cl$_2$ with Glycine Ethyl Ester. A mixture of *cis*-[Co(trien)(H$_2$O)Cl]Cl$_2$ (1.98 g, 6 mmoles) and glycine ethyl ester hydrochloride (0.42 g, 3 mmoles) was made into a paste with 1 ml of water, using a mortar and pestle. The mixture was ground continuously while 0.22 g (3 mmoles) of diethylamine was added. The reaction mixture quickly turned pink-red. After 3 min, the mixture was dissolved in 10 ml of methanol and the insoluble starting material was removed by filtration (1.0 g). To the methanol solution was added acetone to precipitate a pink solid, which was converted into a perchlorate by dissolution in water and precipitation with NaClO$_4$. The yield of pink needles, *cis*-α-[Co(trien)(glyOEt)Cl](ClO$_4$)$_2$ (XV) was 0.2 g, 6%. The infrared spectrum of XV has ester bands at 1740 and 1250 cm^{-1} and no amide bands. The molar conductance (in freshly prepared aqueous solution) is 227 ohm^{-1}.

Anal. Calcd for $[Co(C_6H_{18}N_4)(C_4H_9NO_2)Cl](ClO_4)_2 \cdot 0.5H_2O$: C, 21.77; H. 5.12; N, 12.69. Found: C, 21.82; H, 5.19; N, 12.69.

When the same experiment was repeated using 1.68 g (12 mmoles) instead of 0.42 g (3 mmoles) of glycine ethyl ester hydrochloride, two products, the dipeptide ester complex Vb (0.6 g) as an initial precipitate and the chloroester complex XV (0.26 g) as the second precipitate, were isolated.

Amide Hydrolysis Reactions with β-[Co(trien)(OH)(OH$_2$)]$^{2+}$. Stock solutions of β-[Co(trien)(OH)(OH$_2$)]$^{2+}$ were prepared before each experiment by dissolving a weighed amount of β-[Co(trien)(CO$_3$)]ClO$_4 \cdot 2H_2O$ in 2 equiv of 1 *N* HClO$_4$ and, after 60 min, adjusting the pH to 7.5 using a 10% LiOH solution and heating in a water bath at 50°. An equivalent amount of glycylglycine ethyl ester (or glycine-N,N-diethylamide) hydrochloride was added and the pH readjusted to 7.5. Aliquots were withdrawn at convenient times and their pH's determined, and they were spotted on an Eastman chromatogram sheet (Type K301R2). The chromatograms were run using an upper layer of the mixed solvent, *n*-BuOH (5):H$_2$O (5):concentrated HClO$_4$ (1) as eluent. The dipeptide ester complex Vb and the amide complex VIIId have an approximate R_f of 0.75, whereas the glycinato complex VI exhibits an R_f of 0.35.

As the reactions started, the pH of the solutions increased to maxima (8.5 in *ca.* 10 min for the dipeptide ester reaction; 9.5 in *ca.* 20 min for the gylcinamide reaction) and then gradually decreased. During the time pH was increasing, only the dipeptide ester complex Vb and the glycinamide complex, VIIId were detected on chromatograms. The glycinato complex VI started to appear after the pH's of the solutions reached their maxima.

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