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Cleavage of Peptides and Peptide Esters with *cis*-β-Hydroxoaquo(triethylenetetramine)cobalt(III) Ion[1]

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Received September 12, 1973 AIC30668L

cis-β-Hydroxoaquo(triethylenetetramine)cobalt(III) ion at pH 8.0 and 25° has been shown to be effective in promoting the hydrolysis of amino acid esters, dipeptides, and dipeptide esters. Both the condensation reaction and subsequent peptide bond hydrolysis are stoichiometric and specific for the N-terminal residue as indicated by isolation of the β-[Co(trien)(dipeptide ester)]$(ClO_4)_3$ and β-[Co(trien)(dipeptide)]$(ClO_4)_2$ condensation products and the β-[Co(trien)(amino acid)]$(ClO_4)_2$ hydrolysis products and by quantitative amino acid analysis following displacement of the amino acid from the complex by cyanide ion. The amino acid complexes β-[Co(trien)$(NH_2CHR'COR)](ClO_4)_y$ (where y is 2 or 3, trien is triethylenetetramine, R' is a basic, acidic, or neutral amino acid side chain or a modified basic or acidic side chain, and R is one of the groups O, NHCHR'COO, $NHCHR'COOCH_3$, $NHCHR'COOC_2H_5$, or $NHCHR'COOC_3H_7$), have been prepared independently from the free amino acids, dipeptides, and dipeptide esters. The results show that quantitative total hydrolysis of peptides and proteins using cobalt(III)-activated intermediates is a feasible procedure.

Introduction

We have been examining the usefulness of cobalt(III) complexes in the metal ion promoted hydrolysis of peptides, peptide esters, and proteins. The compounds *cis*-$[Co(N)_4(OH)(OH_2)]^{2+}$ (where $(N)_4$ represents the nitrogen donor atoms in either bidentate or tetradentate ligands) have been shown capable of promoting the hydrolysis of peptide bonds in aqueous solution, using relatively mild conditions of temperature and pH.[3-7] This resulted in the hydrolysis of the N-terminal amino acid residue which appeared chelated in the coordination sphere of cobalt(III). (See Figure 1.)

Tetradentate ligands have been found most useful, since these leave two cis sites available for interaction with the α-amino and carboxyl functional groups of the N-terminal residue of peptides and avoid complications from exchange reactions involving the amine ligands.[7]

An initial investigation of the facility with which the species *cis*-β-$[Co(trien)(OH)(OH_2)]^{2+}$, *cis*-α-$[Co(trien)(OH)(OH_2)]^{2+}$, $[Co(en)_2(OH)(OH_2)]^{2+}$, *cis*-$[Co(tren)(OH)(OH_2)]^{2+}$, and $[Co(NH_3)_4(OH)(OH_2)]^{2+}$ hydrolyze glycyl dipeptides and dipeptide esters has shown that the *cis*-β-$[Co(trien)(OH)(OH_2)]^{2+}$ species promotes hydrolysis at at least 50-fold the rate of any of the other species.[8] For this reason, the trien species has been further investigated to determine its usefulness in peptide sequencing.

It has been suggested[9] that interaction of the hydroxoaquocobalt(III) ion with potentially coordinating side chain functional groups, such as those of arginine, histidine, cysteine, and aspartic acid, might interfere with the application of the method to peptide sequence determination.

The present project had three goals: to prepare and characterize the β-$[Co(trien)(aao)]^{2+}$ complexes, to isolate and characterize the intermediate condensation products following condensation of dipeptides and dipeptide esters with the β-$[Co(trien)(OH)(OH_2)]^{2+}$ ion, and to establish the quantitative nature of the reactions

$$\beta\text{-}[Co(trien)(OH)(OH_2)]^{2+} + \text{dipeptide} \rightarrow \beta\text{-}[Co(trien)(\text{dipeptide})]^{2+} + 2H_2O$$

$$\beta\text{-}[Co(trien)(\text{dipeptide})]^{2+} \xrightarrow{OH^-} \beta\text{-}[Co(trien)(aao_1)]^{2+} + aao_2$$

$$\beta\text{-}[Co(trien)(aao_1)]^{2+} \xrightarrow{CN^-} [Co(trien)(CN)_2]^{+} + aao_1$$

Results

Amino Acids. Reaction of the β-$[Co(trien)(OH)(OH_2)]^{2+}$ ion with amino acids at pH 8.0 and 50° afforded the corresponding amino acid complexes essentially quantitatively. As the reaction proceeds, the color of the solution changes from purple to orange. The products were isolated and characterized as their perchlorate or iodide salts. Yields of pure isolated amino acid complexes varied from 25 to 80%. Because of their greater solubility, the complexes of those amino acids having exposed basic or acidic side chain functional groups were more difficult to obtain crystalline and were recovered in lower yield. The amino acid complexes have been prepared for all of the protein amino acids with the exception of cysteine. Cysteine reacted with $[Co(trien)(OH)(OH_2)]^{2+}$ at pH 8.0 to give a mixture of seven products when examined on Dowex 50W-X4 (eluent 0.5 *M* NaOAc, pH 8.0). Only about 3-5% of the products had the characteristic visible absorption spectra of the β-$[Co(trien)(aao)]^{2+}$ complexes. The remaining brown and black products have not been characterized.

When the sulfur was modified (cysteic acid) or protected (*S*-methyl, *S*-benzyl), chelation occurred rapidly. Complexes of threonine, aspartic acid, asparagine, glutamic acid, and glutamine were recrystallized from a solution of water-sodium perchlorate, methanol-$LiClO_4$, or methanol-LiI. Aspartic acid, asparagine, glutamic acid, and glutamine were purified by ion-exchange chromatography (H^+ form Dowex 50W-X4) before crystallization. All compounds were finally recrystallized from water or 50% water-methanol. The absence of remaining starting materials and impurities was monitored by ion-exchange chromatography of a sample of the amino acid complex on Dowex 50W-X2 resin (H^+ form)

(1) The following abbreviations are used throughout the paper: trien, triethylenetetramine; tren, 2,2',2''-triaminotriethylamine; en, ethylenediamine; aao, amino acid anion. The common symbols for the amino acids are used. The α and β nomenclature for trien refers to the geometric arrangement about the metal atom: G. H. Searle and A. M. Sargeson, *Inorg. Chem.*, 4, 45 (1965). Subscripts 1 and 2 are used to distinguish between the two nonequivalent positions in the β structure.

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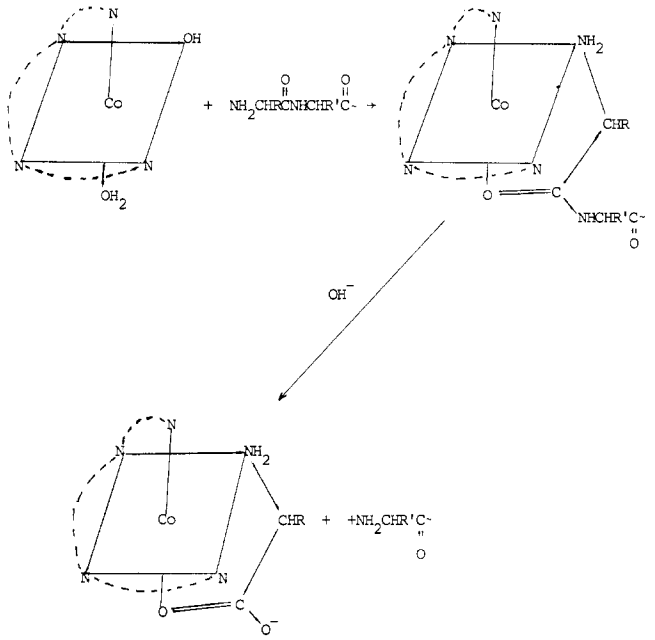
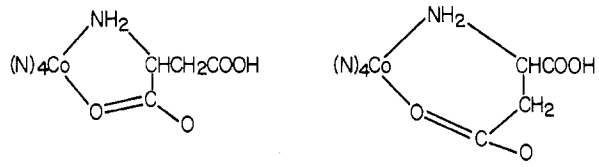


Figure 1.

using sodium perchlorate (1.0 *M*) as eluent. When impurities were detected chromatographically, the complexes were again recrystallized. The visible absorption spectra of the complexes show two symmetrical bands at about 480 and 350 nm. Tyrosine and tryptophan complexes have higher intensities than the other amino acid complexes at 350 nm. This is consistent with parent amino acid absorbances.[10] The position of these bands does not vary appreciably, suggesting a similar octahedral ligand field throughout the series of complexes. The positions and intensities of these bands are similar to those of related octahedral complexes, especially $[Co(en)_2(aao)]^{2+}$ [11] and $[Co(tren)(aao)]^{2+}$.[7] The ir spectra of the β-$[Co(trien)(aao)]^{2+}$ complexes show strong absorptions at 1645 and 1380 cm^{-1} assigned to the symmetric and asymmetric stretching modes of coordinated carboxylates. The β-$[Co(trien)(aao)](ClO_4)_2$ complexes have been assigned the β_2 configuration on the basis of the preparative method used and by comparison of their physical properties (*e.g.*, extinction coefficients, pmr, CD measurements, optical rotation) with those of the authentic glycinato and prolinato complexes.[12-14]

The formation of cobalt(III) complexes with amino acids having side chain functional groups, other than by bidentate α-NH_2, α-COOH chelation, has been investigated. Following the preparation of the N-O bonded species, with the exception of aspartic acid and cysteine, no other amino acid containing species could be observed.

This indicated that only α-NH_2, α-COOH chelates were produced. The reaction of the β-$[Co(trien)(OH)(OH_2)]^{2+}$ species with aspartic acid at pH 8.0 gave a mixture of two N-O bonded orange bidentate products, suggesting that both the five- and six-membered ring complexes had been formed, *i.e.*



The cobalt(III) complex of β-alanine containing the six-membered bidentate complex has been prepared and characterized.

Modified Amino Acids. The cobalt(III) complexes of the side chain protected amino acids L-aspartic β-benzyl ester, *O*-benzyl-L-tyrosine, *N*-ϵ-carbobenzoxy-L-lysine, nitro-L-arginine, *im*-tosyl-L-histidine, *O*-benzyl-L-serine, L-glutamic γ-benzyl ester, *S*-methyl-L-cysteine, *S*-benzyl-L-cysteine, and L-cysteic acid have been prepared by the method above. The complexes of *S*-benzyl-L-cysteine, L-cysteic acid, and *O*-benzyl-L-tyrosine were recovered crystalline. The other complexes were purified by column chromatography using Dowex 50W-X2 (Na^+ form) and elution with LiCl (1.0 *M*, pH 8.0). The complexes were recovered by precipitation with acetone–ethanol (50:50). The complexes were characterized by visible spectra, by pmr, and by amino acid analysis. For amino acid analysis the modified (amino acido)-cobalt(III) complexes were treated with sodium cyanide, and the substituted amino acids identified by their elution characteristics on Beckman AA-15 or PA-35 cation-exchange spherical-bead resins.

α-Amino-Protected Amino Acids. The complex β_2-[Co(trien)(*N*-methylglycinato)]$(ClO_4)_2$ has been prepared and characterized previously.[12] The preparation of the analogous *N*-dimethylglycine has been unsuccessful.

A selection of N-formylated, N-acetylated, and N-benzylated amino acids (*N*-formyl-L-methionine, *N*-acetyl-L-tyrosine ethyl ester, *N*-acetyl-L-leucine, *N*-acetylglycine, *N*-benzyl-L-histidine) were allowed to react with β-$[Co(trien)(OH)(OH_2)]^{2+}$ using the reaction conditions outlined for the preparation of the amino acid complexes. With the exception of *N*-formylmethionine, which gives partial loss of the *N*-formyl group, no complexes of N-acylated amino acid were formed.

In an experiment attempting the preparation of (amino acido)(trien)Co^{III} complexes, using 18-α-*N*-butyloxyamino acid derivatives, no N-O bonded cobalt(III) complexes could be prepared (in each case the α-*N*-*tert*-butyloxyamino acid compounds were initially tested with ninhydrin to ascertain the absence of free amino acid contaminants).

Amino Acid Esters. The reaction of the β-$[Co(trien)(OH)(OH_2)]^{2+}$ ion with amino acid esters $NH_2CHRCOOR'$ (where R = H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, $CH_2C_3N_2H_4$ ($C_3N_2H_4$ = 4-imidazolyl), $(CH_2)_2SCH_3$, $(CH_2)_2COOH$, or CH_2CONH_2 and R' = CH_3, C_2H_5, or C_3H_7) occurs rapidly at 50° and pH 8.0 to yield the β-$[Co(trien)(NH_2CHRCOO)]^{2+}$ complexes. In all reactions, the final amino acid complexes were formed essentially quantitatively. In each case the $[Co(trien)(aao)]^{2+}$ product was isolated as its perchlorate salt following purification with Dowex 50W-X2 (eluent 1.0 *M* HCl) and identified by elemental analysis, visible spectra, pmr, and chromatographic properties.

Peptide Esters. β-$[Co(trien)(OH)(OH_2)]^{2+}$ reacts rapidly with glycylglycine, glycyl-L-leucine, glycyl-L-phenylalanine, glycyl-L-alanine, L-alanylglycine, L-phenylalanylglycine, and L-leucylglycyl-L-phenylalanine methyl, ethyl, or isopropyl esters at pH 8.0 and 40° to give the corresponding $[Co(trien)(peptideOR)]^{3+}$ complexes: β-$[Co(trien)(OH)(OH_2)]^{2+}$ + peptideOR → $[Co(trien)(peptideOR)]^{3+}$ + $2H_2O$.

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These may be isolated crystalline and have been characterized by elemental analysis and pmr. The complexes containing alanine and phenylalanine are extremely soluble in water and were recovered from water-methanol or water-ethanol with $LiClO_4$.

Peptides. The complexes β_2-[Co(trien)(peptido)]$(ClO_4)_2$ have been prepared by acidic ester hydrolysis of the corresponding dipeptide ester complexes.

Stability to Base Hydrolysis of the β_2-[Co(trien)(aao)]$^{2+}$ Species. [Co(trien)(aao)]$^{2+}$ ions decompose in conditions of high alkalinity to give a variety of products. Some of these have been identified as $[Co_2(trien)_2(OH)_2]^{4+}$ and [Co(trien)-$(OH)_2]^+$, while about 30% (pH 13.0) appears as a brown polymeric material of unknown composition. Base-induced decomposition of a variety of cobalt(III)-amino acid complexes (including those having acidic and basic functional groups) at pH 10-12 has been investigated by chromatography on Dowex 50W-X4. The breakdown of the complexes was measured visually by the appearance of other products on elution. All compounds were analyzed for their chromatographic purity before hydrolysis. The results showed that the complexes are stable at pH 11.0 over several hours, but at pH 12.0 other products appear after 60 min (*e.g.*, at pH 11.0 and 360 min none of the amino acid complexes showed any decomposition with the exception of L-threonine (~10%); at pH 12.0 and 60 min, L-alanine showed ~5%, L-methionine ~10%, L-threonine ~40%, and glycine ~10%).

Qualitative Hydrolysis of β_2-[Co(trien)(dipeptido)]$(ClO_4)_2$ Complexes. Base-hydrolyzed samples of the β_2-[Co(trien)-(dipeptido)]$(ClO_4)_2$ complexes in which the N-terminal amino acid was glycine and the C-terminal residue was glycine, alanine, leucine, or phenylalanine were examined by high-voltage electrophoresis and tlc at various temperatures and pH values. For each compound the appropriate amino acid complexes were used as markers. The results showed that hydrolysis of dipeptide complexes, at pH values below 11.0 and temperatures below 45°, gave clear, well-defined products which could be readily separated by the chromatographic systems used. At higher pH values a variety of additional products were produced which have been identified as dipeptides, $[Co(trien)(OH)_2]^+$, and $[Co_2(trien)_2(OH)_2]^{4+}$. A further cobalt(III) species appeared above pH 12.0. This has been tentatively identified as a stable N-N bonded species, presumably arising from an N-O to N-N chelation shift.

Quantitative Hydrolysis of β-[Co(trien)(dipeptide)]$(ClO_4)_2$ Complexes. The alkaline hydrolysis at pH 10.80 of β-[Co(trien)(dipeptido)]$^{2+}$ derivatives of glycylglycine, glycyl-L-alanine, glycyl-DL-leucine, and glycyl-L-phenylalanine was measured by multiple-sampling amino acid analysis. The rate and quantitation of base hydrolysis was measured by the gradual appearance of the C-terminal amino acid. The results are reported in Table I.

Stability of β-[Co(trien)(dipeptide)]$(ClO_4)_2$ Complexes to Alkaline Hydrolysis. β_2-[Co(trien)(aao)]$^{2+}$ ions decompose at pH values above 11.0. The stability of the β-[Co(trien)-(glyX)]$(ClO_4)_2$ complexes (X = gly or ala) in base (NaOH, 0.1 or 0.01 *M*) has been measured quantitatively following recovery of C-terminal glycine or alanine, after hydrolysis for 10 half-lives, by amino acid analysis. Control experiments using the dipeptide were carried out using the same reaction conditions. In the dipeptide experiment, less than 2% of the peptide linkage had been cleaved at pH 13.0.

The dipeptide complex results appear in Table II. The results indicate that a part of the dipeptide was displaced intact from the complex at high pH. The loss of quantitative recovery may be indicative of the formation of stable N-N bonded species. Hydrolysis above pH 11.0 does not give quantitative recovery of the N-terminal amino acid.

Table I. Hydrolysis of β-[Co(trien)(dipeptide)]$^{2+}$ Species at pH 10.8 (μ = 0.1 (ClO_4^-))[a]

Complex	Recovery of C-terminal amino acid, %				
	0	20 min	40 min	60 min	80 min
β-[Co(trien)(glyglyO)]$(ClO_4)_2$	0	64	78	96	
β-[Co(trien)(glyalaO)]$(ClO_4)_2$	0	68	92	100	
β-[Co(trien)(glyleuO)]$(ClO_4)_2$	0	57	77	88	94
β-[Co(trien)(glypheO)]$(ClO_4)_2$	0	80	100		

[a] The results of this table were calculated using ninhydrin values determined under identical conditions (*i.e.*, for glycine 0.5497, 0.5836, 0.5891, 0.5623, 0.5684; for alanine, 0.5629, 0.5578, 0.5663, 0.5491, 0.5544; for leucine, 0.5889, 0.5797, 0.5898, 0.5748; for phenylalanine, 0.5430, 0.5480, 0.5521, 0.5510.

Table II. Stability of β-[Co(trien)(glyX)]$(ClO_4)_2$ to Hydrolysis at pH 10-13 (X = Glycine or Alanine)

pH	Time, min	Amt × 10^8, mol			
		gly	glygly	ala	glyala
10.0	1000	1.04		0.99	
11.0	100	0.97	0.01	0.99	
11.9	10	0.69	0.06	0.78	0.06
12.8	1.0	0.48	0.12	0.50	0.19

Recovery of Amino Acids and Dipeptides Following Displacement from Their Complexes by Sodium Cyanide. Amino acid analysis of solutions of [Co(trien)(aao)]$^{2+}$ species following displacement of the chelated amino acid using NaCN gave a quantitative yield of the amino acid in each case (lys, 1.01; arg, 0.94; phe, 1.00; leu, 1.01; asp, 1.08) (mol × 10^{-8} recovered/mol × 10^{-8} applied). Unbuffered solutions of NaCN gave simultaneous hydrolysis and displacement, from dipeptide complexes resulting in both the amino acid and dipeptide appearing in the products. The use of buffered solution of NaCN (pH 8.0) eliminates this problem, giving quantitative recovery of the dipeptides and no contamination by the C-terminal amino acids. The results are reported in Table III.

Quantitative Hydrolysis and Cyanide Displacement from β-[Co(trien)(dipeptido)]$(ClO_4)_2$. Base hydrolysis and cyanide displacement of amino acids and dipeptides from the cobalt(III)-dipeptide complexes of glycylglycine, glycyl-L-alanine, glycyl-DL-leucine, and glycyl-L-phenylalanine were investigated to ascertain if the sequence

$$[Co(N)_4(\text{dipeptido})]^{2+} \xrightarrow{OH^-} [Co(N)_4(aao_1)]^{2+} + aao_2$$

$[Co(N)_4(\text{dipeptido})]^{2+}$ → analysis 1; $\xrightarrow{CN^-, H^+}$ analysis 2

$[Co(N)_4(aao_1)]^{2+}$ → analysis 3

aao_2 $\xrightarrow{CN^-, H^+}$ analysis 4

could be quantitatively established. The results for amino acid analyses series 1-4 are reported in Tables IV-VII. For analysis 1 (Table IV) the samples were applied at 100-fold the normal sample concentration, to detect the presence of free amino acid impurities in the complexes. These results, together with elemental analyses, and ir and pmr spectra, confirmed the purity of the complexes.

The amount of the peptide bond hydrolysis during the displacement of the dipeptides by acidified NaCN, together with any contamination by the cobalt(III) complexes of the N-terminal amino acids, are shown in Table V. In all cases, displacement by acidified NaCN gave greater than 97% recovery of the dipeptides.

For the quantitation of the base hydrolysis of β_2-[Co(trien)-(dipeptido)]$^{2+}$ species the products following hydrolysis by

Table III. Quantitative Displacement of β-[Co(trien)(dipeptide)]$^{2+}$ Complexes

Complex	% dipeptide recovered[a]
β-[Co(trien)(glygly)]$(ClO_4)_2$	98.7
β-[Co(trien)(glyala)]$(ClO_4)_2$	99.3
β-[Co(trien)(glyleu)]$(ClO_4)_2$	99.8
β-[Co(trien)(glyphe)]$(ClO_4)_2$	98.9

[a] Amino acid contamination by the C-terminal residue of the dipeptide was less than 2% in each case.

Table IV. Quantitative Hydrolysis and Cyanide Displacement of Amino Acids and Dipeptides from β-[Co(trien)(dipeptide)]$(ClO_4)_2$ (Purity of β-[Co(trien)(dipeptide)]$^{2+}$)

Dipeptide complex	Amino acid impurities,[a] %				
	gly	ala	leu	phe	Others (%)
glygly	0.36	0.04			asp (0.37), glu (0.21)
gly-L-ala	0.61	0.74			asp (0.30)
gly-DL-leu	0.48	0.27	0.47		asp (0.43), glu (0.37)
gly-L-phe	0.66	0.31		0.91	asp (0.36), glu (0.26)
glygly[b]	0.69	0.24			asp (0.47), glu (0.41)

[a] No allowance has been made for amino acid impurities in the dilution buffer or the pH 3.25 elution buffer. [b] The ethylenediamine complex was included for comparison with the triethylenetetramine complexes.

Table V. Displacement of Dipeptides from β-[Co(trien)(dipeptide)]$(ClO_4)_2$

Complex	Dipeptide recovery, %	Amino acid impurities (%)[a]
β-[Co(trien)(glygly)]$(ClO_4)_2$	98.3	gly (2.1)
β-[Co(trien)(glyala)]$(ClO_4)_2$	98.7	gly (0.7), ala (0.8)
β-[Co(trien)(glyleu)]$(ClO_4)_2$	101.3	gly (0.4), leu (0.6)
β-[Co(trien)(glyphe)]$(ClO_4)_2$	99.4	gly (1.3), phe (0.7)
β-[Co(en)$_2$(glygly)]$(ClO_4)_2$	97.8	gly (2.3)

[a] Corrected for the amount of these amino acids in the dilution buffer.

Table VI. Base Hydrolysis of β-[Co(trien)(dipeptide)]$(ClO_4)_2$ (pH 10.8)

Complex	C-Terminal amino acid		gly impurity $\times 10^8$, mol
	Amt $\times 10^8$, mol	% recovery	
β-[Co(trien)(glygly)]$^{2+}$	1.08	99.7	
β-[Co(trien)(glyala)]$^{2+}$	0.94	100.4	0.040
β-[Co(trien)(glyleu)]$^{2+}$	1.06	99.2	0.061
β-[Co(trien)(glyphe)]$^{2+}$	1.01	102.1	0.037
[Co(en)$_2$(glygly)]$^{2+}$	0.96	98.9	

Table VII. Cyanide Displacement Following Base Hydrolysis of β-[Co(trien)(dipeptide)]$(ClO_4)_2$[a]

Complex	Amino acid recovery $\times 10^8$, mol	
	N-Terminal	C-Terminal
β-[Co(trien)(glygly)]$^{2+}$	1.07	1.04
β-[Co(trien)(glyala)]$^{2+}$	0.98	0.96
β-[Co(trien)(glyleu)]$^{2+}$	0.98	1.00
β-[Co(trien)(glyphe)]$^{2+}$	1.03	1.02
[Co(en)$_2$(glygly)]$^{2+}$	1.00	0.99

[a] Norleucine (1×10^{-8} mol) was added as an internal reference for all analyses. The values shown have been corrected to the ninhydrin value of norleucine 0.5430.

potentiometric titration were analyzed for their amino acid composition. The quantitative recovery of the C-terminal amino acid was used as a measure of quantitation of base hydrolysis, *i.e.*

$$\beta_2\text{-[Co(trien)(dipeptido)]} \xrightarrow{OH^-} \beta_2\text{-[Co(trien)(aao}_1\text{)]} + \text{aao}_2$$

The results are reported in Table VI.

The quantitative recovery of the N-terminal amino acid was measured following cyanide displacement of the amino acids from the base-hydrolyzed samples. The results appear in Table VII. In all cases, the ratio of N-terminal amino acid to C-terminal amino acid was 1.0 within the limit of experimental error.

Discussion

The β_2-[Co(trien)(aao)]$^{2+}$ species are the alkaline hydrolysis (pH 8–11) products of N-terminally chelated peptides formed by the condensation of the *cis*-β-hydroxoaquo(triethylenetetramine)cobalt(III) ion with peptides and peptide esters. The β_2-[Co(trien)(aao)]$^{2+}$ species were prepared and characterized so as to provide convenient markers for the chromatographic identification of products formed in the more complex ester and dipeptide reactions. The (amino acido)(triethylenetetramine)cobalt(III) complexes of some of the nonpolar amino acids have been prepared previously.[5,15-17] The complexes of this present work were prepared by a modification of the method of Collman, *et al.*[5] Column and paper chromatography proved an easy and rapid method for identification of these ions and the different amino acid complexes could be easily separated from one another. The complex species have a similar R_f ratio to that of the parent amino acids, and since the complexes are highly colored, semiquantitative spectrophotometric estimations of the eluted amino acid complex bands is possible.

Three geometric possibilities exist for the complexes: two have the unsymmetrical β configuration of the triethylenetetramine chelate ring and the other has the symmetrical α structure. Both the method of preparation and the infrared and proton magnetic resonance spectra suggest that the present complexes retain the β configuration by comparison with the glycinato and prolinato complexes studied earlier.[12,13] The infrared spectra of all of the chelated amino acid complexes show bands characteristic of coordinated amino acids.[18,19] Furthermore, all of the complexes show at least four strong absorptions in the 990–1000-cm^{-1} region attributable to the β-trien configuration. (Only two major absorptions might be expected for the α-trien structure.)

It has been suggested[9] that interaction of the β-[Co(trien)(OH)(OH$_2$)]$^{2+}$ ions with potentially ligating side chain functional groups on either the N-terminal amino acid or the penultimate amino acid might interfere with the application of the method to hydrolysis of peptides containing these residues (*e.g.*, arginine, cysteine, histidine, aspartic acid). Ion-exchange chromatography of the products following condensation of amino acids at amino acid esters with β-[Co(trien)(OH)(OH$_2$)]$^{2+}$ show that complications occur only with cysteine and aspartic acid. For cysteine a variety of products are present, none of which have the characteristic N-O visible spectrum of bidentate amino acid complexes. The condensation reaction of β-[Co(trien)(OH)(OH$_2$)]$^{2+}$ with aspartic acid gives rise to both five- and six-membered N-O bonded chelates. The amount of the six-membered species increases with increasing temperature. Alternative hydrolysis procedures have been investigated using cobalt(III) com-

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plexes of diethylenetriamine[20] and these may be essential for peptides containing aspartic acid. The N-terminal specificity of the condensation reaction has been clearly demonstrated by the isolation of the intermediate β-[Co(trien)(dipeptido)]$^{2+}$ species having properties similar to the known [Co(trien)($NH_2CH_2CONHCH_2COOC_2H_5$)]$(ClO_4)_3$ structure.[21] Furthermore, when equimolar concentrations of β_2-[Co(trien)(OH)(OH_2)]$^{2+}$ and dipeptide esters are used, the N-terminal amino acid is selectively removed as β_2-[Co(trien)(aao)]$^{2+}$ and only a trace of the C-terminal amino acid complex is formed.

The requirement for a free N-terminal amino group is also demonstrated by the failure of β-[Co(trien)(OH)(OH_2)]$^{2+}$ to form complexes with *N*-formyl-, *N*-acetyl-, *N*-benzyl-, or *N*-*tert*-butyloxyamino acids.

Examination of the hydrolysis of the dipeptide complexes, by amino acid analysis, for recovery of the C-terminal residue, gave different reaction rates with various peptide bonds. At pH values above 11.0, the desired products could not be recovered quantitatively due to the presence of base-catalyzed side reactions. This occurs in part by the displacement of intact peptide from the complex.

Displacement of amino acids and dipeptides from their bidentate complexes gave quantitative recoveries (>98%) following treatment with acidied NaCN. In an experiment designed to examine the compatibility and overall quantitation of the hydrolysis-displacement reaction sequence, recovery of the C-terminal amino acid was >98% in each case. Following cyanide displacement, the measured ratio N-terminus:C-terminus was 1.0 within analytical error.

The results indicate that quantitative peptide sequence analysis by β-[Co(trien)(OH)(OH_2)]$^{2+}$ should be effective for performically oxidized (cysteine) or carboxymethylated (tryptophan) peptides not containing aspartic acid. Examination of the hydrolysis of larger peptides is at present in progress.

Experimental Section

Instrumentation. Visible and infrared spectra were recorded on a Cary Model 14 spectrophotometer (for 5×10^{-3} *M* aqueous solutions) and a Perkin-Elmer 457 infrared spectrophotometer, respectively.

Cobalt estimations were made using a Techtron AA4 atomic absorption spectrophotometer.

Proton magnetic spectra were recorded on Varian HA 100-MHz or Jeol C-60 HL 60-MHz instruments using external tetramethylsilane or internal sodium 3-(trimethylsilyl)-1-propanesulfonate, respectively, as reference standards.

Amino acid determinations were performed with a Beckman Model 120C amino acid analyzer using norleucine as internal reference standard.

The following Radiometer apparatus was used in the measurement of buffer pH and in pH-Stat titrations: TTA3 electrode assembly, ABU 1 autoburet, TTT-1 titrator, SBR2 titrigraph, and pHA scale expander. In the pH-Stat titrations, the titrant (NaOH) was added under a nitrogen atmosphere to the continuously stirred solution in a thermostated cell (25.0 ± 0.2°). For all reactions involving perchlorates, the glass electrode was protected by a sodium nitrate bridge.

Electrophoresis. High-voltage electrophoresis was performed using a Pherograph flat-bed, cool-plate refrigerated electrophoresis apparatus using 3MM Whatman paper and using either formic acid-acetic acid-water (25:75:400) or pyridine-acetic acid-water (1:2:100) as electrolyte buffers.

Chromatography. For column chromatography the reaction products were separated with either Bio-Rad analytical Dowex 50W 200–400 mesh (cation exchange) or Sephadex (CM-25, SE-25) resins using sodium perchlorate (0.1–1.5 *M*), sodium acetate (0.1–1.0 *M*), or hydrochloric acid (0.1–2.0 *M*) for elution. For thin-layer chromatography with silica gel $11F_{254}$ (Merck), aqueous 2% amino acid complex solutions were spotted on the layers and air-dried. The plates were eluted in the ascending manner in tanks lined with filter paper soaked with the appropriate eluent to aid saturation.

After elution, the spots were detected under ultraviolet light or by spraying with ninhydrin 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.

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, elution with the upper phase 1-butanol-water-glacial acetic acid (5:5:1 v/v); system B, silica gel plate eluted with 1-propanol-water (7:3 v/v); system C, 1-butanol-acetic acid-water-pyridine (100:100:20:2 v/v). For paper chromatography the cobalt-amino acid complexes were eluted in the descending manner using 3MM Whatman paper (57 cm × 23 cm) with formic acid-acetic acid-water (25:75:400) or 1-butanol-acetic acid-water-pyridine (100:100:20:2.2) as the elution buffer.

Materials. Amino acids and peptides were purchased from Mann Research Laboratory Inc. and Cyclo Chemical Co. Wherever possible the compounds were of the natural L configuration and of the highest quality available. All other reagents were of Analar grade and were used throughout without further purification.

Preparation of Complexes. β-*cis*-Carbonato(triethylenetetramine)-cobalt(III) perchlorate hemihydrate was prepared according to the method of Sargeson and Searle.[22] For the present experiments, the products were carefully purified at each step to reduce the possibility of contamination by either amino acids or ammonia.

Preparation of Amino Acid Complexes. β-[Co(trien)CO_3]ClO_4·H_2O (0.05 mol) was converted to [Co(trien)(OH_2)$_2$]$^{3+}$ with 2 equiv of 2.0 *M* $HClO_4$. After the evolution of CO_2 ceased, the solution was heated to 60° for 10 min. On cooling, the pH of the solution was adjusted to about 4 (1.0 *M* NaOH) and the amino acid (0.05 mol) added (total volume 50 ml). The pH of the solution was raised to 8.0 with NaOH. After heating the solution to 50° for 20 min, the solution was quenched to pH 3 with 1.0 *M* $HClO_4$ and allowed to cool. On cooling and addition of $NaClO_4$, some of the solutions gave crystalline products which were filtered off, washed with 20 ml of ice-cold 50% methanol-water, and air-dried. In cases where no solid appeared after the 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 filtration, washed with ethanol and acetone, and recrystallized by dissolving in a minimum of hot aqueous 25% $NaClO_4$ solution. Analytical samples were dried *in vacuo* over P_2O_5 at 100° for 20 hr.

The following are analyses for β-[Co(trien)(amino acid)]$(ClO_4)_2$ complexes (λ^1_{max}, ϵ^1; λ^2_{max}, ϵ^2) (λ in nm and ε in M^{-1} cm^{-1}). *Anal.* Calcd for [Co(trien)(L-ala)]$(ClO_4)_2$·H_2O: C, 21.19; H, 5.14; N, 13.73. Found: C, 20.87; H, 5.07; N, 13.46 (478, 133; 349, 139). Calcd for [Co(trien)(L-arg)]$(ClO_4)_3$·H_2O·$HClO_4$: C, 18.10; H, 4.43; N, 14.07. Found: C, 18.08; H, 4.63; N, 13.46 (478, 156; 349, 118). Calcd for [Co(trien)(L-Asp)]$(ClO_4)_2$·H_2O: C, 21.67; H, 4.73; N, 12.64. Found: C, 21.90; H, 5.02; N, 12.45 (481, 121; 346, 134). Calcd for [Co(trien)(gly)]$(ClO_4)_2$·H_2O: C, 19.33; H, 5.07; N, 14.09. Found: C, 19.67; H, 4.93; N, 13.94 (478, 115; 345, 128). Calcd for [Co(trien)(L-glu)]$(ClO_4)_2$·H_2O: C, 23.25; H, 4.97; N, 12.33. Found: C, 23.29; H, 4.83; N, 12.51 (487, 146; 338, 144). Calcd for [Co(trien)(L-his)]$(ClO_4)_2$·$2H_2O$: C, 24.21; H, 5.25; N, 16.47. Found: C, 24.52; H, 5.41; N, 16.39 (479, 149; 351, 167). Calcd for [Co(trien)(L-ile)]$(ClO_4)_2$·$2H_2O$: C, 25.27; H, 5.66; N, 12.28. Found: C, 25.70; H, 5.67; N, 12.37 (490, 176; 352, 153). Calcd for [Co(trien)(L-leu)]$(ClO_4)_2$·H_2O: C, 26.09; H, 5.48; N, 12.68. Found: C, 25.71; H, 5.57; N, 12.45 (478, 155; 349, 168). Calcd for [Co(trien)(L-lys)]$(ClO_4)_2$·$2H_2O$: C, 24.60; H, 6.03; N, 14.36. Found: C, 24.77; H, 5.58; N, 14.03 (478, 164; 348, 207). Calcd for [Co(trien)(L-met)]$(ClO_4)_2$·H_2O: C, 23.37; H, 5.17; N, 12.39. Found: C, 23.27; H, 5.13; N, 12.43 (491, 164; 348, 135). Calcd for [Co(trien)(L-phe)]$(ClO_4)_2$·H_2O: C, 30.73; H, 5.16; N, 11.95. Found: C, 30.69; H, 5.19; N, 11.99 (478, 148; 345, 169). Calcd for [Co(trien)(L-pro)]$(ClO_4)_2$·H_2O: C, 24.63; H, 5.26; N, 13.06. Found: C, 25.06; H, 5.36; N, 12.38 (486, 153; 352, 173). Calcd for [Co(trien)(sar)]$(ClO_4)_2$·H_2O: C, 21.19; H, 5.14; N, 13.73. Found: C, 21.24; H, 5.21; N, 13.94 (485, 149; 352, 179). Calcd for [Co(trien)(L-ser)]$(ClO_4)_2$·H_2O: C, 20.54; H, 4.98; N, 13.31.

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Found: C, 20.86; H, 4.82; N, 13.67 (478, 124; 349, 141). Calcd for $[Co(trien)(L\text{-thr})](ClO_4)_2$: C, 22.44; H, 5.09; N, 13.09. Found: C, 22.63; H, 5.28; N, 13.19 (482, 137; 351, 164). Calcd for $[Co(trien)(L\text{-tyr})](ClO_4)_2 \cdot H_2O$: C, 29.91; H, 5.02; N, 11.63. Found: C, 29.59; H, 4.86; N, 11.59 (478, 183; 344, 252). Calcd for $[Co(trien)(L\text{-val})](ClO_4)_2 \cdot H_2O$: C, 24.54; H, 5.62; N, 13.01. Found: C, 24.22; H, 5.46; N, 13.00 (479, 153; 349, 160).

Amino Acid Esters. The amino acid ester hydrochloride salts were prepared by the method of Brenner and Huber.[23] The hydrochlorides were converted to the perchlorate salts by twice recrystallizing them from $LiClO_4$–methanol using $AgNO_3$ as indicator. The products were identified by pmr, ir, and elemental analyses. The free esters were prepared by adding the salts to a large excess of ice-cold saturated NaOH solution and extracting the solution three times with ether. The ether extracts were combined, dried over anhydrous K_2CO_3, filtered, and reduced to a small volume.

The amino acido-β-triethylenetetraminecobalt(III) salts were prepared as before by addition of the appropriate ester or ester perchlorate salt.

The β-$[Co(trien)(aao)](ClO_4)_2$ complexes were dissolved in water and their visible spectra compared with the spectra of the authentic materials prepared earlier. The use of ester hydrochloride salts was attempted, but these gave a range of impurities on Dowex 50W, including $[Co(trien)Cl(H_2O)]^{2+}$ and $[Co(trien)Cl_2]^+$.

Modified Amino Acids. The cobalt(III) complexes of the side chain protected amino acids have been prepared by the method above. The complexes of *S*-benzyl-L-cysteine, cysteic acid, and *O*-benzyl-L-tyrosine were recovered crystalline and recrystallized from hot methanol–water (50:50 v/v) by addition of $LiClO_4$. *Anal.* Calcd for $[Co(C_6H_{18}N_4)(C_3H_7NO_3S)](ClO_4)_2 \cdot H_2O$: C, 18.26; H, 4.60; N, 11.89. Found: C, 18.15; H, 4.79; N, 12.03. Calcd for $[Co(C_6H_{18}N_4)(C_{10}H_{13}NO_2S)](ClO_4)_2$: C, 31.22; H, 5.08; N, 11.40. Found: C, 31.86; H, 5.21; N, 11.18. Calcd for $[Co(C_6H_{18}N_4)(C_{16}H_{17}NO_3)](ClO_4)_2 \cdot 0.5H_2O$: C, 38.59; H, 5.30; N, 10.27. Found: C, 37.93; H, 5.44; N, 10.51.

The other complexes were purified by column chromatography (Dowex 50W-X4) and precipitated with acetone–ether (50:50). The complexes were characterized by visible spectra, by pmr spectra, and by amino acid analysis.

Visible Spectra. λ^1_{max} and λ^2_{max} values (nm) for $[Co(trien)(\text{modified amino acid})]^{2+}$ complexes are as follows: *N*-ϵ-carbobenzoxy-L-lysine, 481, 347; nitro-L-arginine, 478, 351; *O*-benzyl-L-tyrosine, 478, 350; *im*-benzyl-L-histidine, 479, 348; L-glutamic γ-benzyl ester, 483, 348; *O*-benzyl-L-serine, 477, 343; *S*-benzyl-L-cysteine, 478, 348; L-aspartic β-benzyl ester, 480, 346.

For amino acid analysis the ninhydrin values and retention times were determined using standard Beckman 120C analyzer conditions but with extended elution. Long column: buffer pH 3.25, 0.2 *M* Na^+, 70 min; buffer pH 4.28; 0.20 *M* Na^+, 120–600 min (the samples contained 1×10^{-8} mol of norleucine). Short column: buffer pH 5.25, 0.35 *M* Na^+, 170 min. (See Table VIII.)

For amino acid analysis an aliquot of each solution was taken and diluted to 1×10^{-8} mol/ml. Two milliliters of this solution was used directly for amino acid analysis. The absence of amino acids confirmed the total reaction of the derivatives with the hydroxoaquo(triethylenetetramine)cobalt(III) species. Solid sodium cyanide (final concentration 0.01 *M*) was added to the cobalt–amino acid complex (2×10^{-8} mol) in a well-ventilated fume hood and the solution warmed to 40° for 4 hr with stirring to liberate the free amino acid from the complex. After titration of the solution to pH 2.2 with 1.0 *M* HCl and removal of the released hydrogen cyanide by freeze-drying, the residue was made up to 2.0 ml (pH 2.71) and the sample analyzed for the side chain protected amino acids using modified 120C analyzer conditions. The results showed that quantitative condensation had occurred and hence provided unequivocal identification of the side chain protected amino acid–cobalt complexes.

Reaction of Hydroxoaquo(triethylenetetramine)cobalt(III) Ion with Peptide Esters. The peptide ester hydrochloride salts were prepared by the method of Brenner and Huber.[23] The hydrochloride salts were converted to the hydroperchlorates as above.

β-$[Co(trien)(\text{dipeptide ester})](ClO_4)_3$ complexes were prepared by addition of the peptide ester hydroperchlorate salt (0.01–0.001 mol) to a solution (2–20 ml) of β-*cis*-$[Co(trien)(OH_2)_2]^{3+}$ and adjusting the pH to 8.0 with 1.0 *M* NaOH. The solutions were heated to about 60° for 120 min. On cooling and addition of sodium perchlorate, orange crystals of the β-$[Co(trien)(\text{dipeptide ester})](ClO_4)_3$ sometimes formed (glycylglycine, glycyl-L-alanine). These products were collected, washed with a small amount of ice-cold water, and air-dried. In most cases the products did not crystallize out and were purified using Sephadex CM-25 (eluent 0.1 *M* $NaClO_4$) or Sephadex G10 (eluent H_2O). The orange 2+ fraction was collected and evaporated to dryness *in vacuo* at 45°. The residue, after evaporation, was taken up in a small volume of methanol–water (90:10) at 80°. Addition of $LiClO_4$ and allowing the solution to cool slowly gave orange crystalline precipitates of the required products. The compounds were identified by ir, pmr, visible extinctions, and elemental analysis.

Table VIII

Compd	Retention time, min: Compd	NH_3	nle	Ninhydrin value: R	nle
N-ϵ-Carbobenzoxy-L-lysine	19.0	36.0		0.5208	
Nitro-L-arginine	116.5		136.0	0.5589	0.5241
O-Benzyl-L-tyrosine	65.3	34.7		0.9132	
im-Benzyl-L-histidine	163.0	34.5		0.4148	
L-Glutamic γ-benzyl ester	233.5		136.4	0.5041	0.5268
O-Benzyl-L-serine	141.5		135.7	0.5624	0.5194
L-Aspartic β-benzyl ester	182.0		134.5	0.1150	0.5336
S-Benzyl-L-cysteine	194.5		134.2	0.5352	0.5185

The following are analyses for β-$[Co(trien)(\text{dipeptide ester})](ClO_4)_3$ complexes (λ^1_{max}, ϵ^1; λ^2_{max}, ϵ^2) (λ in nm and ϵ in M^{-1} cm^{-1}). *Anal.* Calcd for glycylglycine methyl ester: C, 19.79; H, 4.53; N, 12.59. Found: C, 19.62; H, 4.61; N, 12.47 (478, 134; 347, 149). Calcd for glycylglycine ethyl ester: C, 21.14; H, 4.73; N, 12.33. Found: C, 20.70; H, 4.51; N, 11.95 (478, 135; 347, 152). Calcd for glycylglycine isopropyl ester: C, 23.03; H, 4.67; N, 12.40. Found: C, 22.65; H, 4.50; N, 11.96 (480, 143; 347, 158).

Acidic Hydrolysis of β-$[Co(trien)(\text{dipeptide ester})](ClO_4)_2$ Complexes. To each of the dipeptide ester complexes was added 50 ml of 6.0 *M* HCl and the samples were rotary evaporated at 50° to dryness. This procedure was repeated twice. The product was taken up in a minimum volume of hot water and reduced in volume until crystallization commenced. The samples were cooled in an ice bath, filtered, washed with a minimum volume of ice-cold methanol–water (90:10), and air-dried. The following are analyses for β-$[Co(trien)(\text{dipeptide})](ClO_4)_2$ complexes (λ^1_{max}, ϵ^1; λ^2_{max}, ϵ^2) (λ in nm and ϵ in M^{-1} cm^{-1}). *Anal.* Calcd for glycylglycine: C, 22.44; H, 4.71; N, 15.70. Found: C, 22.35; H, 4.68; N, 15.57 (478, 141; 347, 153). Calcd for glycylalanine: C, 23.21; H, 5.49; N, 14.76. Found: C, 22.51; H, 5.76; N, 13.73 (482, 139; 350, 161). Calcd for glycylleucine: C, 28.44; H, 5.62; N, 14.22. Found: C, 28.91; H, 5.40; N, 13.72 (476, 151; 348, 163). Calcd for glycylphenylalanine: C, 32.65; H, 5.00; N, 13.44. Found: C, 32.36; H, 5.17; N, 13.19 (478, 134; 348, 152).

Hydrolysis of β_2-$[Co(trien)(glyglyOR)](ClO_4)_2$ with Hydroxide Ion. β_2-$[Co(trien)(glyglyOR)](ClO_4)_3$ was dissolved in a minimum volume of hot water (10 ml). To the pink solution, originally at acidic pH, was added 0.1 M NaOH dropwise to adjust the pH to about 8, whereupon the solution had become orange. Orange crystals precipitated following the addition of $NaClO_4$ and cooling at 0°. The infrared and visible spectra are identical with those of β_2-$[Co(trien)(gly)](ClO_4)_2$.

Stability to Base Hydrolysis of the β_2-$[Co(trien)]^{2+}$ Species. Samples of each of the β_2-(amino acido)(triethylenetetramine)cobalt(III) complexes (15 mg) were taken up in water (5 ml) and their pH's adjusted by potentiometric titration to pH 10, 11, and 12. After the required time intervals had elapsed the samples were quenched to pH 8.0 with dilute HCl. The samples were sorbed into Dowex 5W-X2 and eluted with 1.0 *M* sodium perchlorate. The breakdown of complexes was measured by the visual appearance of other products on elution. All compounds were analyzed for their chromatographic purity before hydrolysis.

Quantitative Hydrolysis of β-$[Co(trien)(\text{dipeptide})](ClO_4)_2$ Complexes. The alkaline hydrolysis at pH 10.80 of β-$[Co(trien)(\text{dipeptide})]^{2+}$ derivatives of glycylglycine, glycyl-DL-leucine, and glycyl-L-phenylalanine was measured by multiple-sampling amino acid analysis. For multiple-sampling analysis, aliquots of the complexes at pH 10.8 (1×10^{-7} mol/ml) were taken, quenched to pH 2 with HCl (0.5 *M*), and made up to volume with pH 2.2 dilution buffer. The samples (1×10^{-8} mol) were applied to the appropriate amino acid analyzer column at 10-min intervals, and the concentration of amino acid present was determined by comparison with a series of standard amino acids applied at known concentrations and in an identical manner.

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Stability of β-[Co(trien)(dipeptide)](ClO$_4$)$_2$ Complexes to Alkaline Hydrolysis. Samples of each of the β-[Co(trien)(dipeptide)](ClO$_4$)$_2$ complexes (15 mg) were taken up in water (5 ml) and their pH's adjusted by potentiometric titration to pH 10, 11, 12, and 13, using 0.01 *M* NaOH. After the required time intervals had elapsed, the samples were quenched to pH 8.0 with dilute $HClO_4$.

A sample of each of the products was diluted to a final concentration of 1×10^{-6} mol/ml with pH 2.2 dilution buffer (0.2 *M* NaCit). One milliliter of each of the samples was used for amino acid analysis in the normal manner.

Recovery of Amino Acids and Dipeptides Following Displacement from Their Complexes by Sodium Cyanide. [Co(trien)(amino acid)]$^{2+}$ species (lys, arg, phe, leu, asp) (1×10^{-6} mol) were dissolved in a minimum volume of hot water and, on cooling, slightly greater than 2 equiv of solid sodium cyanide was added. The solutions were warmed to 40° for 15 min before being allowed to cool and diluted to 100 ml with pH 2.2 dilution buffer. Amino acid analysis of 1.0 ml of solution gave a quantitative yield of the amino acid in each case (lys, 1.01; arg, 0.94; phe, 1.00; leu, 1.01; asp, 1.08).

The dipeptides were recovered using a similar procedure to that outlined above. Initial experiments for dipeptide displacement by NaCN gave mixtures of dipeptide and amino acid products, indicating simultaneous hydrolysis and displacement (*e.g.*, for β-[Co(trien)(glygly)]$^{2+}$ at 40° in, NaCN (0.1 *M*), pH 10.64: glycine, 37.3%; glycylglycine, 62.7%). The use of buffered solutions of NaCN or NaCN-H^+ eliminated this problem, giving quantitative recovery of the dipeptides and no contamination by the C-terminal amino acids.

Quantitative Hydrolysis and Cyanide Displacement from β-[Co(trien)(dipeptide)](ClO$_4$)$_2$. The cobalt(III)–dipeptide complexes of glycylglycine, glycyl-L-alanine, glycyl-DL-leucine, and glycyl-L-phenylalanine at 100 times the normal sample concentration for amino acid analysis (*i.e.*, 1×10^{-6} mol/ml) were applied to the resin bed to detect the presence of free amino acid impurities (Table II). To a further sample of each of the complexes was added acidified NaCN (0.1 *M*) and the samples were warmed to 40° for 2 hr. The excess cyanide was removed by freeze-drying and the remaining samples were diluted to 1×10^{-8} mol/ml for amino acid analysis.

A sample (10 mg) of each of the cobalt(III)–peptide complexes was hydrolyzed by adding 20 ml of Na_2HPO_4 (0.5 *M*) and sufficient NaOH (1.0 *M*) to give a final pH of 10.8 ± 0.1. The samples were left to stand at room temperature for 150 min ($>9t_{1/2}$ for hydrolysis), before diluting the samples to a final concentration of 1×10^{-8} mol/ml with 0.2 *M* NaCit. The quantitative recovery of the C-terminal amino acid was used as a measure of quantitation of base hydrolysis.

The quantitative recovery of the N-terminal amino acid was measured following cyanide displacement of the amino acids from the base-hydrolyzed samples. To a 10-ml sample of the hydrolyzed solution of the complexes was added a 10-fold excess of solid NaCN and the solutions were acidified with HCl (1.0 *M*). The solutions were warmed at 40° for 3 hr before removing excess HCN *in vacuo*. The samples were made up to 95 ml with dilution buffer, the pH adjusted to ~2 with NaOH (1.0 *M*) and the volume made up to 100 ml. A sample (10 ml) was made up to 100 ml with pH 2.2 dilution buffer to give a final concentration of 1×10^{-8} mol/ml for analysis.

Amino acid analysis in the presence of cobalt(III)–tetramine complexes of amino acids and dipeptides causes the accumulation of these products on the resin bed. The complexes cannot be removed using the normal Beckman regeneration cycle (*i.e.*, NaOH, 0.2 *M*, 30 min, 30 psi; NaOH, 0.2 *M*, Na^+, pH 3.25, 60 min, 200 psi). The columns can be regenerated by pumping with 1.0 *M* sodium citrate buffer (pH 4.3, 6 hr, 225 psi) and NaOH (0.2 *M*, 60 min, 30 psi) and reequilibrated with sodium citrate buffer (0.2 *M*, pH 3.25, 175 psi, 180 min).

Acknowledgment. This work was supported in part by the award of an Australian National University Scholarship to K. W. B. The Authors wish to thank the Microanalytical Unit of The Australian National University for the Co, C, H, N, and halogen analyses. We wish to thank Drs. D. A. Buckingham and A. M. Sargeson for helpful discussion.

Registry No. *cis*-β$_2$-[Co(trien)(L-ala)](ClO$_4$)$_2$, 51151-90-7; *cis*-β$_2$-[Co(trien)(L-arg)](ClO$_4$)$_2$·HClO$_4$, 50859-42-2; *cis*-β$_2$-[Co(trien)(L-asp)](ClO$_4$)$_2$ (5-ring), 50932-68-8; *cis*-β$_2$-[Co(trien)(gly)](ClO$_4$)$_2$, 51022-67-4; *cis*-β$_2$-[Co(trien)(L-glu)](ClO$_4$)$_2$, 50859-44-4; *cis*-β$_2$-[Co(trien)(L-his)](ClO$_4$)$_2$, 50859-46-6; *cis*-β$_2$-[Co(trien)(L-ile)](ClO$_4$)$_2$, 51095-79-5; *cis*-β$_2$-[Co(trien)(L-leu)](ClO$_4$)$_2$, 51151-98-5; *cis*-β$_2$[Co(trien)(L-lys)](ClO$_4$)$_2$, 50859-48-8; *cis*-β$_2$-[Co(trien)(L-met)](ClO$_4$)$_2$, 50859-50-2; *cis*-β$_2$-[Co(trien)(L-phe)](ClO$_4$)$_2$, 51151-92-9; *cis*-β$_2$-[Co(trien)(L-pro)](ClO$_4$)$_2$, 51095-81-9; *cis*-β$_2$-[Co(trien)(sar)](ClO$_4$)$_2$, 15053-83-5; *cis*-β$_2$-[Co(trien)(L-ser)](ClO$_4$)$_2$, 50859-52-4; *cis*-β$_2$-[Co(trien)(L-thr)](ClO$_4$)$_2$, 50859-54-6; *cis*-β$_2$-[Co(trien)(L-tyr)](ClO$_4$)$_2$, 51095-83-1; *cis*-β$_2$-[Co(trien)(L-val)](ClO$_4$)$_2$, 51152-00-2; *cis*-β$_2$-[Co(trien)(*S*-benzyl-L-cys)](ClO$_4$)$_2$, 50859-56-8; *cis*-β$_2$-[Co(trien)(cysteic acid)](ClO$_4$)$_2$, 50932-70-2; *cis*-β$_2$-[Co(trien)(*O*-benzyl-L-tyr)](ClO$_4$)$_2$, 50859-58-0; *cis*-β$_2$-[Co(trien)(*N*-ε-carbobenzoxy-L-lys)]$^{2+}$, 50859-59-1; *cis*-β$_2$-[Co(trien)(nitro-L-arg)]$^{2+}$, 50859-60-4; *cis*-β$_2$-[Co(trien)(*im*-benzyl-L-his)]$^{2+}$, 50859-61-5; *cis*-β$_2$-[Co(trien)(L-glu γ-benzyl ester)]$^{2+}$, 50859-62-6; *cis*-β$_2$-[Co(trien)(*O*-benzyl-L-ser)]$^{2+}$, 50859-63-7; *cis*-β$_2$-[Co(trien)(L-asp β-benzyl ester)]$^{2+}$, 50859-64-8; *cis*-β$_2$-[Co(trien)(glygly methyl ester)](ClO$_4$)$_3$, 51063-11-7; *cis*-β$_2$-[Co(trien)(glygly ethyl ester)](ClO$_4$)$_3$, 51019-38-6; *cis*-β$_2$-[Co(trien)(glygly isopropyl ester)](ClO$_4$)$_3$, 51019-40-0; *cis*-β$_2$-[Co(trien)(glygly)](ClO$_4$)$_2$, 51019-42-2; *cis*-β$_2$-[Co(trien)(gly-L-ala)](ClO$_4$)$_2$, 50859-66-0; *cis*-β$_2$-[Co(trien)(gly-DL-leu)](ClO$_4$)$_2$, 50859-68-2; *cis*-β$_2$-[Co(trien)(gly-L-phe)](ClO$_4$)$_2$, 50859-70-6; *cis*-β$_2$-[Co(trien)(L-asp)](ClO$_4$)$_2$ (6-ring), 50859-72-8; *cis*-β$_2$-[Co(trien)(OH)(OH$_2$)]$^{2+}$, 50859-73-9.

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Dissymmetric Arsine Complexes. Preparation and Properties of a Series of Cobalt(III) Complexes Containing a Linear Quadridentate Arsine

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Received September 24, 1973 AIC30704G

The linear quadridentate arsine tetars, $(CH_3)_2As(CH_2)_3As(C_6H_5)(CH_2)_2As(C_6H_5)(CH_2)_3As(CH_3)_2$, exists in meso and racemic forms which form stable cobalt(III) complexes. An extensive series of these complexes of both ligands have been prepared in their various topological isomers as well as the optically active forms of the stereospecifically coordinated racemic ligand. Both thermodynamic and kinetic methods have been used to prepare the various isomers and the isomeric equilibria have been measured in all cases. It was found that the meso ligand generally prefers the trans topology, whereas the racemic ligand is flexible in the isomers it can form. All the systems are very prone to catalytic substitution and topological equilibration which occur *via* labile Co(II) species. These problems are discussed in detail and methods for dealing with these are given.

Hitherto the study of the relationships between the circular dichroism spectra and the absolute configurations of transition metal complexes has been restricted to complexes containing "hard" donor atoms such as nitrogen and oxygen. While, except for the exciton circular dichroism of interligand transitions,[1] these relationships are far from clear, some gen-