CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, NORTH CAROLINA,
AND THE FACULTY OF PHARMACEUTICAL SCIENCES, UNIVERSITY OF TOKYO, TOKYO, JAPAN

Cleavage of Amino Acid Esters and Peptides with Hydroxoaquo(2,2′,2″-triaminotriethylamine)cobalt(III) Ion[1]

BY EIICHI KIMURA, STEFAN YOUNG,[2a] AND JAMES P. COLLMAN[2b]

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Hydroxoaquo(2,2′,2″-triaminotriethylamine)cobalt(III) ion at pH 7.5 and 60° has been shown to be effective in promoting the hydrolysis of amino acid esters, dipeptides, and tripeptides. The reaction is stoichiometric and specific for N-terminal amino acids as was indicated by the isolation of the triaminotriethylaminecobalt(III)–amino acid chelate formed and following the appearance of the cleavage products using thin layer chromatography. The amino acid complexes, Co(tren)-AA^{2+}, were prepared independently from the free amino acids. The hydrolysis reaction of peptides with Co(tren)(OH)-$(H_2O)^{2+}$ is slower than with β-Co(trien)$(OH)(H_2O)^{2+}$.

Introduction

We have been studying the scope and mechanism of the metal ion promoted hydrolysis of amino acid esters, amides, and peptides. The model compounds which we chose for this purpose are $CoN_4(OH)(H_2O)^{2+}$ where N_4 represents the nitrogen donor atoms in a tetradentate ligand. Tetradentate ligands were used in order to leave two *cis* sites available for interaction with the peptide or amino acid ester and to avoid complications from exchange reactions involving the amine ligands. In aqueous solution cationic chelates of this type were shown to react with peptides in such a way that the N-terminal amino acid residue is hydrolytically cleaved from the peptide to form a chelate ring. In the reaction with amino acid esters, coordination with the metal activates the ester carbonyl to promote not only its hydrolysis but also the formation of peptide bonds.[3-5]

Complexes formed from two bidentate amines were found to be less selective. While *cis*-$Co(en)_2(OH)(H_2O)^{2+}$ hydrolyzes glycine esters to yield a single product, $Co(en)_2gly^{2+}$, the reaction with glycylglycine is rather complex, giving a mixture of $Co(en)_2gly^{2+}$, $Co(en)gly_2^{+}$, and $Co(en)_3^{3+}$. Presumably, these products arise from *cis–trans* equilibrium of the aquohydroxo complex.[6] By contrast, the trien analog reacts smoothly with amino acid esters and amides under mild conditions forming β-Co(trien)$(AA)^{2+}$. These peptide hydrolysis reactions involve the removal of N-terminal amino acid residues, and in favorable cases the amino acids of small peptide molecules can be cleaved in a sequential manner.[7,8]

As an extension of this work, we examined another tetradentate cobalt(III) complex, Co(tren)(OH)-$(H_2O)^{2+}$, which was expected to be suitable for peptide hydrolysis since the tren ligand leaves two *cis* octahedral positions unsubstituted. The present project had two goals: to explore the possible advantages Co(tren)$(OH)(H_2O)^{2+}$ may have over other tetramine ligands for sequential peptide analysis and to understand the mechanism by which the tren influences the reactivity of the other two coordination sites.

Results

Amino Acids.—Reaction of the Co(tren)(OH)-$(H_2O)^{2+}$ ion with amino acids at pH 7.5 and 60° afforded the corresponding amino acid complexes. As the reaction proceeds, the color of the solution changes from purple to orange. The products were isolated and characterized as their perchlorates. Yields of pure isolated amino acid complexes ranged from 30 to 60%. Introduction of a heteroatom such as sulfur, oxygen, or nitrogen into amino acids complicates this reaction. Cysteine which contains a free mercaptide group in the side chain gave an intractable product. When the sulfur was protected by methyl as in $S–CH_3$ cysteine, chelation occurred smoothly. Serine and threonine (alcoholic side chains) react in a normal manner except that the complex derived from the former must be precipitated by adding alcohol. Lysine smoothly formed a chelate, and the product was recrystallized from an acidic solution in order to protonate the ε-amino group. Aspartic acid and arginine appeared to react, but the product complexes did not give the expected elemental analysis. Repeated recrystallization from acidic or basic solutions failed to afford crystalline materials, although the spectral properties of these products indicated that complexation had taken place, as in

$$Co(tren)(OH)(H_2O)^{2+} + AAH \longrightarrow Co(tren)AA^{2+} + 2H_2O$$

Reactions of Co(tren)$(OH)(H_2O)^{2+}$ ions with D-, L- and DL-alanine afforded Co(tren)(D-ala)$^{2+}$ ($[\alpha]^{16}D$

(1) The following abbreviations are used throughout the paper: tren, 2,2′,2″-triaminotriethylamine; en, ethylenediamine; trien, triethylenetetramine; AA, amino acid anion. The common symbols for the amino acids are used.

(2) (a) Taken in part from the Ph.D. dissertation of S. Young, University of North Carolina, 1967 (deceased). (b) Department of Chemistry, Stanford University, Stanford, Calif. 94305. Author to whom inquiries should be addressed.

(3) J. P. Collman and E. Kimura, *J. Am. Chem. Soc.*, **89**, 6096 (1967).

(4) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *ibid.*, **89**, 4539 (1967).

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

(6) D. A. Buckingham and J. P. Collman, *Inorg. Chem.*, **6**, 1803 (1967).

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

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

TABLE I

(AMINO ACIDO)(2,2′,2″-TRIAMINOTRIETHYLAMINE)COBALT(III) PERCHLORATES

	Anal., %								Visible spectral data (in H_2O)
	C		H		N		S		
	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	λ_{max}, nm (ϵ_{max})
$[Co(tren)(gly)](ClO_4)_2$	20.09	20.16	4.64	4.64	14.65	14.80			471 (105), 342 (100)
$[Co(tren)(L\text{-ala})](ClO_4)_2$	21.96	21.89	4.92	4.92	14.23	14.27			471 (105), 342 (91)
$[Co(tren)(D\text{-ala})](ClO_4)_2$	21.96	22.18	4.92	4.96	14.23	14.13			480 (111), 346 (110)
$[Co(tren)(leu)](ClO_4)_2$	26.98	26.94	5.66	5.70	13.11	13.10			471 (126), 343 (118)
$[Co(tren)(val)](ClO_4)_2$	25.39	25.55	5.42	5.46	13.46	13.76			472 (124), 343 (110)
$[Co(tren)(phen)](ClO_4)_2$	31.70	31.69	4.97	4.99	12.32	12.32			471 (122), 343 (115)
$[Co(tren)(pro)](ClO_4)_2$	25.49	25.54	5.06	5.17	13.52	13.32			476, 343
$[Co(tren)(try)](ClO_4)_2$	33.61	33.77	4.81	4.81	13.87	13.63			470 (118), 350
$[Co(tren)(his)](ClO_4)_2$	25.82	25.83	4.69	4.74	17.56	17.69			471 (119), 338 (126)
$[Co(tren)(ser)](ClO_4)_2$	21.27	21.51	4.76	4.91	13.78	13.62			471 (118), 343 (122)
$[Co(tren)(thre)](ClO_4)_2$	23.00	22.75	5.02	5.49	13.41	13.12			470 (110), 341 (103)
$[Co(tren)(cysCH_3)](ClO_4)_2$	22.23	22.25	5.05	4.87	12.99	13.17	5.95	6.15	470 (128), 340
$[Co(tren)(cySO_3)]ClO_4$	22.91	23.29	4.91	4.93	14.84	14.67	6.80	7.07	471 (116), 343 (110)
$[Co(tren)(met)](ClO_4)_2$	23.92	23.98	5.11	5.10	12.68	12.55	5.80	5.73	463 (161), 400
$[Co(tren)(lys)](ClO_4)_2$	22.22	22.37	4.96	4.97	12.93	12.81			472 (120), 343 (117)
$[Co(tren)(arg)](ClO_4)_2$	24.77	22.24	5.14	5.15	19.41	14.15			473, 340
$[Co(tren)(arg)](ClO_4)_3$	21.27	15.64	4.76	3.74	16.53	10.24			
$[Co(tren)(asp)](ClO_4)_2$	22.40	24.50	4.51	4.45	13.06	10.68			472, 344
$[Co(tren)(asp)]ClO_4$	27.57	28.21	5.32	5.60	16.07	14.65			
$[Co(tren)(sar)](ClO_4)_2 \cdot H_2O$	21.19	21.41	5.12	5.56	13.73	13.34			477 (103), 345 (108)

+15°), Co(tren)(L-ala)$^{2+}$ ($[\alpha]^{20}D$ −26°), and Co(tren)-(DL-ala)$^{2+}$, respectively. Although some optical activity of the amino acid is retained upon complexation, the degree of racemization was not determined.

The visible and ultraviolet absorption of the complexes show two symmetrical bands at 470 and ~343 nm. The positions of these bands do not vary appreciably, suggesting a similar octahedral ligand field throughout the series of complexes (Table I). The positions and intensities of these bands are similar to those of related octahedral complexes, especially Co-(en)$_2$AA^{2+} [9] and Co(trien)AA^{2+}.[10] The infrared spectra of these amino acid complexes show strong absorptions at 1660 and 1380 cm^{-1} assigned to the asymmetric and symmetric stretching modes of coordinated carboxylate. These physical properties are consistent with coordination of the amino acids as bidentate ligands. Ascending paper or thin layer chromatography on cellulose proved an excellent method for identification of the product complexes. The movement of the amino acid complexes closely resembles the parent amino acid on a similar tlc system.

Amino Acid Esters.—Glycine, L-alanine, and valine esters react rapidly with Co(tren)(OH)$(H_2O)^{2+}$ at 60° and pH 7.5 to yield Co(tren)(gly)$^{2+}$, Co(tren)-(L-ala)$^{2+}$, and Co(tren)(val)$^{2+}$, respectively. The reaction of glycine ester gave two isomeric glycinato complexes as described below. In all reactions, the final amino acid complexes are formed essentially quantitatively. No evidence has been obtained for any intermediate involving a chelated ester group. L-Alanine ester is hydrolyzed smoothly without serious racemization, giving Co(tren)(L-ala)$^{2+}$ ion, whose specific rotation was $[\alpha]^{23}D$ −29°, close to the value $[\alpha]^{20}D$ −26° measured for the sample prepared independently from Co(tren)(OH)$(H_2O)^{2+}$ and L-alanine. Similarly Co(tren)Cl_2^+, having two reactive sites, was shown to hydrolyze amino acid esters at pH 7–8 and 60° to give Co(tren)(AA)$^{2+}$. In this case it was necessary to add a weak base such as diethylamine to control the pH. An apparent intermediate containing

$$Co(tren)(OH)(H_2O)^{2+} + glyOR \longrightarrow Co(tren)(gly)^{2+} + H_2O + ROH$$

$$Co(tren)Cl_2^+ + glyOR \longrightarrow Co(tren)(gly)^{2+} + ROH + 2Cl^- + H^+$$

the amino acid ester as a monodentate such as Co-(tren)(glyOR)Cl^{2+} was isolated and fully characterized.[3] Aqueous solutions of Co(tren)(glyOR)Cl^{2+}, upon adjustment of the pH to 7.5 at 50°, immediately changed from pink to orange to yield the hydrolyzed product, Co(tren)(gly)$^{2+}$, in quantitative yield. Likewise, this hydrolysis occurred promptly in 0.1 *M* $HClO_4$ solution in the presence of Hg^{2+} ion. Reaction of Co(tren)Cl_2^+ with L-alanine ester gave very hygroscopic Co(tren)(L-alaOR)Cl^{2+}, which, without further purification, underwent Hg^{2+}-catalyzed hydrolysis giving Co(tren)(L-ala)$^{2+}$ ($[\alpha]D$ −31°).

Peptides.—The final products formed, upon treating Co(tren)(OH)$(H_2O)^{2+}$ ions with several dipeptides and tripeptides at pH 7.5 and 60°, are given in Table II. In each case, the products were analyzed by tlc using appropriate amino acid complex ions as markers and in most cases the complexes were separated as their perchlorates and characterized by elemental analysis and ir and nmr spectra. When the reaction mixture was spotted on a silica gel plate at convenient time intervals and eluted with *n*-C_3H_7OH–H_2O (7:3), the cobalt-containing species remained on the starting line but the peptides and amino acids moved in the normal manner. In this way it was possible to detect the time of appearance of the cleavage product. The

(9) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964).
(10) B. E. Bryant, H. J. Hu, and W. H. Glaze, *ibid.*, **5**, 1373 (1966).

tlc analysis indicated that cleavage always occurs at the N-terminal position

$$Co(tren)(OH)(H_2O)^{2+} + NH_2CHRC(=O)NHR' \longrightarrow Co(tren)(NH_2CHRCO_2)^{2+} + NH_2R'$$

In a few cases a small amount of the complex from the C-terminal amino acid was detected. When glycylphenylalanine was treated with an equimolar concentration of $Co(tren)(OH)(H_2O)^{2+}$ at pH 7.5 and 60°, $Co(tren)(gly)^{2+}$ and phenylalanine were detected. When treated with 2 equiv of $Co(tren)(OH)(H_2O)^{2+}$, both $Co(tren)(gly)^{2+}$ and $Co(tren)(phe)^{2+}$ were detected. This experiment demonstrates the stepwise nature of the reaction. A number of tripeptides were studied in the same manner; the results are given in Table II. Analysis of the reaction mixture by tlc again demonstrated the N-terminal selectivity of the process. The appearance of the first amino acid chelate was obscured because the amino acid complex and the hydroxoaquo complex have very similar R_f values. A rough comparison of the rate of the cleavage reactions was made between $Co(tren)(OH)(H_2O)^{2+}$ and $Co(trien)(OH)(H_2O)^{2+}$ under the similar reaction conditions. The results of these competitive experiments are given in Table II. In all cases the cleavage

TABLE II

PEPTIDE-CLEAVAGE REACTIONS USING $Co(tren)(OH)(H_2O)^{2+}$ AND $Co(trien)(OH)(H_2O)^{2+}$ AT pH 7.5 AND 60°

Peptide	Procedure[a]	[Complex], M	[Peptide], M	Cleavage product detected by tlc system C
		tren System		
gly-phe	A	0.04	0.04	phe (3–4 hr)
phe-gly	A	0.04	0.04	gly (2–3 hr)
phe-gly	B	0.01	0.01	gly (1–2 hr)
phe-gly-gly	A	0.04	0.04	gly-gly (2–3 hr)
gly-phe-gly	B	0.01	0.01	phe-gly
gly-gly-phe	A	0.033	0.033	gly-phe (2–3 hr)
gly-gly-phe	B	0.01	0.01	gly-phe (3–4 hr)
		trien System		
gly-phe	A	0.05	0.05	phe (30–40 min)
phe-gly	B	0.01	0.012	gly (15–30 min)
phe-gly-gly	A	0.04	0.04	gly-gly (15–30 min)
phe-gly-gly	B	0.016	0.016	gly-gly (15–30 min)
gly-phe-gly	B	0.01	0.01	phe-gly
gly-gly-phe	A	0.033	0.033	gly-phe (15–30 min)
gly-gly-phe	B	0.01	0.01	gly-phe (40–60 min)

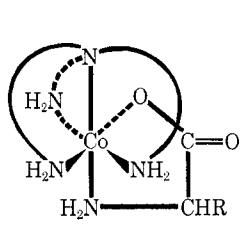
[a] Procedure A in unbuffered solution at 60 ± 5°, and procedure B in buffered solution with 2,4,6-collidine at 60 ± 1°. Tlc on silica gel eluted with 1-propanol–water (70:30; v/v).

of peptide by the trien system was considerably faster than by the tren system, as judged from time of appearance of the cleaved amino acid or peptide on the chromatogram and from the color change which accompanies the formation of amino acid complex. Similarly coordination of the free amino acids with $Co(tren)(OH)(H_2O)^{2+}$ was found to be slow compared with $Co(trien)(OH)(OH_2)^{2+}$.

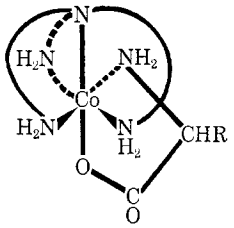
Discussion

In the pH range 7.5–8.0, amino acids react with $Co(tren)(OH)(H_2O)^{2+}$ to form the corresponding amino acid complexes $Co(tren)(AA)^{2+}$. These complex ions were prepared and characterized so as to provide marker complexes for the chromatographic analysis of the peptide hydrolysis products.

Two geometric possibilities exist for the $Co(tren)(AA)^{2+}$ ions; one has the amino acid nitrogen *trans* to the tertiary nitrogen of the tren ligand (A) and the other has it *trans* to the primary nitrogen of the tren ligand (B). This situation is analogous to that of $Co(trien)(AA)^{2+}$, in which two isomeric glycine complexes, β_1 and β_2, were isolated and characterized.[11]



A

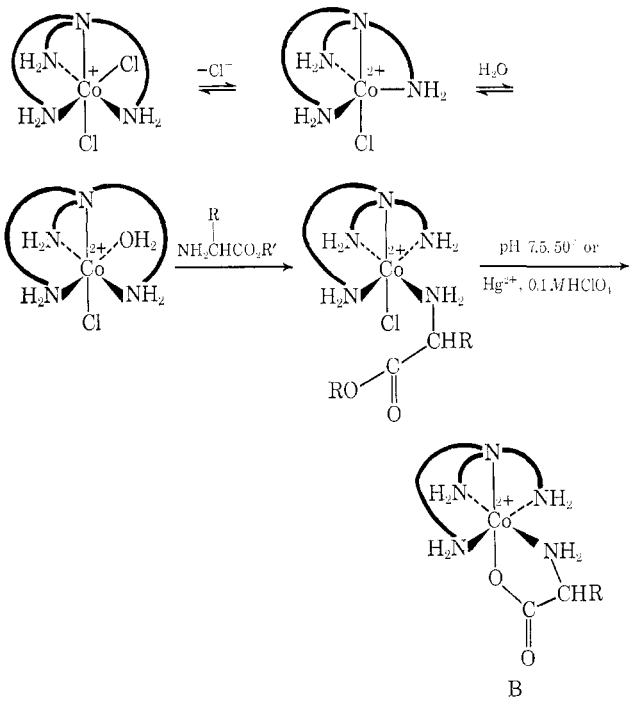


B

Specific formation of β_1- and β_2-$Co(trien)(gly)^{2+}$ ions in the reaction of $Co(trien)(OH)(H_2O)^{2+}$ with glycine ester and glycine, respectively, suggested that the water molecule coordinated *trans* to the primary nitrogen of the trien is more readily substituted by the amino group of the glycine ester or the carboxylate of glycine. In the reaction of $Co(trien)Cl(H_2O)^{2+}$ with glycine or glycine ester, the initial replacement of the water molecule by the carboxylate or the amino group is thought to determine the structure of the product. An analogy might be applicable in the tren system. The aquation rate of $Co(trien)Cl_2^+$ (k_1 = 2.96×10^{-3} sec^{-1} at 28°) is greater than those of *cis*-$Co(NH_3)_4Cl_2^+$, *cis*-$Co(en)(NH_3)_2Cl_2^+$, *cis*-$Co(en)_2Cl_2^+$, or *cis*-$Co(trien)Cl_2^+$. This observation, along with other kinetic data supporting an SN1 type mechanism, was rationalized on the basis of steric strain rather than electronic or inductive effects.[12] It was proposed that the chloride ion *trans* to the primary amine of the tren should be more easily aquated, since the elimination of this chloride ion would release the steric strain in the dichloro complex, forming a rather stable trigonal bipyramid prior to aquation (Scheme I). The water molecule should be readily displaced by the nitrogen of amino acid esters, yielding $Co(tren)(NH_2C(R)HCO_2R')Cl^{2+}$ which would determine the structure of the ultimately hydrolyzed amino acid complex as (B). Our observation that $Co(tren)(glyOR)Cl^{2+}$ shows always one spot on the tlc plate in various eluent systems seems to support this hypothesis. Treatment of $Co(tren)(glyOR)Cl^{2+}$ with OH^- at pH 7.5 or with Hg^{2+} resulted in the rapid hydrolysis of the ester, giving orange $Co(tren)(gly)^{2+}$, whose homogeneity was established by the tlc. The Hg^{2+}-

(11) L. G. Marzilli and D. A. Buckingham, *Inorg. Chem.*, **6**, 1042 (1967).
(12) S. K. Madan, W. M. Ruff, and J. C. Bailar, Jr., *ibid.*, **4**, 1366 (1965).

SCHEME I



catalyzed reaction probably proceeds by the mechanism proposed by Alexander and Busch.[13] The glycinato complex obtained in this manner was identical with the one prepared from Co(tren)(OH)$(H_2O)^{2+}$ and glycine. On the basis of the preceding arguments, we tentatively assign structure B to the orange glycine complex, although at present there are no structural data to support this supposition.

Careful treatment of glycine ester with Co(tren)-(OH)$(H_2O)^{2+}$ gave two isomeric glycinato complexes as orange and red crystalline perchlorates. The less soluble orange glycinato complex is identical with the one described above, having visible absorption bands at 472 nm (ϵ 105) and 342 nm (ϵ 100). The more soluble red glycinato complex has visible absorption bands at 499 nm (ϵ 92) and 347 nm (ϵ 75) and infrared C=O antisymmetrical and symmetrical stretching frequencies at 1670 and 1380 cm^{-1}, values similar to those found with the orange complex. The nmr spectra of both complexes show a methylene resonance for the chelated glycine at 3.7 ppm. These isomers can be distinguished by their behavior on tlc. Structure A is tentatively assigned to the red glycinato complex. Since both complexes are stable under these reaction conditions, they must be kinetic products.

In the reaction of alanine ester with Co(tren)(OH)-$(H_2O)^{2+}$, we observed two products on a chromatogram: a slow-moving orange spot (λ_{max} 482 and 346 nm) as the major product and a faster moving red spot (λ_{max} 500 and 347 nm) as a minor product. After several recrystallizations we were able to isolate only the orange alaninato product. The physical properties of this product are quite similar to those of the orange glycinato complex and hence this alanine complex has been assigned structure B. The red product may be the isomeric alaninato complex A.

Amino acid esters are rapidly hydrolyzed at pH 7.5 and 60° in the presence of Co(tren)(OH)$(H_2O)^{2+}$ in a manner analogous to Co(trien)(OH)$(H_2O)^{2+}$. Unlike Co(trien)XY^{n+},[14] the tren complex ions contain a plane of symmetry and the dissymmetry of the amino acid complexes is due to the asymmetric center on the amino acid ligand. Thus the tren complex is a better model for studying the hydrolysis of optically active amino acid esters and peptides by metal ion catalyzed reactions. The specific rotation of Co(tren)-(L-ala)$^{2+}$ resulting from the hydrolysis of L-alanine ester by Co(tren)(OH)$(H_2O)^{2+}$ is almost the same as that obtained by treatment of Co(tren)(L-alaOR)Cl^{2+} with Hg^{2+} ion in 0.1 M $HClO_4$ solution, where less racemization might be expected. It may become important that no significant racemization of the optically active center was observed during the hydrolysis by the Co^{3+} ion, in comparison with other chemical degradation methods which are usually accompanied by racemization. Our results in this regard are preliminary inasmuch as we do not know the specific rotation for the pure enantiomeric cationic amino acid chelate or the geometrical isomer (A or B) that we are dealing with.

Hydrolysis of dipeptides and tripeptides by Co(tren)-(OH)$(H_2O)^{2+}$ is both facile and essentially quantitative. The N-terminal specificity of the reactions is clearly demonstrated by the amino acid complexes formed using glycylphenylalanine and phenylalanylglycine, as well as glycylleucine and leucylglycine. When equimolar concentrations of the cobalt complex and dipeptide are used, the N-terminal amino acid is selectively removed as Co(tren)AA^{2+} and only a trace of the C-terminal amino acid complex is detected in a few cases.

We have not isolated the anticipated intermediate Co(tren)(glyglyOR)$^{3+}$ as we did in the case of the trien complex,[3] but our attempts to find such an intermediate were not strenuous. In view of the selectivity of the tren reagent such an intermediate must be formed so that the hydrolyzed (C-terminal) amino acid fragment from the peptide does not have an opportunity to react with Co(tren)(OH)$(OH_2)^{2+}$. Glycylglycine rapidly coordinates with Co(tren)Cl_2^+ in aqueous solution to form Co(tren)(glyglyH)Cl^{2+} ion in which the dipeptide is probably coordinated as a monodentate through the carboxyl oxygen.

The rate of reaction of Co(tren)(OH)$(H_2O)^{2+}$ with peptides is slower than that of β-Co(trien)(OH)$(H_2O)^{2+}$, as shown in Table II. In this regard, the tren system has no particular advantage over the trien system for the cleavage of peptides. The mechanism for the peptide hydrolysis by the trien system involves the replacement of a coordinated water molecule by the terminal amino group of the peptide as the rate-

(13) M. D. Alexander and D. H. Busch, *J. Am. Chem. Soc.*, **88**, 1130 1966).

(14) For example, β-Co(trien)(OH)$(H_2O)^{2+}$ reacts with L-alanine to give Dβ_1-Co(trien)(L-ala)$^{2+}$ and Lβ_2-Co(trien)(L-ala)$^{2+}$. With L-alanine ester, the main product was Dβ_1-Co(trien)(L-ala)$^{2+}$.

determining step, followed by activation of the carbonyl group to attack by external hydroxide or water through prior coordination of the carbonyl oxgen.[3,8] An analogous mechanism is probable in the tren system, but there may be differences in relative rates of the first and second stages of the peptide or amino acid ester hydrolysis reaction. In the case of Co(tren)(OH)$(H_2O)^{2+}$, initial coordination of the N-terminal peptide amine may be rapid compared with the formation of an intermediate chelate which leads to hydrolysis. This would explain the greater selectivity of the tren complex for N-terminal hydrolysis compared with the analogous trien complex.

Further support for this argument concerning differences between the tren and trien complexes comes from a comparison of reactions involving the chloro complexes. Reaction of Co(trien)(glyOR)Cl^{2+} with glycine ester under mild conditions gives rise to Co(trien)(glyglyOR)$^{3+}$, probably by way of a coordinated ester intermediate,[3] a fact which demonstrates the relatively labile character of the coordinated chloride ion in Co(trien)(glyOR)Cl^{2+}. By contrast, under similar conditions the chloride ion in Co(tren)(glyOR)Cl^{2+} was found to be inert. Similarly, β-Co(trien)Cl_2^+ easily reacts with N-methylglycinamide to form β-Co(trien)(glyNHCH$_3$)$^{3+}$ in which both chloride ions of the starting material are substituted by amine and amide carbonyl.[3] The same reaction with Co(tren)Cl_2^+ afforded only a hygroscopic impure pink solid having $\nu_{C=O}$ at 1670 cm^{-1}. This result suggests that only one of the two coordinated chloride ions is labile under the reaction conditions employed.[15] These results suggest that the slower peptide hydrolysis in the tren system may be due to the difficulty in coordinating the amide carbonyl group. Once such coordination occurs, nucleophilic attack of hydroxide ion should follow rapidly, as indicated by the fact that Co(tren)(glyOR)Cl^{2+} is very rapidly hydrolyzed in the presence of Hg^{2+}.

Experimental Section

Instrumentation.—Infrared spectra were recorded on a Perkin-Elmer Model 421 or Model 237-B grating spectrophotometer as KBr disks.

Visible spectra were recorded on a Cary Model 14 spectrophotometer for 5×10^{-3} *M* aqueous solutions.

Proton magnetic resonance spectra were recorded on a Varian A-60 (60 Mcps) at 37°, the internal temperature of the probe. The samples were dissolved in 99.7% deuterium oxide. When an acidic solution was desired, nitric acid or phosphorus pentoxide was added. A small amount of sodium 3-(trimethylsilyl)-1-propanesulfonate (Brinkmann Instruments, Inc.) was added to serve as the internal standard.

Measurements of pH were made using a Leeds and Northrup pH indicator, No. 7664, with the miniature electrode assembly, No. 124138, standardized against a reference buffer solution, No. 103-1-0-2, pH 6.85.

Conductivity measurements were made on an Industrial Instruments conductivity bridge, Model 16B2, equipped with an external cell. The cell constant was determined using 0.10 *M* potassium chloride. The samples were dissolved in distilled water to obtain 10^{-3} *M* solutions.

The RD and CD curves were measured on a Shimadzu spectrophotometer.

Thin Layer Chromatography.—The adsorbents were purchased from Brinkmann Instruments, Inc., Westbury, N. Y. In order to coat five 20 × 20 cm or twenty 5 × 20 cm glass plates, a slurry was prepared by mixing 15 g of MN 300 G cellulose in 90 ml of water or 30 g of silica gel H in 60–65 ml of water in a mechanical blender for about 1 min at high speed. The slurry was coated onto the plates with an applicator whose exit gate setting was 0.25 mm. In order to coat two 20 × 40 cm glass plates for preparative separation, a slurry was prepared by mixing 100 g of silica gel PF_{254} with 250 ml of water in a mechanical blender for about 1 min. The slurry was coated onto the plates, one at a time, with an applicator whose exit gate setting was 0.75 mm. The 0.25-mm layers were normally dried at room temperature overnight and then at 110° for 10–30 min. The 0.75-mm preparative layers were dried overnight at room temperature and then at 110° for 3–4 hr.

For normal analysis, aqueous 2% amino acid complex solutions were spotted on the layers with capillaries and air dried. The glass plates were eluted in the ascending manner in tanks lined with filter paper soaked with the appropriate eluent to aid saturation. The precoated sheets were eluted in an Eastman chromatogram developing apparatus. For preparative work, a solution of 50 mg of complex in 1–2 ml of water was applied to the layer in a continuous streak with a Desaga electromechanical sample applicator (Brinkmann) and air dried. The chromatogram was eluted in the ascending manner as above.

After elution, the spots were detected under ultraviolet light or by spraying with ninhydrin spray reagent (Brinkmann) and heating at 100°. Amino acids and peptides appeared after heating for a few minutes, but amino acid complexes also appeared after heating for 10–20 min. Adsorbents which contained fluorescent indicators were sometimes used. Eluents which contained perchloric acid destroyed the fluorescence and caused plastic sheets sprayed with ninhydrin to turn black on heating.

The following systems were employed: system A, cellulose plate eluted with the upper phase 1-butanol–water–60% perchloric acid (5:5:1; v/v); system B, cellulose plate eluted with the upper phase 1-butanol–water–glacial acetic acid (5:5:1; v/v); system C, silica gel plate eluted with 1-propanol–water (70:30; v/v); system D, silica gel plate eluted with the upper phase of 1-butanol–water–60% perchloric acid (5:5:1; v/v); system E, silica gel plate eluted with the upper phase 1-butanol–water–glacial acetic acid (5:5:1; v/v); system F, cellulose plate eluted with ether–methanol–water–hydrochloric acid (50:30:20:2; v/v); system G, cellulose plate eluted with ether–methanol–water–60% perchloric acid (50:30:20:2; v/v).

Materials.—Amino acids and peptides were purchased from Mann Research Lab, Inc., and Cyclo Chemical Co. Wherever possible the compounds were of the natural L configuration and of the highest quality available.

Preparation of the Ligand.—2,2′,2″-Trihydroxytriethylamine was commercially available (Eastman) and was used without further purification.

(a) **2,2′,2″-Trichlorotriethylamine Hydrochloride.**—This compound was prepared by the method of McCombie and Purdie[16] with the exception that more chloroform was used to help moderate the exothermic reaction. A solution of 89 ml (100 g, 0.67 mol) of triethanolamine in 300 ml of dry chloroform was placed in a two-neck 2-l. flask equipped with condenser, dropping funnel, and magnetic stirrer. A solution of 163 ml (270 g, 2.27 mol) of thionyl chloride (12% excess) in 150 ml of dry chloroform was added dropwise to the stirred and cooled reaction mixture. The addition must be made slowly; otherwise a violent exothermic reaction with rapid evolution of gases will occur.

(15) It should be mentioned here that *trans*-Co(en)$_2$Cl$_2^+$ reacts with N-methylglycinamide under the same conditions to yield only *cis*-Co(en)$_2$(glyNHCH$_3$)Cl^{2+} in which the glycinamide coordinates as a monodentate ligand: E. Kimura, Ph.D. thesis, University of North Carolina, 1967. Preparation of Co(en)$_2$(glyNHCH$_3$)$^{3+}$ appears to require Hg^{2+} ion to displace the chloride ion by the amide oxygen.[5]

(16) M. McCombie and P. Purdie, *J. Chem. Soc.*, 1217 (1935).

After complete addition, the mixture was heated at reflux. During reflux when the contents of the flask suddenly solidified, more chloroform was added and reflux was maintained until evolution of sulfur dioxide and hydrochloric acid was no longer visible. After cooling, the white crystals were collected by vacuum filtration and washed liberally with chloroform. The product weighed 138 g (85%); mp 127–129°, lit.[16] mp 133°. The product used was without further purification. *Note. This water-soluble mustard hydrochloride is a powerful vesicant.*[17,18] *Gloves should be worn at all times and extreme care should be used in handling the material.*

(b) **2,2′,2″-Triphthalimidotriethylamine.**—A mixture of 48.2 g (0.2 mol) of 2,2′,2″-trichlorotriethylamine hydrochloride, excess potassium phthalimide (185.2 g, 1.0 mol), and 350 ml of dimethylformamide was heated and the temperature was maintained at 65–70° for 24 hr. After cooling, the contents were poured into 1600 ml of iced 1% sodium carbonate solution and stirred for 1 hr. The solid product was allowed to settle so that the major portion of liquid could be decanted to ease filtration of the finely divided solid. The white solid was washed with boiling ethanol and air dried; mp 187–189°, lit.[19] mp 193°. Yields ranged from 75% to greater than theory due to the presence of impurities. Since these impurities were destroyed in subsequent hydrolysis, no purification was attempted.

(c) **2,2′,2″-Triaminetriethylamine Trihydrochloride (tren·3HCl).**—2,2′2″-Triphthalimidotriethylamine was hydrolyzed by the method of Mann and Pope[19] with the exception that larger amounts were used and *n*-octyl alcohol was added to reduce foaming of the solution.

A mixture of 45 g (0.085 mol) of 2,2′,2″-triphthalimidotriethylamine and 900 ml of 1:1 hydrochloric acid was heated at reflux. A few drops of *n*-octyl alcohol was added to reduce foaming. The solid dissolved after about 1 hr, but reflux was maintained for 3–4 hr. On cooling phthalic acid precipitated and was removed by filtration. The filtrate was evaporated nearly to dryness and after cooling yielded 20 g of white crystals. The crystals were collected by filtration and washed with ethanol. This compound is reported to be the 4-hydrochloride monohydrate.[19] The solid can then be recrystallized from an ethanol-water mixture to obtain the trihydrochloride. Alternately, alcohol can be added to the hot concentrated solution to precipitate the trihydrochloride. The average yield of pure product was around 80%. *Anal.* Calcd for $C_6H_{21}N_4Cl_3$: C, 28.19; H, 8.58; N, 21.92. Found: C, 28.14; H, 8.23; N, 21.43.

Preparation of the Complexes. (a) **Dinitro(2,2′,2″-triaminetriethylamine)cobalt(III) Chloride.**—To an ice-cold solution of 47.2 g (0.2 mol) of cobalt(II) chloride hexahydrate and 27.2 g (0.4 mol) of sodium nitrite in water was added dropwise tren·HCl [51.8 g (0.2 mol) of tren·3HCl and 16 g (0.4 mol) of sodium hydroxide in 75 ml of water], while aerating the solution vigorously by passing air through a coarse fritted glass filter stick. A few drops of *n*-octyl alcohol were added to reduce foaming. After 4 hr, the solid was collected by vacuum filtration on a glass funnel of medium porosity and washed with a small amount of ice-cold 1 *N* hydrochloric acid, followed by generous amounts of alcohol and acetone. After being air dried, the crude yellow-brown crystals weighed 40 g (60%). The product was recrystallized from a minimum of hot water and gave 32 g of pure product. A second crop could be obtained by concentration of the mother liquor. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(NO_2)_2]Cl$: C, 21.66; H, 5.46; N, 25.27. Found: C, 21.72; H, 5.51; N, 25.36.

(b) **Dichloro(2,2′,2″-triaminotriethylamine)cobalt(III) Chloride Monohydrate.**—A mixture of excess concentrated hydrochloric acid (85 ml) and 32 g (0.91 mol) of $[Co(tren)(NO_2)_2]Cl$ was evaporated to dryness on a steam bath. The solution was stirred occasionally to ensure that all the dinitro compound was hydrolyzed. The blue-purple solid was ground with alcohol, collected by filtration, and washed with alcohol and acetone. The solid weighed 28.3 g (95%) after being air dried. The product could be recrystallized by dissolving it in a minimum of hot 3 *N* hydrochloric acid. *Anal.* Calcd for $[Co(C_6H_{18}N_4)Cl_2]Cl\cdot H_2O$: C, 21.86; H, 6.12; N, 17.00. Found: C, 21.91; H, 5.96; N, 16.93.

(c) **Carbonato(2,2′,2″-triaminotriethylamine)cobalt(III) Chloride Sesquihydrate.**—A mixture of $[Co(tren)Cl_2]Cl\cdot H_2O$ (28.3 g, 0.086 mol) and excess lithium carbonate (8.2 g, 0.111 mol) in 70 ml of water was heated on a steam bath and the blue-purple color changed to red in about 15 min. After 1 hr the pH was determined to ensure that the solution was basic, the excess lithium carbonate was removed by filtration of the hot solution, 1 g of calcium chloride was added, and the solution was allowed to stand overnight. Calcium carbonate was removed by filtration, the filtrate was warmed to 50°, and alcohol was added slowly until turbid. A red oil formed on standing in a refrigerator overnight, and after scratching a solid product was obtained. The liquid was decanted and acetone was added. The crude product was collected by filtration and air dried, 23.4 g (83%). The product was recrystallized by dissolving it in a minimum of hot water and adding alcohol until turbid; on standing in a refrigerator overnight, red crystals were formed. The product was collected by filtration and washed with alcohol and acetone. *Anal.* Calcd for $[Co(C_6H_{18}N_4)CO_3]Cl\cdot 1.5H_2O$: C, 25.65; H, 6.46; N, 17.10. Found: C, 25.79; H, 6.49; N, 17.07.

(d) **Carbonato(2,2′,2″-triaminotriethylamine)cobalt(III) Perchlorate Hemihydrate.**—To a warm solution of 16.5 g (0.05 mol) of $[Co(tren)CO_3]Cl\cdot 1.5H_2O$ in 30 ml of water was added an excess of sodium perchlorate (8 g, 0.065 mol). On standing, red crystals of product were formed. The product was collected by filtration, washed with cold aqueous sodium perchlorate solution, alcohol, and acetone, and air dried. The product gave a negative test for chloride ion with silver nitrate. *Anal.* Calcd for $[Co(C_6H_{18}N_4)CO_3]ClO_4\cdot 0.5H_2O$: C, 22.50; H, 5.12; N, 15.00. Found: C, 22.47; H, 4.90; N, 14.77.

(e) **Diaquo(2,2′,2″-triaminotriethylamine)cobalt(III) Perchlorate Monohydrate.**—A 10% excess of perchloric acid (2.2 ml of 5 *N*) was added dropwise to solid $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$ (1.91 g, 0.005 mol) cooled in ice. Effervescence was allowed to subside before each addition. After complete addition of acid, the solution was placed in a vacuum desiccator and water was removed slowly by pumping. After solid formed, still moist, it was collected by filtration and washed with ether. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(H_2O)_2]ClO_4\cdot H_2O$: C, 12.92; H, 4.34; N, 10.05. Found: C, 13.09; H, 4.65; N, 9.90.

(f) **Hydroxoaquo(2,2′,2″-triaminotriethylamine)cobalt(III) Perchlorate.**—To a solution 1.2 g of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$ in 10 ml of water was added 5 *N* perchloric acid until the evolution of carbon dioxide ceased and the pH was less than 1. Nitrogen was bubbled through the solution to expel dissolved carbon dioxide. A 10% aqueous solution of lithium hydroxide was added slowly to the solution and a red-purple solid precipitated at pH 4.5. The product was collected by filtration and washed with acetone. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(OH)(H_2O)](ClO_4)_2$: C, 16.41; H, 4.82; N, 12.76. Found: C, 16.60; H, 4.69; N, 12.72.

Preparation of Amino Acid Complexes. (a) **Reaction of Hydroxoaquo(2,2′,2″-triaminotriethylamine)cobalt(III) Ion with Amino Acids. General Procedure.**—Perchloric acid (2 ml of 60%) was added dropwise to 1.9 g (5.1 mmol) of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$ in 20–30 ml of water. Effervescence was observed as carbon dioxide was lost, and the resulting solution of $Co(tren)(H_2O)_2^{3+}$ had a pH of less than 1. The solution was allowed to stand for 30 min to ensure complete reaction, and excess carbon dioxide was flushed with a stream of nitrogen. Freshly prepared aqueous solution of 10% lithium hydroxide was added to adjust the pH to 7.5, and then the amino acid (6.0 mmol) was added to the purple solution of $Co(tren)(OH)(H_2O)^{2+}$. The total volume was brought to 50 ml, and the pH was readjusted to 7.5. The mixture was heated for several hours at 60 ± 5°; during this period the color changed to orange. Sodium perchlorate monohydrate (1–5 g) was added and the warm solution was allowed to

(17) J. Van Alphen, *Rec. Trav. Chim.*, **56**, 1007 (1937).
(18) K. Ward, *J. Am. Chem. Soc.*, **57**, 914 (1935).
(19) F. G. Mann and W. J. Pope, *Proc. Roy. Soc.* (London), **A109**, 44 (1925).

evaporate under a stream of air until crystalline material was observed. The solution was cooled to room temperature and allowed to stand for several hours. In cases where no solid appeared after volume was reduced to 10 ml, precipitation was induced by cooling in an ice bath or by adding ethanol or acetone. The product was collected by vacuum filtration, washed with ethanol and acetone, and recrystallized by dissolving in a minimum amount of hot aqueous 25% sodium perchlorate solution. Analytical samples were dried *in vacuo* over phosphorus pentoxide at 100° for 20 hr.

(b) **Reaction of Hydroxoaquo(2,2′,2″-triaminotriethylamine)-cobalt(III) Ion with Amino Acid Esters. (1) Preparation of Amino Acid Ethyl Ester Free Base.**—L-Alanine ethyl ester hydrochloride (5 g) was added to a saturated aqueous solution of potassium carbonate. The solution was extracted with three 25-ml portions of chloroform. The combined extracts were dried over potassium carbonate overnight. The solution was filtered and chloroform was removed *in vacuo*. The remaining oil was vacuum distilled and the fraction of bp 46–48° (10–11 mm) was collected; lit.[20] bp 48° (11 mm).

Glycine ethyl ester was prepared in the same manner and the fraction of bp 46–48° (10 mm) was collected; lit[20] bp 43–44° (11 mm).[20]

(2) **Glycine Ethyl Ester.**—The pH of a solution of 2.23 g (4.0 mmol) of $[Co(tren)(H_2O)_2](ClO_4)_3 \cdot H_2O$ in 10 ml of water was adjusted to 7.5 with freshly prepared aqueous 10% lithium hydroxide solution. Glycine ethyl ester (1.04 g, 8 mmol) was added, the volume was increased to 30 ml, and then was heated overnight at 50 ± 5°. The solution was evaporated to 10 ml, 5 g of sodium perchlorate monohydrate was added, and the solution was placed in a refrigerator overnight. Scratching the side of the beaker with a glass rod induced crystallization. The solid was collected by filtration, washed with acetone and ether, and air dried; yield 0.8 g (42%). A portion was recrystallized by dissolving in a minimum amount of hot aqueous 25% sodium perchlorate solution. Tlc (system A) of the product showed one spot of the same R_f compared to known glycine complex prepared from acid. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_2H_4NO_2)]Cl(ClO_4)$: C, 23.20; H, 5.35; N, 16.91. Found: C, 23.23; H, 5.37; N, 16.82.

Concentration of the mother liquor after elimination of the orange solid precipitated red crystals; yield 0.7 g. The analytical sample was recrystallized from H_2O. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_2H_4NO_2)](ClO_4)_2$: C, 20.10; H, 4.64; N, 14.16. Found: C, 20.35; H, 4.64; N, 14.73.

(3) **L-Alanine Ethyl Ester.**—To a solution of 2.23 g (4.0 mmol) of $[Co(tren)(H_2O)_2](ClO_4)_3 \cdot H_2O$ in 10 ml of water was added 0.94 g (8.0 mmol) of alanine ethyl ester. The pH of the resulting solution was 7.5, and a purple solid similar to that observed when base was added to the diaquo compound was formed. The solid dissolved after increasing the volume to 30 ml and heating to 60°. The solution was heated for a few hours at 60 ± 5° and then evaporated to 10 ml; solid formed on the addition of 5 g of sodium perchlorate monohydrate. A little water was added and the solution was heated to 80° to redissolve the solid. The product crystallized on cooling to room temperature. The product was collected by filtration, washed consecutively with ice-cold aqueous 33% sodium perchlorate solution, ethanol, and acetone, and air dried; yield 1.83 g (93%). A portion was recrystallized by dissolving in a minimum of hot aqueous 33% sodium perchlorate solution. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_3H_6NO_2)](ClO_4)_2$: C, 21.96; H, 4.91; N, 14.23. Found: C, 21.98; H, 4.98; N, 14.57.

The visible spectrum of the product dissolved in water had bands at λ 482 and 346 nm. Tlc (systems A and B) showed only one spot compared to the alanine complex prepared from the acid.

Preparative tlc on silica gel PF (Brinkmann) and elution with the upper layer 1-butanol–water–60% perchloric acid (5:5:1; v/v) gave a slow-moving orange band and faster moving red band. The bands were scraped from the plate, stirred with warm water, and filtered to remove silica gel, and the filtrate was concentrated to a small volume. The red and orange liquids were neutralized with base and 1 *N* sodium cyanide solution was added. The solutions were spotted on tlc (system C) and both bands showed the presence of alanine.

(4) **L-Valine Methyl Ester Hydrochloride.**—Perchloric acid (1 ml, 60%) was added to 0.748 g (20 mmol) of $[Co(tren)CO_3]ClO_4 \cdot 0.5H_2O$ dissolved in water. The solution was allowed to stand for 45 min to ensure complete reaction, and carbon dioxide was flushed out with a stream of nitrogen. The pH was adjusted to 7.5 by the addition of freshly prepared aqueous solution of lithium hydroxide. L-Valine methyl ester hydrochloride (0.335 g. 2.0, mmol) was added and the volume was brought to about 50 ml. The solution was heated overnight at 60 ± 5° on a hot plate. Sodium perchlorate monohydrate (2.0 g) was added and the solution was allowed to evaporate to 20 ml. No product crystallized on cooling, but the addition of 2 g of sodium perchlorate monohydrate caused precipitation. The solution was heated until all solid dissolved and was allowed to cool to room temperature. The solid that crystallized was removed by filtration, washed with ethanol and acetone, and recrystallized from a 25% sodium perchlorate aqueous solution. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_5H_{10}NO_2)](ClO_4)_2$: C, 25.40; H, 5.42; N, 13.46. Found: C, 25.43; H, 5.42; N, 13.37.

A second crop of crystals was obtained by concentrating the filtrate to 10 ml. The product was collected by filtration, washed with ethanol and acetone, and recrystallized from an aqueous solution of 25% sodium perchlorate monohydrate; total yield ~0.8 g. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_5H_{10}NO_2)](ClO_4)_2$: C, 25.40; H, 5.42; N, 13.46. Found: C, 25.65; H, 5.38; N, 13.59.

Treatment of $[Co(tren)(glyOR)Cl](ClO_4)_2$ with Hg^{2+} ion in 0.1 *M* $HClO_4$ Solution.—To $[Co(tren)(glyOC_2H_5)Cl](ClO_4)_2 \cdot H_2O$[3] (560 mg) dissolved in 10 ml of warm 0.1 *M* $HClO_4$ solution, was added $Hg(OAc)_2$ (160 mg). The original pink solution immediately turned orange. Upon concentration, orange needles precipitated; yield 350 mg. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_2H_4NO_2)](ClO_4)_2$: C, 20.10; H, 4.64; N, 14.65. Found: C, 19.91; H, 4.67; N, 14.57. The infrared, visible, and nmr spectra were identical with those of the sample prepared from $Co(tren)(OH)(H_2O)^{2+}$ and glycine.

Treatment of $[Co(tren)(L\text{-}alaOC_2H_5)Cl]Cl_2$ with Hg^{2+} Ion.—$[Co(tren)Cl_2]Cl$ (2.0 g) was ground to a fine powder in a mortar with L-alanine ethyl ester hydrochloride (1.0 g). Water (5 ml) was added and then diethylamine (0.8 ml) was added dropwise with continuous mixing. The mixture gradually turned pink when allowed to stand at room temperature overnight. Attempts to crystallize the product failed. The pink solid obtained by trituration in ethanol was very hygroscopic. The crude $[Co(tren)(L\text{-}alaOC_2H_5)Cl]Cl_2$ ($\nu_{C=O}$ at 1730 cm^{-1}) was mixed with $Hg(OAc)_2$ (excess amount) in 10 ml of 0.1 *M* $HClO_4$ solution. The initially pink solution turned orange in a few minutes when warmed to ~50°, and then orange needles precipitated. After recrystallization from hot water, the yield was 1.0 g. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_3H_6NO_2)]HgCl_4$: C, 17.00; H, 3.80; N, 11.02. Found: C, 17.65; H, 4.05; N, 11.21. Visible spectrum: 473 nm (ε 106) and 343 nm (ε 96). The infrared spectrum exhibits $\nu_{C=O}$ at 1660 cm^{-1}.

Hydrolysis of $Co(tren)(glyOC_2H_5)Cl^{2+}$ with Hydroxide Ion.—$[Co(tren)(glyOC_2H_5)Cl](ClO_4)_2H_2O$ was dissolved in a minimum amount of hot water. To the pink solution, originally at acidic pH, was added 0.1 *M* NaOH solution dropwise to adjust the pH to ~7.5, whereupon the solution had become orange. Orange prisms precipitated when the solution was cooled in ice. The yield was quantitative. The infrared and the visible spectra are identical with those of $[Co(tren)(gly)](ClO_4)_2$.

Dipeptide Cleavage. Reaction of Hydroxoaquo(2,2′,2″-triaminotriethylamine)cobalt(III) Ion with Dipeptides. General Procedure.—Perchloric acid (1 ml, 60%) was added dropwise to a solution of 0.748 g (2.0 mmol) of $[Co(tren)CO_3]ClO_4 \cdot 5H_2O$ in 30–40 ml of water. Carbon dioxide was evolved, and the resulting soultion had a pH of less than 1. The solution was al-

(20) E. Fisher, *Ber.*, **34**, 433 (1901).

lowed to stand for 30 min to ensure complete reaction, and carbon dioxide was flushed out with a stream of nitrogen. A freshly prepared aqueous solution of 10% lithium hydroxide was added until the pH was 7.5. The dipeptide (2.0 mmol) was added and the volume and pH were brought to about 100 ml and 7.5, respectively. The concentrations of both cobalt complex and dipeptide were approximately 0.02 *M*. The solution was covered with a watch glass and then heated overnight at 60 ± 5° on a hot plate.

Glycyl-L-phenylalanine.—Sodium perchlorate monohydrate was added and the solution was allowed to evaporate to 15 ml. A solid precipitated on cooling. The product was collected by filtration, washed with ethanol and acetone, and recrystallized from an aqueous solution of 25% sodium perchlorate monohydrate. Tlc (system A) analysis showed only one spot which corresponded to the marker complex prepared from glycine. Tlc (system C) showed a gradual increase of phenylalanine and a gradual decrease of glycylphenylalanine. When excess Co(tren)(OH)$(H_2O)^{2+}$ was used, both Co(tren)$(gly)^{2+}$ and Co(tren)·$(phe)^{2+}$ were detected on tlc (system A). *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_2H_4NO_2)](ClO_4)_2$: C, 20.10; H, 4.64; N, 14.64. Found: C, 20.30; H, 4.86; N, 14.75.

Other Dipeptides.—Glycylglycine, glycyl-L-leucine, L-phenylalanylglycine, L-leucylglycine, L-tryptophenylglycine, L-histidylglycine, L-lysylglycine, L-serylglycine, L-prolylglycine, and L-alanylglycine were also cleaved under conditions similar to those given above. In all but the last two examples the tren complex of the N-terminal amino acid residue was isolated and characterized by elemental analyses. Tlc analysis of the crude reaction mixtures revealed only the complex formed from the N-terminal residue in all cases except L-prolylglycine, where the two apparently isomeric proline complexes and the glycine complex were all detected, and phenylalanylglycine, where some glycine complex was detected. System A was used for tlc analysis in all but pro-gly, ala-gly, and phe-gly where systems D, D, and a combination of A and F were employed, respectively.

Peptide-Cleavage Reactions Followed by Tlc. Procedure A.—Perchloric acid (1 ml 60%) was added to 1.0 mmol of the carbonato complex dissolved in a small volume of water. The mixture was allowed to stand for 30 min to ensure complete reaction and carbon dioxide was flushed out by bubbling nitrogen through the solution. The pH was adjusted to 7.5 by the addition of a freshly prepared aqueous solution of 10% lithium hydroxide. The peptide (1.0 mmol) was added, the volume was brought to 20–30 ml, and the pH was readjusted to 7.5 with lithium hydroxide. The mixture was heated to 60 ± 5° on a hot plate. Small samples were withdrawn at convenient time intervals, spotted on silica gel coated plates (Eastman chromatogram sheet), and air dried. The plate was eluted with 1-propanol–water (70:30; v/v) and the spots were detected by spraying with ninhydrin and heated at 100° for 5–10 minutes. The cobalt complexes remained at the origin, but amino acids and peptides moved in the normal manner.

The peptides studied in this way are given below along with the experimental variations, concentration, and total volume. Results are summarized in Table II.

(a) **Glycyl-DL-phenylalanine.** (1) **tren System.**—Procedure A was followed using 0.374 g (1.0 mmol) of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$, 0.222 g (1.0 mmol) of glycyl-DL-phenylalanine, and a total volume of 25 ml.

(2) **trien System.**—Procedure A was followed except that 0.328 g (1.0 mmol) of $[Co(trien)CO_3]ClO_4\cdot 1.5H_2O$ was hydrolyzed with concentrated hydrochloric acid. After adjustment of pH as in procedure A, 0.222 g (1.0 mmol) of glycyl-D,L-phenylalanine was added and the total volume was brought to 20 ml.

(b) **L-Phenylalanylglycine. tren System.**—Procedure A was followed using 0.374 g (1.0 mmol) of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$, 0.222 g (1.0 mmol) of L-phenylalanylglycine, and a total volume of 25 ml.

(c) **L-Phenylalanylglycylglycine.** (1) **tren System.**—Procedure A was followed using 0.374 g (1.0 mmol) of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$, 0.279 g (1.0 mmol), of L-phenylalanylglycylglycine, and a total volume of 25 ml.

(2) **trien System.**—Procedure A was followed using 0.38 g (1.0 mmol) of $[Co(trien)CO_3]ClO_4\cdot H_2O$, 0.279 g (1.0 mmol) of L-phenylalanylglycylglycine, and a total volume of 25 ml.

(d) **Glycylglycyl-L-phenylalanine.** (1) **tren System.**—Procedure A was followed using 0.374 g (1.0 mmol) of $[Co(tren)CO_3]ClO_4\cdot 0.5H_2O$, 0.279 g (1.0) of glycylglycyl-L-phenylalanine, and a total volume of 30 ml.

(2) **trien System.**—Procedure A was followed using 0.38 g (1.0 mmol) of $[Co(trien)CO_3]ClO_4\cdot H_2O$, 0.28 g (1.0 mmol) of glycylglycyl-L-phenylalanine, and a total volume of 30 ml.

Procedure B.—Perchloric acid (5 ml, 1 *N*) was added to 1.667 mmol of carbonato complex (0.623 g of $[Co(tren)CO_3]ClO_4\cdot H_2O$ or 0.638 g of $[Co(trien)CO_3]ClO_4\cdot H_2O$) dissolved in about 20 ml of water in a 50-ml volumetric flask. The solution was warmed to ensure complete reaction and carbon dioxide was flushed out with a stream of nitrogen. The pH was adjusted to 7.5 with an aqueous solution of sodium hydroxide. Water was added to bring the volume to the mark to give 0.033 *M* solutions.

A buffer solution of pH 7.5 was prepared by adding 60% perchloric acid to a solution of 1.2 g of 2,4,6-collidine in 75 ml of water until pH 7.5 was obtained. The volume was brought to 100 ml.

A 0.033 *M* solution of peptide was prepared by dissolving the appropriate amount of the peptide in water and diluting to the mark in a 10-ml volumetric flask.

The cleavage reaction was carried out pipetting 3 ml of a 0.033 *M* cobalt complex solution, 3 ml of a 0.033 *M* peptide solution, and 4 ml of the buffer solution into a 10-ml volumetric flask and then heating the mixture at 60 ± 1° in a water bath. Small portions were withdrawn and spotted on silica gel coated plates (Eastman chromatographic sheet) at convenient time intervals. It was found necessary to spot several times in order to obtain a concentration of the cleaved species that could be easily detected. The plates were eluted with 1-propanol–water (70:30; v/v), and spots detected by spraying with ninhydrin and heating at 100° for 5–10 min.

Peptides studied using procedure B are given below along with variations and amounts of peptide used in each case. The results are given in Table II.

(a) **L-Phenylalanylglycine.**—L-Phenylalanylglycine monohydrate (0.099 g, 0.412 mmol) was dissolved in water and diluted to the mark in a 10-ml volumetric flask to give a 0.0412 *M* solution.

(1) **tren System.**—Procedure B was followed using 3 ml of 0.033 *M* $trenCo^{III}$ species, 3 ml of 0.0412 *M* L-phenylalanylglycine, and 4 ml of 2,4,6-collidine buffer.

(2) **trien System.**—Procedure B was followed using 3 ml of 0.033 *M* $trienCo^{III}$ species, 3 ml of 0.0412 *M* L-phenylalanylglycine, and 4 ml of 2,4,6-collidine buffer.

(b) **L-Phenylalanylglycylglycine.**—L-Phenylalanylglycylglycine (0.093 g, 0.33 mmol) was dissolved in 5 ml of 2,4,6-collidine buffer and diluted to the mark in a 10-ml volumetric flask with distilled water to give a 0.033 *M* solution.

trien System.—Procedure B was followed using 5 ml of 0.033 *M* $trienCo^{III}$ species and 5 ml of 0.033 *M* L-phenylalanylglycylglycine.

(c) **Glycyl-L-phenylalanylglycine.**—Glycyl-L-phenylalanylglycine (0.093 g, 0.33 mmol) was dissolved in 4 ml of 2,4,6-collidine buffer and diluted to the mark in a 10-ml volumetric flask with water to give a 0.033 *M* solution.

(1) **tren System.**—Procedure B was followed using 3 ml of 0.033 *M* $trenCo^{III}$ species, 3 ml of 0.033 *M* glycyl-L-phenylalanylglycine, and 4 ml of 2,4,6-collidine buffer. It was difficult to detect the appearance of L-phenylalanylglycine because of the very similar R_f of the glycyl-L-phenylalanylglycine.

(2) **trien System.**—Procedure B was followed using 3 ml of 0.033 *M* $trienCo^{III}$ species, 3 ml of 0.033 *M* glycyl-L-phenylalanylglycine, and 4 ml of 2,4,6-collidine buffer. Again it was difficult to detect the appearance of the dipeptide in the presence of tripeptide due to their similar R_f values.

(d) **Glycylglycyl-L-phenylalanine.**—Glycylglycyl-L-phenylalanine (0.93 g, 0.33 mmol) was dissolved in water and diluted

to the mark of a 10-ml volumetric flask with water to give a 0.033 *M* solution.

(1) **tren System.**—Procedure B was followed using 3 ml of 0.033 *M* trenCo^{III} species, 3 ml of 0.033 *M* glycylglycyl-L-phenylalanine, and 4 ml of 2,4,6-collidine buffer.

(2) **trien System.**—Procedure B was followed using 3 ml of 0.033 *M* trienCo^{III} species, 3 ml of glycylglycyl-L-phenylalanine, and 4 ml of 2,4,6-collidine buffer.

(3) **Blank.**—Procedure B was followed using 3 ml of 0.033 *M* glycylglycyl-L-phenylalanine and 3 ml of 2,4,6-collidine buffer and diluting to the mark in a 10-ml volumetric flask with water.

Reaction of Co(tren)Cl_2^+ with Glycylglycine.—Glycylglycine (300 mg) and [Co(tren)Cl_2]Cl (660 mg) were dissolved in 2 ml of hot water. After the solution turned pink, excess $NaClO_4$ was added and the whole mixture was allowed to stand at room temperature to crystallize the violet product. Addition of dilute $HClO_4$ to the filtrate increased the yield to a total of 500 mg. The analytical sample was recrystallized from water. *Anal.* Calcd for [Co($C_6H_{18}N_4$)($C_4H_8N_2O_2$)Cl](ClO_4)$_2$·H_2O: C, 20.42; H, 4.80; N, 14.13. Found: C, 20.18; H, 4.87; N, 14.14. The visible spectrum shows λ_{max} 503 nm (ϵ 102) and 358 nm (ϵ 93). The nmr spectrum contains CH_2 singlet (4.2 ppm), CH_2 singlet (4.0 ppm), and 12 H of the tren CH_2 as a broad multiplet. The infrared spectrum shows the amide carbonyl at 1695 cm^{-1} and the carboxylate at 1600 (b) and 1390 cm^{-1}.

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CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ILLINOIS AT CHICAGO CIRCLE, CHICAGO, ILLINOIS

Isomers of the Bis(L-2,4-diaminobutyrato)cobalt(III) Ion and Conformation of the Chelate Rings

BY WADE A. FREEMAN AND CHUI FAN LIU

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The three optically active geometric isomers of the bis(L-2,4-diaminobutyrato)cobalt(III) ion were prepared, separated by ion-exchange chromatography, and identified. The identification of the three geometries is based upon the elution order of the complexes, the pmr spectra, the visible–ultraviolet spectra, the circular dichroism spectra, and comparison of these properties with those of an analogous series consisting of the three isomers of the bis(L-2,3-diaminopropionato)cobalt(III) ion. Pmr evidence indicates that the six-membered nitrogen–cobalt–nitrogen chelate rings in the bis(2,4-diaminobutyrato)cobalt(III) complexes are in rapid equilibrium between boat and chair conformations at room temperature. The effect of the flexible and flexing chelate ring system on the circular dichroism spectra of the complexes is discussed.

Introduction

In an effort to learn more about the contribution of ligand conformations to the circular dichroism of the d–d transitions in optically active metal ion complexes, the three possible geometrical isomers of bis(L-2,4-diaminobutyrato)cobalt(III) have been prepared and identified. These isomers are analogous to the three isomers of the bis(L-2,3-diaminopropionato)cobalt(III) ion which were prepared and identified in a previous report.[1,2] The bis(L-2,4-diaminobutyrato)cobalt(III) ion isomers, however, each contain two six-membered nitrogen–cobalt–nitrogen chelate rings. These rings are nonrigid and can assume either boat or chair conformations. This is in contrast to the case of the bis(L-2,3-diaminopropionato)cobalt(III) ions where the chelate ring systems are entirely rigid. It is this contrast that makes these new compounds of interest since identification of all the isomers of both series lends insight into the effects of ligand conformations on circular dichroism and other spectra. Like L-2,3-diaminopropionic acid, L-2,4-diaminobutyric acid is constrained to form facial isomers only when coordinated about cobalt(III). If, however, *rac*-2,4-diaminobutyric acid is coordinated to cobalt(III), five geometrical bis isomers are possible since the two ligands in the complex then may have opposite absolute configurations. These considerations have been explored for the 2,3-diaminopropionic case in an earlier paper.[1]

(1) W. A. Freeman and C. F. Liu, *Inorg. Chem.*, **7**, 764 (1968).
(2) C. F. Liu and J. A. Ibers, *ibid.*, **8**, 1911 (1969).

Experimental Section

Reagents.—Commercial reagent grade chemicals were used throughout, except where otherwise indicated.

Ligand.—L-2,4-Diaminobutyric acid–dihydrochloride was purchased from Mann Research Laboratories, Inc., New York, N. Y. This compound was listed as homogeneous upon paper chromatography. *Anal.* Calcd for $C_4H_{10}N_2O_2$·2HCl: N, 14.66. Found: N, 14.7. $[\alpha]_D$ 14.3°. It was used as received.

Preparation of Complexes.—The mixture of the isomeric cobalt(III) complexes was prepared by the slow addition of a stoichiometric amount of freshly prepared sodium tris(carbonato)-cobaltate(III) trihydrate to 5.00 g of 2,4-diaminobutyric acid–dihydrochloride dissolved in 20 ml of warm water. This solution also contained a suspension of about 0.5 g of activated charcoal. The mixture was heated on the steam bath for 4 hr after the completion of the addition of the sodium tris(carbonato)cobaltate-(III) trihydrate. It was then filtered hot from the charcoal and the charcoal was carefully washed with hot water. The washings and filtrate were combined and, after cooling, were immediately loaded on the ion-exchange column.

Separation of Isomers.—Separation was effected on a 5.0 ×