Cobalt(III)-Promoted Hydrolysis of Chelated Glycine Amides, Glycylglycine, and Glycylglycine Esters. Kinetics and Mechanism

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Abstract: A variety of N–O chelated glycine amide and peptide complexes of the type $[CoN_4(glyNR_1R_2)]^{3+}$ have been prepared and their rates of base hydrolysis measured. The kinetic data are consistent with a rate law of the the form $v = k'K_b[Co][^-OH]/(K_b + [^-OH])$, where K_b is the base dissociation constant associated with the amide proton. k' values for the complexes vary from 1 to 25 l^{-1} sec^{-1} at 25°, $\mu = 1.0$, corresponding to rate enhancements over the uncoordinated substrates of *ca.* 10^4–10^6. The results are consistent with attack of solvent hydroxide at the carbonyl carbon of the chelated amide, and require the amide conjugate base to be unreactive. Their relevance to cobalt(III)-promoted hydrolysis of peptides and to metal-ion-activated enzymic hydrolysis is discussed.

This study of the kinetics and mechanism of hydrolysis and cleavage of chelated glycine amides, glycylglycine, and glycylglycine esters was undertaken as part of a general program to determine the effect of metal ions on the reactions of amino acid derivatives. A detailed examination of the mechanism was prompted by earlier studies which showed that $[Co^{III}(tetramine)(OH)OH_2]^{2+}$-type complexes[1] enhanced the cleavage of dipeptides.[2,3]

The activation was attributed to an intermediate formed by chelation of the terminal NH_2– group and the carbonyl oxygen atom, and in at least one instance a complex of this nature was isolated from the reaction mixture.[4] Moreover, recent studies on chelated amino acid esters have shown that this type of coordination greatly enhances nucleophilic attack at the coordinated carbonyl moiety.[5–7] We wished to see if similar rate enhancements occurred with analogous amide and peptide complexes and if these rates were consistent with the >C=O chelated species being the *reactive* intermediates in the $[Co^{III}(tetramine)(OH)OH_2]^{2+}$-promoted reactions.

Experimental Section

Analar reagents were used throughout without further purification. Pmr spectra were recorded on a Varian HA 100-MHz instrument (using either external tetramethylsilane (TMS) or internal *t*-butyl alcohol (*t*BA) as reference). Electronic spectra and pK_b measurements were recorded on a Cary 14 spectrophotometer. Uv and visible rate data were collected using Cary 14 and Gilford 2400 recording spectrophotometers. Some cobalt estimations were made with a Techtron AA4 atomic absorption spectrophotometer. Rates of base uptake were determined at constant pH and temperature by pH-Stat titration with the following Radiometer apparatus; TTA_3 electrode assembly, ABU 1 autoburet, TTT1 titrator, and SBR_2 titrigraph; pH measurements were made using the TTT1 titrator in conjunction with a pHA 630T scale expander. For D_2O solutions, the pH was measured with a glass electrode, and the pD evaluated from the empirical expression pD = pH + 0.4.[8] Bio-Rad analytical Dowex 50W × 2 (200–400 mesh) cation exchange resin was used for chromatographic separations.

Preparation of Complexes. $[Co(en)_2glyNH_2)](NO_3)_2ClO_4$. *cis*-$[Co(en)_2Br(glyNH_2)](ClO_4)_2$[9] (1.05 g) was dissolved in the minimum volume of warm water containing 1 drop of 70% $HClO_4$. $AgNO_3$ (0.4 g) in H_2O (10 ml) was added, and after 10 min a further 0.1 g of $AgNO_3$, followed by 0.2 g of LiCl, was added to precipitate excess Ag^+. After filtration, $LiNO_3$ was added to the filtrate, and on cooling, orange crystals formed. These were collected and recrystallized from dilute perchloric acid by addition of $LiNO_3$ and cooling. The product was washed with methanol and dried in an evacuated desiccator: yield, 0.5 g; absorption maxima, 487 (ϵ 97), 343 (ϵ 111), and 206 nm (ϵ 39,200) in H_2O at 25°.

Anal. Calcd for $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$: C, 14.84; H, 4.77; N, 23.08. Found: C, 15.12; H, 4.65; N, 23.51.

$[Co(en)_2(glyNHCH_3)](NO_3)_2ClO_4 \cdot H_2O$ was prepared as described above by treatment of *cis*-$[(Co(en)_2Br(glyNHCH_3)](ClO_4)_2$[9] (1.1 g) with $AgNO_3$ (0.4 g) in dilute perchloric acid (5 ml): yield, 0.4 g; absorption maxima, 487 (ϵ 116) and 345 nm (ϵ 104) in H_2O at 25°.

Anal. Calcd for $[Co(en)_2(glyNHCH_3)](NO_3)_2ClO_4 \cdot H_2O$: C, 16.53; H, 4.76; N, 22.03. Found: C, 16.51; H, 4.68; N, 21.83.

$[Co(en)_2(glyN(CH_3)_2)]I_2ClO_4$ was prepared from *cis*-$[Co(en)_2Br(glyN(CH_3)_2)]Br_2$[9] (0.8 g) by treatment with $AgNO_3$ (0.4 g) in dilute perchloric acid (5 ml) and addition of NaI to the filtrate after removal of the insoluble silver salts. The product was recrystallized from dilute perchloric acid by addition of NaI and cooling, washed with methanol, and dried in an evacuated desiccator: yield, 0.4 g; absorption maximum, 487 nm (ϵ 109) in H_2O at 25°.

Anal. Calcd for $[Co(en)_2(glyN(CH_3)_2)]I_2 \cdot ClO_4$: C, 15.13; H, 4.13; N, 13.24. Found: C, 14.82; H, 4.10; N, 13.57.

$[Co(en)_2(glyglyO)](ClO_4)_2 \cdot H_2O$. $[Co(en)_2(glyOCH_3)](ClO_4)_3$[7] (*ca.* 10 g) was dissolved in anhydrous acetone and treated with a threefold excess of freshly prepared methyl glycinate. The product was precipitated with ether, dissolved in dilute HCl (pH 3), and sorbed on a cation exchange resin (H^+). The tripositive band on elution with 3 *M* HCl was allowed to stand in the eluate for 24 hr and was then evaporated to dryness. The residue was dissolved in methanol, neutralized (pH 7–8) with LiOH–MeOH solution, and the chloride salt crystallized by addition of acetone and standing. The product was converted to the perchlorate salt by passage through a perchlorate-form Bio-Rad AG1-X8 (200–400 mesh) anion exchange resin and the eluate reduced to dryness. The product was twice recrystallized from water by addition of $LiClO_4$ and cooling and air dried (6.8 g). Absorption maxima occurred at 487 (ϵ 93) and 343 nm (ϵ 104) in H_2O at 25°.

(1) Shorthand notations used in this article are as follows: en, ethylenediamine; trien, triethylenetetramine; N_4, trien or bis(en); glyglyO, glycylglycinate anion; glyglyOH, O-protonated glycylglycine; glyglyOR, glycylglycine esters; gly, glycinate anion; $glyNR_2$, glycine amides. The β nomenclature is described by L. G. Marzilli and D. A. Buckingham, *Inorg. Chem.*, **6**, 1042 (1967).

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

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

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

(5) M. D. Alexander and D. H. Busch, *ibid.*, **88**, 1130 (1966).

(6) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *ibid.*, **90**, 6032 (1968).

(7) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *ibid.*, **92**, 5701 (1970).

(8) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

(9) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, submitted for publication.

Anal. Calcd for $[Co(en)_2(glyglyO)](ClO_4)_2 \cdot H_2O$: C, 18.23; H, 4.78; N, 15.94; Co, 11.18. Found: C, 18.27; H, 4.62; N, 15.79; Co, 11.36.

β_2-[Co(trien)(glyNHCH$_3$)](ClO$_4$)$_3$. β-$[Co(trien)Cl_2]Cl$ (6.2 g) and glycinemethylamide hydrobromide (3.5 g) were mixed with water (3 ml) and N-methylethylenediamine (1.5 g) was added dropwise with continual trituration over 1 hr. Methanol was added and the unreacted starting material removed. The methanol extracts were reduced to dryness by rotary evaporation, and the residue was dissolved in dilute HCl (pH 4) and then sorbed on the resin (H^+) and eluted with 3 *M* HCl. The eluate containing the tripositive ions was collected, reduced to dryness, and several times redissolved in water and reduced to dryness to remove acid. On dissolution in water (15 ml) and addition of $NaClO_4$ and cooling, crystallization occurred. The product was twice recrystallized from dilute $HClO_4$, washed with methanol, and dried in an evacuated desiccator (2.8 g). Absorption maxima occurred at 478 nm (ϵ 143) and 347 nm (ϵ 156) in H_2O at 25°.

Anal. Calcd for $[Co(trien)(glyNHCH_3)](ClO_4)_3$: C, 18.21; H, 4.75; N, 14.16. Found: C, 18.30; H, 4.48; N, 14.39.

β_2-[Co(trien)(glyglyOCH$_3$)](ClO$_4$)$_3$·H$_2$O. β-$[Co(trien)CO_3]ClO_4 \cdot H_2O$ (3.8 g) was dissolved in water (10 ml), and $HClO_4$ (8 ml, 3 *M*) was added. Glycylglycine methyl ester hydrochloride (2 g) was added, and the solution was brought to pH 7–8 with 2 *M* NaOH solution and allowed to stand for 1 hr. The orange crystals which formed were collected. A second crop was obtained by adding $NaClO_4$ to the filtrate and allowing the solution to stand overnight at 5°. The two fractions were combined and recrystallized from water. The product was washed with ethanol and dried in an evacuated desiccator (1.7 g). Absorption maxima occurred at 478 (ϵ 133) and 347 nm (ϵ 147) in H_2O at 25°.

Anal. Calcd for $[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$: C, 19.79; H, 4.53; N, 12.59. Found: C, 19.94; H, 4.41; N, 12.38.

β_2-[Co(trien)(glyglyOC$_2$H$_5$)](ClO$_4$)$_3$·2H$_2$O was prepared as described above from β-$[Co(trien)CO_3]ClO_4 \cdot H_2O$ (3.8 g) and glycylglycine ethyl ester hydrochloride (2.0 g): yield, 1.5 g; absorption maxima, 478 (ϵ 140) and 347 nm (ϵ 153) in H_2O at 25°.

Anal. Calcd for $[Co(trien)(glyglyOC_2H_5)](ClO_4)_3 \cdot 2H_2O$: C, 20.63; H, 12.03; N, 4.90. Found: C, 20.54; H, 12.00; N, 4.60.

β_2-[Co(trien)(glyglyOC$_3$H$_7$)](ClO$_4$)$_3$ was prepared as above from β-$[Co(trien)CO_3]ClO_4 \cdot H_2O$ (3.8 g) and glycylglycine isopropyl ester hydrochloride (3 g): yield, 1.8 g; absorption maxima, 478 (ϵ 132) and 345 nm (ϵ 156) in H_2O at 25°.

Anal. Calcd for $[Co(trien)(glyglyOCH(CH_3)_2)](ClO_4)_3$: C, 23.03; H, 4.76; N, 12.40. Found: C, 22.61; H, 4.77; N, 12.34.

β_2-[Co(trien)(glyglyOH)](ClO$_4$)$_3$. β_2-$[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$ (2 g) was dissolved in $HClO_4$ (15 ml, 70%) and stood at ambient temperature (*ca.* 20°) for 3 days. On cooling and addition of water (10 ml), crystals formed. Two crops were combined and recrystallized from dilute perchloric acid (pH 1). The product was washed with methanol and dried in an evacuated desiccator (1.5 g). Pmr spectrometry indicated the presence of ~5% unreacted starting material, and the following preparation was preferred for kinetic experiments.

Anal. Calcd for $[Co(trien)(glyglyOH)](ClO_4)_3$: C, 18.84; H, 4.43; N, 13.18. Found: C, 19.01; H, 4.57; N, 12.77.

β_2-[Co(trien)(glyglyO)](ClO$_4$)$_2$·H$_2$O. β_2-$[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$ (3 g) was converted to the chloride form on an anion exchange resin, and the eluate was taken to dryness and then redissolved in HCl (*ca.* 30 ml, 11.6 *N*) and left at room temperature for 88 hr. The solution was reduced to dryness, taken up in the minimum volume of water, and neutralized (pH 7–8) with 2 *M* NaOH. On addition of methanol and cooling, crystallization of the chloride salt occurred. This was collected, washed with methanol, and converted to the perchlorate by dissolution in water and addition of $NaClO_4$. The product was recrystallized from water by addition of $NaClO_4$ and cooling, washed with methanol, and dried in an evacuated desiccator. Absorption maxima occurred at 478 (ϵ ~141) and 347 nm (ϵ ~153) in H_2O at 25°.

Anal. Calcd for $[Co(trien)(glyglyO)](ClO_4)_2 \cdot H_2O$: C, 21.73; H, 4.92; N, 15.19. Found: C, 21.74; H, 4.80; N, 14.93.

Kinetic Measurements. The base hydrolysis of all complexes was followed spectrophotometrically. A weighed quantity of complex was dissolved in water and allowed to equilibrate at 25° in one premixing chamber of a stopped-flow reactor.[10] In the other premixing chamber was NaOH of known concentration (μ = 2.0). After mixing, the absorbance change was followed in the region 280–310 nm. Slower rates were followed by dissolving the complex in glycine buffer at 25° and μ = 1.0, and quickly transferring the solution to a thermostated cell. pK_b values were calculated from plots of the initial absorbance of each solution against pH.

The base hydrolysis of $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ was also followed by pH-Stat titration of 0.2 *M* NaOH using the radiometer apparatus. A weighed quantity of complex (*ca.* 0.2 g) was dissolved in 0.1 *M* KNO_3 (20 ml) and transferred to the thermostated reaction vessel, which was continuously stirred as the titrant was added under a nitrogen atmosphere.

The base hydrolysis of some complexes was also followed qualitatively in the pmr spectrometer at 34.2°. A quantity of the complex was dissolved in 2 *M* Na_2CO_3 buffer in D_2O (pD 10.14) and the resulting spectrum was scanned at spaced time intervals until no further change was apparent. The products of the reaction were identified by comparison of their chemical shifts and coupling constants with those of authentic samples run under the same conditions.

Product Analysis from Base Hydrolysis. $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ **and** $[Co(en)_2(glyNHCH_3)](NO_3)_2ClO_4$. The complex (~0.15 g) was dissolved in water (200 ml) and an equal volume of 0.2 *M* glycine buffer (pH 10.55) was added. After standing for 8 hr at 25°, the solution was sorbed on the cation exchange resin (Na^+ form) and eluted successively with 1 *M* $NaClO_4$, 1 *M* HCl, and 2 *M* HCl. The sorbed species was homogeneous for all eluents and the eluate gave ~97% recovery of cobalt (atomic absorption spectroscopy and visible spectroscopy). In a separate experiment, $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ (0.2 g/20 ml of 0.1 *M* KNO_3) was titrated against 0.2 *M* NaOH at pH 10.00. When base uptake had ceased, the solution was quenched with HCl and sorbed and eluted from an H^+-form resin using 1 *M* HCl as eluent. The eluate was evaporated to dryness, redissolved in water (2 ml), and NaI was added. The iodide salt was collected, recrystallized from water by cooling, and dried in an evacuated desiccator. The pmr spectrum of the isolated complex was identical with that of an authentic sample of $[Co(en)_2gly]I_2$.

Anal. Calcd for $[Co(en)_2gly]I_2$: C, 14.19; H, 3.96; N, 13.79. Found: C, 14.09; H, 4.14; N, 13.74.

$[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ (*ca.* 0.1 g) was dissolved in 2 *M* NaOH (10 ml) at 20° and quenched with 70% $HClO_4$ after 2 min. The solution was diluted, then sorbed and eluted from an H^+-form resin with 1 *M* $NaClO_4$, pH 5–6. Only one product band was observed consistent with $[Co(en)_2gly]^{2+}$. The same result was obtained when $[Co(en)_2(glyNHCH_3)](NO_3)_2ClO_4$ (0.1 g) was treated in an identical manner.

β_2-$[Co(trien)(glyglyOC_3H_7)](ClO_4)_3$ (1 g) was hydrolyzed for *ca.* 15 min in 2 *M* NaOH at 20°, quenched with 70% $HClO_4$, diluted with water, sorbed on the cation exchange resin (H^+), and eluted with 1 *M* $NaClO_4$ (pH 5). Four distinct bands were observed. The eluate fractions were collected (2 *M* HCl was used as eluent after elution of the first band) and examined by visible spectroscopy and atomic absorption spectroscopy for Co. The first eluate fraction (6.5%, ϵ_{485} 150) was red in appearance and its visible spectrum was unaltered by treatment with 1 *M* NaOH for 8 hr or with Ag^+. On acidification to pH 1 the solution turned orange, and the red band was not observed when 2 *M* HCl was used as eluent. The red band separated from a larger scale reaction (2 g of complex, 2 *M* NaOH, 5 min) was resorbed on the resin (H^+ form) and eluted with 3 *M* HCl. The orange eluate was reduced to dryness and the pmr spectrum run in D_2O solution. Band 2 (6.5%, ϵ_{487} 145) and bands 3 (18%, ϵ_{480} 135) were assigned, respectively, as the (*RS* + *SR*) and (*RR* + *SS*) diastereoisomers of β_2-$[Co(trien)gly]^{2+}$ by comparison with authentic compounds.[11] These ions differ only in the configuration about the "planar" secondary N atom of the triethylenetetramine chelate ring. Band 4 (66.5%, ϵ_{507} 122) was assigned as β-$[Co(trien)Cl(OH_2)]^{2+}$, formed by anation of β-$[Co(trien)(OH_2)_2]^{3+}$ by the HCl eluent (total Co recovery 97%). Hydrolysis of *ca.* 0.3 g of complex for 3 min in 0.5 *M* NaOH at 20° gave 17% band 1, 72% bands 2 plus 3, and 5% band 4; after reaction for 3 min in 0.1 *M* NaOH at 20° no band 1 was observed and band 4 contributed <5%; after 24 hr in glycine buffer, pH 8.6, band 1 was absent and traces of band 4 were still present.

β_2-$[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$. A similar product distribution to that described above was observed after chromatog-

(10) Y. Inoue and D. D. Perrin, *J. Phys. Chem.*, **66**, 1689 (1962).

(11) D. A. Buckingham, D. M. Foster, L. G. Marzilli, and A. M. Sargeson, *Inorg. Chem.*, **9**, 11 (1970); D. A. Buckingham, L. G. Marzilli, K. R. Turnbull, and A. M. Sargeson, unpublished results.

Table I. Assignments of Pmr Absorptions of Chelated Amides and Peptides[a]

Cation	$\alpha = CH_2$	$\beta = CH_2$	Ester or amide alkyl group[b]
$[Co(en)_2glyNH_2]^{3+}$	4.45		
$[Co(en)_2glyNHCH_3]^{3+}$	4.50		3.30 (CH_3, s)
$[Co(en)_2glyN(CH_3)_2]^{3+}$	4.62		3.37 (CH_3, s), 3.40 (CH_3, s)
$[Co(en)_2(glyglyO)]^{2+}$	4.53	4.33	
β_2-$[Co(trien)(glyNHCH_3)]^{3+}$	4.47		3.38 (CH_3, s)
β_2-$[Co(trien)(glyglyOCH_3)]^{3+}$	4.62	4.77	4.27 (CH_3, s)
β_2-$[Co(trien)(glyglyOC_2H_5)]^{3+}$	4.57	4.70	1.70 (CH_3, t), 4.70 (CH_2, q)
β_2-$[Co(trien)(glyglyOCH(CH_3)_2)]^{3+}$	4.58	4.68	1.73 (*gem*-CH_3, d), 5.50 (CH, qi)
β_2-$[Co(trien)(glyglyO)]^{2+}$	4.56	4.36	

[a] Ppm downfield from TMS (100 MHz). [b] s = singlet, d = doublet, t = triplet, q = quartet, qi = quintet.

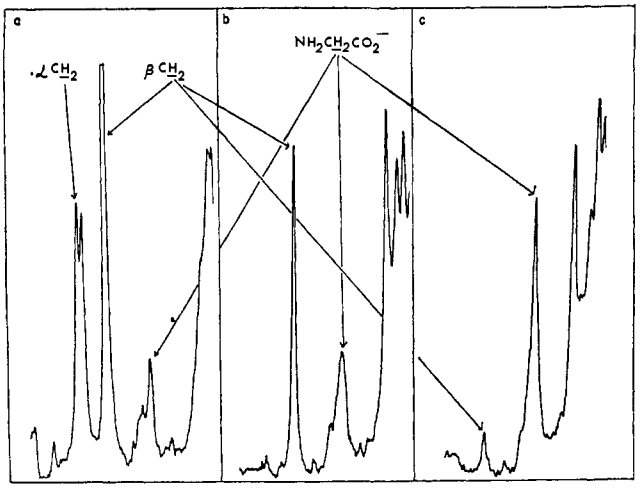


Figure 1. Pmr spectrum (100 MHz) of β_2-$[Co(trien)(glyglyO)](ClO_4)_2 \cdot H_2O$ in (a) D_2O and in 2 *M* Na_2CO_3–D_2O buffer (pD 10.14) after (b) 10 min; (c) 30 min; from *ca.* 2.0 to 3.2 ppm downfield from *t*-butyl alcohol (internal). Part c is the infinity spectrum for peptide hydrolysis.

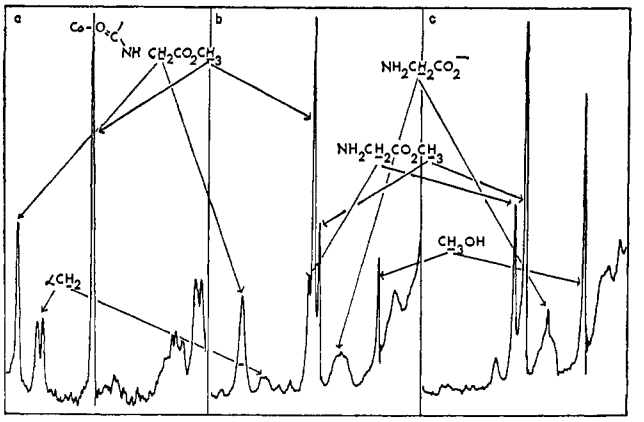


Figure 2. Pmr spectrum (100 MHz) of β_2-$[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$ in (a) D_2O and in 2 *M* Na_2CO_3–D_2O buffer (pD 10.14) after (b) 10 min; (c) 30 min; from *ca.* 1.8 to 3.2 ppm downfield from *t*-butyl alcohol. Part c is the infinity spectrum for peptide hydrolysis.

raphy of the reaction products following base hydrolysis in 2 *M* NaOH at 20° for 5 min (0.3 g of complex) (15% band 1, 60% bands 2 plus 3, and 25% band 4).

β_2-$[Co(trien)(glyNHCH_3)](ClO_4)_3$ (1 g) in 2 *M* NaOH at 20° for 5 min gave four product bands, identical in elution speed and appearance with those observed in the base hydrolysis of the peptide esters. Eluate fraction 1 was isolated and its pmr spectrum examined in D_2O.

Elution of the reaction products from hydrolysis of β_2-$[Co(trien)Cl(glyOC_2H_5)](ClO_4)_2$,[11] in 2 *M* NaOH for 5 min at 20°, gave 75% β-$[Co(trien)gly]^{2+}$ and 25% β-$[Co(trien)(OH_2)_2]^{3+}$. β_2-$[Co(trien)gly]I_2$, on standing for 5 min in 2 *M* NaOH gave, on elution, 11% β-$[Co(trien)(OH_2)_2]^{3+}$

Results

Pmr Spectra. Chemical shift data (100 MHz) for the amide and peptide complexes are summarized in Table I. Some of the trien complexes have previously been described and their pmr spectra compared with those of the uncoordinated ligands.[4] The present results agree with the previous assignments. The ethylene protons of triethylenetetramine and ethylenediamine exhibit broad resonances in the region 3.0–3.7 ppm downfield from TMS. In the peptide complexes the α-CH_2 protons of the glycine chelate ring are upfield from the β-CH_2 protons in the esters, but downfield in the acid anion; for the O-protonated anion, the α- and β-CH_2 signals superpose.[7] The assignments are based upon the lability of α-CH_2 protons in alkaline D_2O.[12]

(12) D. H. Williams and D. H. Busch, *J. Amer. Chem. Soc.*, **87**, 4644 (1965); D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *ibid.*, **89**, 5133 (1967).

All amine and amide protons deuterate rapidly in neutral or alkaline D_2O solutions.

The chemical shifts and coupling constants of the ester and amide alkyl groups in the coordinated molecule differ from those of the hydrolysis products. This allowed a kinetic study of the hydrolysis by pmr. The alkaline hydrolyses of β_2-$[Co(trien)(glyglyOR)](ClO_4)_3$ (R = H, CH_3, C_2H_5, and $CH(CH_3)_2$) and $[Co(en)_2(glyglyO)](ClO_4)_2$ were followed in 2 *M* Na_2CO_3 buffer in D_2O, pD 10.14 (Figures 1–5). Integration of the peaks formed by the reaction products, where practicable, was consistent with the resonance assignments made on the basis of comparison with the chemical shifts and coupling constants of the authentic compounds under the same conditions.

Figure 1 shows the pmr spectra during hydrolysis of β_2-$[Co(trien)(glyglyO)](ClO_4)_2 \cdot H_2O$. Panels b and c are examples of spectra at different times and are different in total cobalt concentration from panel a, which was a separate solution; the same comment is true of Figures 2–5. The α-CH_2 was rapidly deuterated (complete in 10 min), as demonstrated by the collapse of the CH_2 absorption and a concomitant increase in the HOD absorption (*ca.* 3.6 ppm). The β-CH_2 signal slowly decreased in intensity synchronous with the appearance of free glycine, which does not deuterate appreciably on carbon under the conditions used. This latter change gave an approximate measure of the rate of peptide hydrolysis, $t_{1/2} \sim 20$ min (pD 10.14, 34.2°). The hydrolysis of $[Co(en)_2glyglyO](ClO_4)_2 \cdot H_2O$ (Figure 5) was analogous.

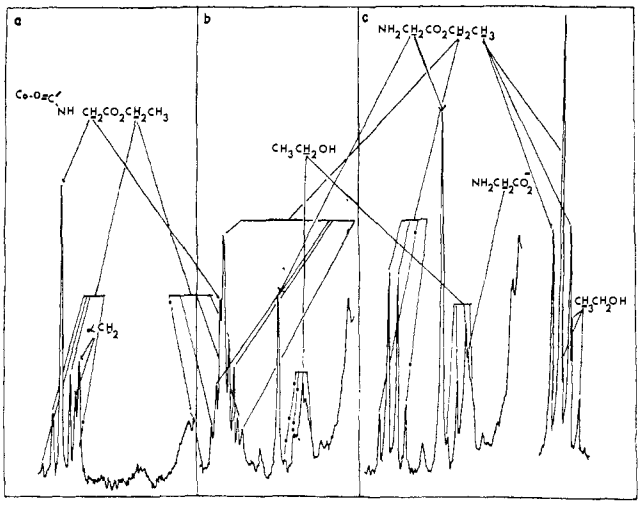


Figure 3. Pmr spectrum (100 MHz) of β_2-[Co(trien)(glyglyOC$_2$H$_5$)]-(ClO$_4$)$_3$·2H$_2$O in (a) D$_2$O and in 2 *M* Na$_2$CO$_3$–D$_2$O buffer (pD 10.14) after (b) 10 min; (c) 30 min; from *ca.* 3.6 to 4.9 ppm downfield from TMS (external). The inset ethyl triplets in (c) are *ca.* 1.6–1.8 ppm downfield from TMS. Part c is the infinity spectrum for peptide hydrolysis.

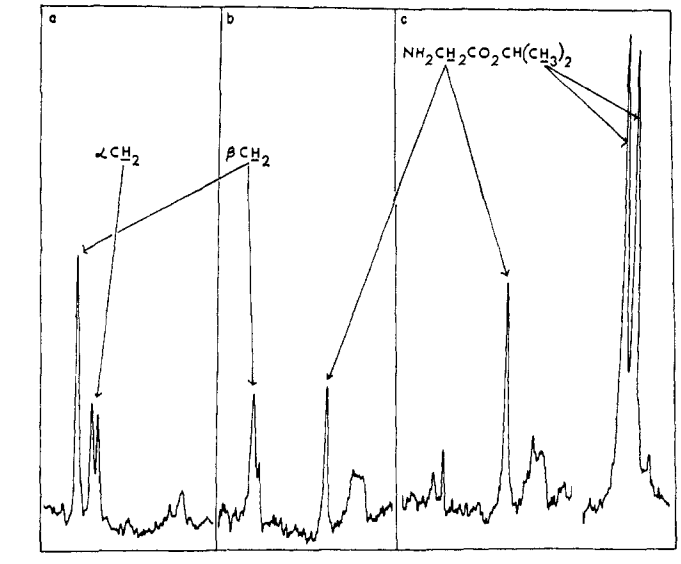


Figure 4. Pmr spectrum (100 MHz) of β_2-[Co(trien)(glyglyOCH-(CH$_3$)$_2$](ClO$_4$)$_3$·H$_2$O in (a) D$_2$O and in 2*M* Na$_2$CO$_3$–D$_2$O buffer (pD 10.14) after (b) 10 min; (c) 30 min; from *ca.* 3.6 to 4.9 ppm downfield from TMS (external). The inset isopropyl alcohol methyl doublet in (c) is *ca.* 1.6–1.9 ppm downfield from TMS. Part c is the infinity spectrum for peptide hydrolysis.

For the chelated peptide esters (R = CH$_3$, C$_2$H$_5$), both ester and peptide hydrolysis occurred, resulting in a complex set of products. For β_2-[Co(trien)-(glyglyOCH$_3$)](ClO$_4$)$_3$·H$_2$O (Figure 2), the α-CH$_2$ rapidly deuterated, while the β-CH$_2$ signal slowly decreased with concomitant formation of the CH$_2$ signals of glycine methyl ester and glycine. The CH$_2$ signal of glycine coincides with a broad methylene resonance of triethylenetetramine and a direct measure of the peptide-to-ester hydrolysis ratio was difficult to obtain from this comparison. However, an estimate was obtained from the ratio of the peak heights of the methyl signals of methyl glycinate and methanol, Figure 2c (k(peptide)/k(ester) ~ 3/2). Alkaline hydrolysis of uncoordinated methyl glycinate was

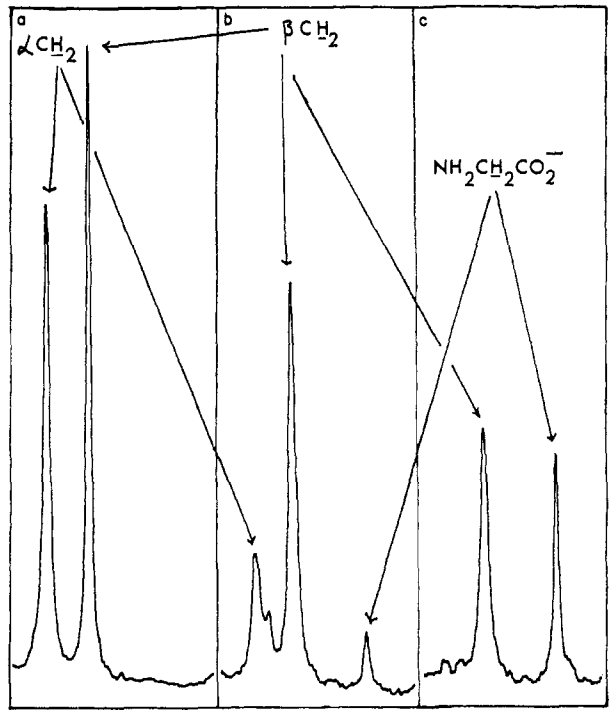


Figure 5. Pmr spectrum (100 MHz) of [Co(en)$_2$(glyglyO)](ClO$_4$)$_2$·H$_2$O in (a) D$_2$O and in 2 *M* Na$_2$CO$_3$–D$_2$O buffer (pD 10.14) after (b) 10 min; (c) 20 min; from *ca.* 4.0 to 4.8 ppm downfield from TMS (external). Part c is not the infinity spectrum for peptide hydrolysis.

shown by pmr spectroscopy to be comparatively slow under the experimental conditions. However, ester hydrolysis of ethyl glycylglycinate was rapid under the conditions, and in 2 *M* Na$_2$CO$_3$ buffer in D$_2$O (pD 10.14) was complete within 10 min. The products were identified by pmr as 2,5-diketopiperazine, ethanol, and a trace (*ca.* 5%) of glycylglycine, by comparison with the authentic compounds.

Alkaline hydrolysis of β_2-[Co(trien)(glyglyOC$_2$H$_5$)]-(ClO$_4$)$_3$ in Na$_2$CO$_3$ buffer in D$_2$O (pD 10.14), Figure 3, was similar to that for the methyl ester with the formation of β_2-[Co(trien)gly]$^{2+}$, glyOC$_2$H$_5$, glyO$^-$, and C$_2$H$_5$OH. The methyl signals of the coordinated dipeptide ester and ethylglycinate in (c) coincide. However, the methyl resonance of ethyl glycinate is slightly downfield from that for ethanol, and the constant 4/1 ratio of the peak heights for the outermost absorptions of the two triplets, Figure 3c, is a measure of the relative rates of peptide-to-ester hydrolysis in the chelated dipeptide ester.

For β_2-[Co(trien)(glyglyOC$_3$H$_7$)](ClO$_4$)$_3$·H$_2$O, Figure 4 shows that base hydrolysis (pD 10.14, 34.2°) does not result in any detectable ester hydrolysis (<2%) and that peptide hydrolysis is almost complete after 30 min. The methyl signals of free isopropyl alcohol absorb ~0.1 ppm upfield from the same signals of isopropyl glycinate. The β-CH$_2$ signal of the chelated dipeptide ester disappeared at the same rate as the appearance of the methylene signal of the free ester, and deuteration of the α-CH$_2$ was again rapid.

Kinetic Data. Base-catalyzed hydrolysis of the chelated glycine amides and dipeptides was followed spectrophotometrically in the region 280–340 nm. At these wavelengths concomitant ester hydrolysis in β_2-[Co(trien)(glyglyOCH$_3$)](ClO$_4$)$_3$ was not observed;

for the bis(ethylenediamine) complexes product analysis and pmr studies established that only amide bond cleavage was involved. At high pH, linear plots of log $(D_t - D_\infty)$ *vs.* time were obtained for the complete reaction, but only for $2t_{1/2}$ in some instances at low pH. The observed rate constants are given in Tables II and III for the bis(en) and trien species, respectively. The observed rates fit a rate law of the form

$$k_{obsd} = \frac{a[OH^-]}{b + [OH^-]}$$

where a and b are constants.

Table II. Spectrophotometric Rate Data (25°, μ = 1.0, 280–310 nm, [complex] $\simeq 7.5 \times 10^{-4}$ *M*)

[OH⁻], *M*	k_{obsd}, sec⁻¹	k_{obsd}/OH^-, M^{-1} sec⁻¹
(a) $[Co(en)_2(glyglyO)](ClO_4)_2 \cdot H_2O$		
1.0	1.58×10^{-2}	0.016
5×10^{-1}	1.65×10^{-2}	0.033
2.5×10^{-1}	1.73×10^{-2}	0.069
5×10^{-2}	1.58×10^{-2}	0.32
2.5×10^{-2}	1.28×10^{-2}	0.51
1×10^{-2}	1.02×10^{-2}	1.0
5×10^{-3}	4.74×10^{-3}	0.95
1.85×10^{-4} [a,f]	5.19×10^{-4}	2.81 (2.7[c])
4.3×10^{-5} [e,h]	1.15×10^{-4}	2.67 (2.4[c])
8.0×10^{-6} [e,h]	3.53×10^{-5}	4.41 (2.9[c])
2.5×10^{-6} [e,h]	1.86×10^{-5}	7.44 (2.6[c])
(b) $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$		
1.0	6.02×10^{-2}	0.06
1.0[i]	5.08×10^{-2}	0.05
5×10^{-1}	6.6×10^{-2}	0.13
1×10^{-1}	6.36×10^{-2}	0.63
5×10^{-2}	5.78×10^{-2}	1.2
2×10^{-2}	5.13×10^{-2}	2.6
1×10^{-2}	4.32×10^{-2}	4.3
5×10^{-3}	3.08×10^{-2}	6.2
7.95×10^{-4} [e,g]	1.93×10^{-2}	24.1
4.7×10^{-4} [a]	7.97×10^{-3}	17.1
4.5×10^{-4} [b]	7.96×10^{-3}	17.8
2.9×10^{-4} [d]	6.74×10^{-3}	23.4
1.3×10^{-4} [b]	3.37×10^{-3}	26.1
1.0×10^{-4} [e,g]	2.98×10^{-3}	24.8
7.1×10^{-5} [b]	1.84×10^{-3}	26.1
5.1×10^{-5} [e,h]	1.28×10^{-3}	25.1
3.3×10^{-5} [b]	8.67×10^{-4}	26.2
2.4×10^{-6} [e, h]	6.19×10^{-5}	25.8
8.0×10^{-6} [e,h]	2.02×10^{-4}	25.3
(c) $[Co(en)_2(glyNHCH_3)](NO_3)_2ClO_4 \cdot H_2O$		
1.0	4.7×10^{-2}	0.05
1.0[a]	4.5×10^{-2}	0.05
5×10^{-1}	4.8×10^{-2}	0.10
1×10^{-1}	3.7×10^{-2}	0.37
5×10^{-2}	2.66×10^{-2}	0.53
2×10^{-2}	1.54×10^{-2}	0.77
1×10^{-2}	7.7×10^{-3}	0.77
5×10^{-3}	4.8×10^{-3}	0.96
4.5×10^{-4} [a]	8.3×10^{-4}	1.86
1.7×10^{-4} [a]	2.5×10^{-4}	1.49
1.0×10^{-4} [a]	1.5×10^{-4}	1.50
(d) $[Co(en)_2(glyN(CH_3)_2)]I_2ClO_4$		
2×10^{-1}	2.24×10^{-1}	1.12
1×10^{-1}	1.03×10^{-1}	1.03
7.5×10^{-2}	7.3×10^{-2}	0.97
5×10^{-2}	5.55×10^{-2}	1.11
2.5×10^{-2}	2.48×10^{-2}	0.99
5×10^{-3}	4.6×10^{-3}	0.92
1.04×10^{-3} [j]	1.54×10^{-3}	1.34

[a] 0.2 *M* glycine buffer. [b] 0.1 *M* glycine buffer. [c] Calculated on subtraction of 1.2×10^{-5} sec⁻¹ from k_{obsd}. [d] 0.05 *M* glycine buffer. [e] [Complex] $\simeq 2 \times 10^{-2}$ *M*. [f] [Complex] = 1.5×10^{-3} *M*. [g] Radiometer data, μ = 0.1 (KNO_3). [h] 1.0 *M* glycine buffer. [i] μ = 2.0 ($NaClO_4$). [j] 0.1 and 0.4 *M* dimethylamine buffer.

Paths independent of $[H^+]$ and first order in $[H^+]$ were not observed for amide hydrolysis. This was demonstrated by the failure to detect any $[Co(en)_2gly]^{2+}$ on chromatography of a solution of $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ after standing at *ca.* 20° for 5 days in 1 *M* $HClO_4$, or by a similar analysis of $[Co(en)_2(glygly\text{-}OH)](ClO_4)_3$ after 4 days in 3 *M* HCl, at *ca.* 20°. Two rate constants are included in Table II, obtained by pH-stat titration of $[Co(en)_2(glyNH_2)](NO_3)_2ClO_4$ with 0.2 *M* NaOH, μ = 0.1 (KNO_3). Base uptake was 1.0 and 0.93 mol/mol of complex at pH 10.0 and 10.9, respectively, consistent with the formation of $[Co(en)_2gly]^{2+}$ and NH_3. The same rate constant for hydrolysis

Table III. Spectrophotometric Rate Data (25°, μ = 1.0, 290–300 nm, [complex] $\simeq 7.5 \times 10^{-4}$–$1.5 \times 10^{-3}$ *M*)

[OH⁻], *M*	k_{obsd}, sec⁻¹	$k_{obsd}/[OH^-]$, M^{-1}sec⁻¹
(a) β_2-$[Co(trien)(glyNHCH_3)](ClO_4)_3$		
1.0	8.66×10^{-2}	0.087
5×10^{-1}	5.78×10^{-2}	0.116
2.5×10^{-1}	4.47×10^{-2}	0.179
5×10^{-2}	2.57×10^{-2}	0.514
2.5×10^{-2}	1.98×10^{-2}	0.792
1×10^{-2}	1.50×10^{-2}	1.50
5×10^{-3}	7.29×10^{-3}	1.46
4.0×10^{-4} [a,b]	7.07×10^{-4}	1.73 (1.6[d])
8.5×10^{-5} [a]	2.22×10^{-4}	2.61 (2.3[d])
8.0×10^{-6} [a]	4.92×10^{-5}	6.15 (2.1[d])
2.4×10^{-6} [a]	3.55×10^{-5}	14.8 (2.3[d])
(b) β_2-$[Co(trien)(glyglyO)](ClO_4)_2 \cdot H_2O$		
1.0	4.2×10^{-2}	0.042
5.0×10^{-1}	3.55×10^{-2}	0.071
2.5×10^{-1}	3.33×10^{-2}	0.132
5.0×10^{-2}	2.10×10^{-2}	0.420
1.0×10^{-2}	1.11×10^{-2}	1.11
5.0×10^{-3}	5.02×10^{-3}	1.00
6.03×10^{-4} [a]	6.9×10^{-4}	1.14
1.74×10^{-4} [a]	2.2×10^{-4}	1.26
4.3×10^{-5} [a]	1.37×10^{-4}	3.19 (2.5[c])
1.13×10^{-5} [a]	6.7×10^{-5}	5.93 (3.3[c])
8.0×10^{-6} [d,e]	1.6×10^{-5}	2.0
2.4×10^{-6} [d,e]	5.7×10^{-6}	2.4
(c) β_2-$[Co(trien)(glyglyOCH_3)](ClO_4)_3 \cdot H_2O$		
1.0	4.3×10^{-2}	0.043
5×10^{-1}	3.4×10^{-2}	0.069
1×10^{-1}	2.1×10^{-2}	0.21
5×10^{-2}	1.3×10^{-2}	0.26
2.5×10^{-2}	9.1×10^{-3}	0.36
1.0×10^{-2}	4.7×10^{-3}	0.47
5.0×10^{-3}	2.4×10^{-3}	0.48
6.0×10^{-4} [a]	9.9×10^{-4}	1.65
1.75×10^{-4} [a]	6.4×10^{-4}	3.66
1.05×10^{-4} [a]	5.9×10^{-4}	5.59 (5.3[c])
4.3×10^{-5} [a]	2.7×10^{-4}	6.25 (5.6[c])
3.9×10^{-6} [a]	5.3×10^{-5}	13.7 (5.9[c])
2.4×10^{-6} [d]	1.17×10^{-5}	4.9
(d) β_2-$[Co(trien)(glyglyOC_3H_7)](ClO_4)_3$		
1.0	4.2×10^{-2}	0.042
5×10^{-1}	2.89×10^{-2}	0.058
2.5×10^{-1}	1.87×10^{-2}	0.075
5×10^{-2}	8.05×10^{-3}	0.161
2.5×10^{-2}	5.41×10^{-3}	0.216
5.0×10^{-3}	1.60×10^{-3}	0.32
6.03×10^{-4} [a]	8.02×10^{-4}	1.33
1.74×10^{-4} [a]	4.20×10^{-4}	2.42
7.59×10^{-5} [a]	2.46×10^{-4}	3.25 (2.9[c])
8.9×10^{-6} [a]	6.0×10^{-5}	6.7 (3.4[c])

[a] 0.2 *M* glycine buffer. [b] 360 nm. [c] Calculated on subtraction of 3×10^{-5} sec⁻¹ from k_{obsd}. [d] 1.0 *M* glycine buffer. [e] [Complex] $\sim 1 \times 10^{-2}$ *M*.

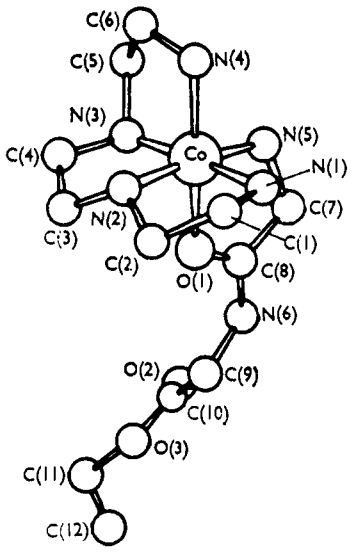


Figure 6. Structure of the β_2-[Co(trien)(glyglyOC$_2$H$_5$)]$^{3+}$ molecular ion from the crystal structure.[14]

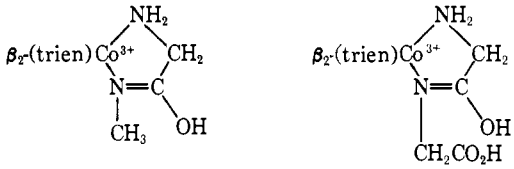
was obtained at $\mu = 0.1$ (pH-stat titration) and at $\mu = 1.0$ (spectrophotometry) (Table IIb).

For the trien complexes (Table III) and for [Co(en)$_2$(glyglyO)]$^{2+}$ (Table IIa) experimental difficulties were encountered at pH <10, resulting from a combination of the small optical density change (~0.01 OD unit), the slow rate, and a slower decomposition of the [Co(en)$_2$gly]$^{2+}$. In some instances the slower rate was subtracted from the observed first-order rates in calculating the derived rate constants (k_{obsd}/[$^-$OH]) for these ions, and in other cases Guggenheim plots[13] were used over the first two or three half-lives. For [Co(en)$_2$(glyNH$_2$)]$^{3+}$, hydrolysis was sufficiently rapid that the slow decomposition rate did not interfere, while for [Co(en)$_2$(glyNHCH$_3$)]$^{3+}$ the observed rate law was established at higher base strengths, pH 10–11.

Measured base dissociation constants (pK_b) for the amide proton at 25°, $\mu = 1.0$, are as follows: 2.8 ± 0.2, [Co(en)$_2$(glyglyO)]$^{2+}$; 2.6 ± 0.1, [Co(en)$_2$(glyNH$_2$)]$^{3+}$; 1.6 ± 0.1, [Co(en)$_2$(glyNHCH$_3$)]$^{3+}$; 2.8 ± 0.2, β_2-[Co(trien)(glyglyO)]$^{2+}$; 1.7 ± 0.1, β_2-[Co(trien)(glyNHCH$_3$)]$^{3+}$; 3.9 ± 0.2, β_2-[Co(trien)(glyglyOCH$_3$)]$^{3+}$; 3.6 ± 0.1, β_2-[Co(trien)(glyglyOC$_3$H$_7$)]$^{3+}$. The [Co(en)$_2$(glyN(CH$_3$)$_2$)]$^{3+}$ ion showed no proton dissociation in the pH range 9–14. These values were obtained spectrophotometrically from the kinetic runs by plotting the extrapolated optical density (280–320 nm) at $t = 0$ *vs.* [OH$^-$]. Optical density changes at these pH's were in the region of 0.05–0.15 OD unit, and since the solutions were mixed using a stopped-flow reactor, extrapolation was only over the first 1–2 sec of the hydrolysis reaction. The value for β_2-[Co(trien)(glyglyOCH$_3$)]$^{3+}$ may be compared with a pK_a value of 9.45 ± 0.09 (pK_b ~ 4.5) obtained by titration with NaOH at ~27°, $\mu = 0.1$.[4] The discrepancy could result from base hydrolysis of the complex during titration.

Products of Co(trien)-Promoted Amide Hydrolysis. Base hydrolysis of the trien amide and peptide complexes was not stoichiometric at high pH and in this respect differed from the bis(en) reactions. Two other cobalt-containing products besides the β_2-[Co(trien)(gly)]$^{2+}$ ions were formed in increasing amounts as the pH was raised. One of these was identified as the β-[Co(trien)(OH)$_2$]$^+$ ion, and other experiments demonstrated that similar amounts of this complex were formed from β_2-[Co(trien)gly]$^{2+}$ under similar conditions. This fact requires β-[Co(trien)(OH)$_2$]$^+$ to be formed from the β_2-[Co(trien)gly]$^{2+}$ product and possibly also from base-catalyzed dissociation of the starting material.

The other major products formed at high pH eluted before the glycinato product in neutral solution and as orange 3+ ions in 3 *M* acid. Visible spectra and chromatographic behavior were unchanged on standing for approximately 8 hr in 0.5 *M* NaOH and 3 *M* HCl. Similar products were not formed by analogous treatment of β_2-[Co(trien)gly]$^{2+}$, β_2-[Co(trien)Cl(glyOC$_2$H$_5$)]$^{2+}$,[11] and β_2-[Co(trien)NH$_3$Cl]$^{2+}$. Therefore, these products do not form from the normal hydrolysis product, and require participation of the amide residue. For β_2-[Co(trien)(glyNHCH$_3$)]$^{3+}$, the pmr spectrum of this isolated product gave absorptions at 2.65 (triplet), 1.6 (singlet), and 1.4–2.1 (broad) ppm downfield from *t*BA in acidic D$_2$O, integrating 2:3:12; on addition of Na$_2$CO$_3$ the triplet collapsed to a singlet. For β_2-[Co(trien)(glyglyOCH$_3$)]$^{3+}$ the product gave absorptions at 3.0 (triplet), 3.0 (singlet), and 1.4–2.1 (broad) ppm from *t*BA (2:2:12), the triplet collapsing to a singlet in alkaline solution. These results are consistent with assignment as N,N-chelated amide and peptide complexes, respectively. It is proposed that these com-



plexes arise from the base-catalyzed dissociation of the amide oxygen atom and reanation by amide nitrogen anion. Similar N,N-chelated amides are known to be stable in acid and alkaline solution.

Discussion

The glycine amide and dipeptide complexes used for the kinetic studies are chelated to the metal *via* the terminal nitrogen and carbonyl oxygen atoms. Evidence for this comes from the crystal structure of β_2-[Co(trien)(glyglyOC$_2$H$_5$)](ClO$_4$)$_3$,[14] Figure 6, the similarity in the electronic spectra to those of [Co(en)$_2$gly]$^{2+}$ and β_2-[Co(trien)gly]$^{2+}$, and the relatively high pK_a (10–12) of the amide proton. The N-bound imide moiety in [Co(NH$_3$)$_4$(glyNH)]$^{2+}$ (pK_b ~ 13.6),[15] [Co(glyglyO)$_2$]$^-$,[16] and [Co(NH$_3$)$_3$(glyglyO)]$^+$[17] is a weakly basic center, and these ions exist in their conjugate base form in neutral or even weakly acidic solutions. Furthermore, protonation in strong acid of [Co(NH$_3$)$_4$(glyNH)]$^{2+}$ [15] and [Co(glyglyO)$_2$]$^-$[18] has been shown to occur at the peptide oxygen atom rather than

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(17) D. A. Buckingham, unpublished results.

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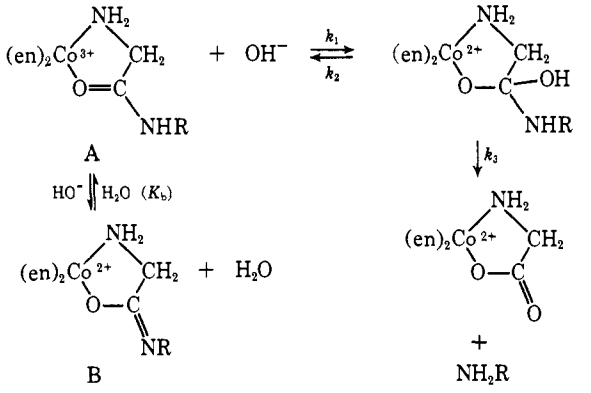
at the imide nitrogen, further demonstrating the very weak basic properties of imide nitrogen coordinated to Co(III). Other studies[19,20] have demonstrated the stability to hydrolysis of the peptide bond when coordination through the imide nitrogen occurs, and this is also true of the proposed N,N products formed from the Co(trien) systems in strong base.

The results establish that hydrolysis in the bis(en) systems consumes 1 equiv of base at high pH to produce $[Co(en)_2gly]^{2+}$ and NH_2R (R = H, CH_3, $CH_2CO_2^-$, CH_2CO_2R). For the bis(en) system, the following mechanism is consistent with the observed rate law (Table II and Figure 7), and the stoichiometry is obeyed in the pH range 9–14.



Assuming a steady-state concentration for the amidol intermediate the derived rate law is

$$v_{[Co]} = \frac{k_3k_1}{k_2 + k_3}\frac{K_b[Co][OH^-]}{(K_b + [OH^-])} \qquad (1)$$

where [Co] = [A] + [B], which reduces to $v_{[Co]} = k_3k_1/(k_2 + k_3)[Co][OH^-] = k'[Co][OH^-]$ when $K_b \gg [OH^-]$ and $v_{[Co]} = k_3k_1/(k_2 + k_3)K_b[Co] = k'K_b[Co]$ when $K_b \ll [OH^-]$.

For the $[Co(en)_2(glyNH_2)]^{3+}$ ion the data at high and low basicities give $k' = 25\ M^{-1}\ sec^{-1}$ and $k'K_b = 6 \times 10^{-2}\ sec^{-1}$, from which $pK_b = 2.6$. The same value was obtained spectroscopically. Similar agreement was obtained between the kinetic (k) and spectroscopic (s) pK_b values for other complexes; $[Co(en)_2(glyglyO)]^{2+}$, 2.6 (k), 2.8 ± 0.2 (s); $[Co(en)_2(glyNHCH_3)]^{3+}$, 1.5 (k), 1.6 ± 0.1 (s). The change in rate law from a first-order dependence on $[OH^-]$ at low pH to independence of $[OH^-]$ at high pH is clearly apparent from Figure 7. However, for the $[Co(en)_2(glyN(CH_3)_2)]^{3+}$ ion, a first-order dependence on $[OH^-]$ was observed at all pH's and this is consistent with the absence of amide deprotonation in this compound. These two observations are inconsistent with deprotonation of an ethylenediamine amine function accounting for the observed rate law for these complexes. Also, the rates for the base-dependent path were similar for all four complexes and the inference is therefore that the imide does not contribute to the hydrolysis.

The kinetic data for the β_2-$[Co(trien)(glyNHR)]^{3+}$ and β_2-$[Co(trien)(glyglyOR)]^{3+}$ (R = H, CH_3, C_3H_7) ions are also consistent with the proposed mechanism at low OH^- concentrations. The similarity between the k' (Table III) and pK_b values for the trien and bis(en) systems suggests that the rates of base hydrolysis and acidities of the chelated amides and dipeptides are relatively insensitive to the nonreacting amine function. At higher OH^- concentrations significant deviations from the rate law occur, resulting from a breakdown in stoichiometry. The products formed under these conditions are dealt with in the Results section.

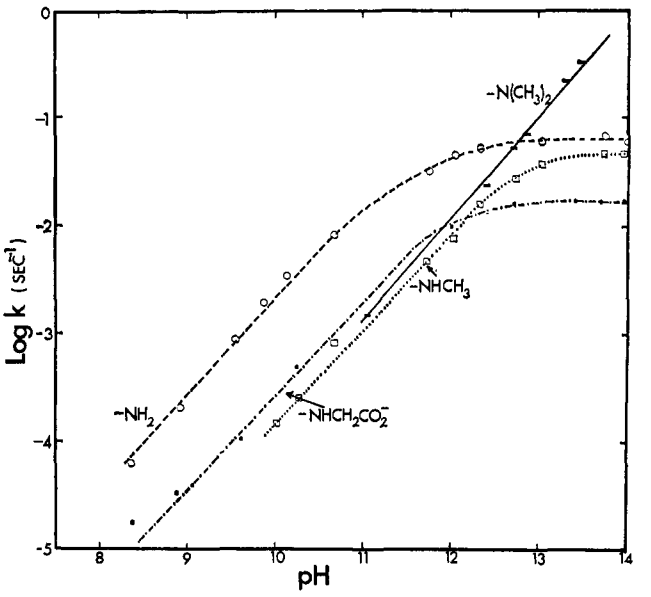


Figure 7. pH profile of first-order rate constants observed in the base hydrolysis of $[Co(en)_2(gly)R)]^{3+,2+}$ ions (R = NH_2, $NHCH_3$, $N(CH_3)_2$, $NHCH_2CO_2^-$); 25°, μ = 1.0.

The rate data for β_2-$[Co(trien)(glyglyOCH_3)]^{3+}$ agree with those obtained by Hay and Morris[21] using the pH-stat technique in the pH range 10–11 and published after the present study had been completed. However, these authors did not investigate the rate law over a sufficiently wide pH range and incorrectly interpreted the data they obtained. Ester hydrolysis was held to occur rapidly prior to attaining the desired pH, and the rate of subsequent base consumption was ascribed to both the β_2-$[Co(trien)(glyglyO)]^{2+}$ ion and its conjugate base. However, from their quoted pK_a of 9.41 for the chelated dipeptide acid[21] and that for glycine (9.75), it is clear that no base consumption can occur after ester hydrolysis; even using the pK_a of 11.2 obtained in the present study the conjugate base of β_2-$[Co(trien)(glyglyO)]^{2+}$ does not consume base and therefore a term involving this ion should not appear in their rate expression. Finally, to demonstrate that ester hydrolysis does not occur rapidly on titration to pH 11.1, the conditions used by Hay and Morris were repeated except that the reaction was quenched on reaching pH 11.1 (*ca.* 30 sec) by adding acid to pH 7. The products were sorbed onto and eluted from an ion exchange resin (Na^+ form) using 1 *M* $NaClO_4$ (pH ~6), and the 3+ band was recovered and estimated spectrophotometrically (71%). A blank experiment showed that $[Co(trien)(glyglyOH)]^{3+}$ and $[Co(trien)(glyglyOCH_3)]^{3+}$ eluted as 2+ and 3+ ions, respectively, under these conditions.

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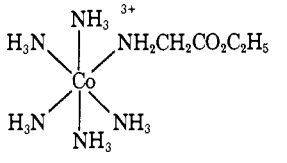
Table IV. Comparison of Second-Order Rate Constants for Hydroxide-Ion-Catalyzed Ester, Amide, and Peptide Hydrolysis Promoted by Co(III) (25°, μ = 1.0)

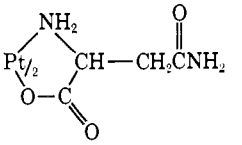
Complex	k',[a] M^{-1} sec^{-1}
$[Co(en)_2(glyOC_3H_7)]^{3+}$	$1.5 \pm 0.5 \times 10^6$ [b]
$[Co(en)_2(glyNH_2)]^{3+}$	25 ± 1
$[Co(en)_2(glyNHCH_3)]^{3+}$	1.6 ± 0.2
$[Co(en)_2(glyN(CH_3)_2)]^{3+}$	1.1 ± 0.2
$[Co(en)_2(glyglyO)]^{2+}$	2.6 ± 0.2
β_2-$[Co(trien)(glyNHCH_3)]^{3+}$	2 ± 1
β_2-$[Co(trien)(glyglyOCH_3)]^{3+}$	5 ± 1
β_2-$[Co(trien)(glyglyOC_3H_7)]^{3+}$	3 ± 1
β_2-$[Co(trien)(glyglyO)]^{2+}$	3 ± 1

[a] $k' = k_1k_3/(k_2 + k_3)$. [b] Reference 7.

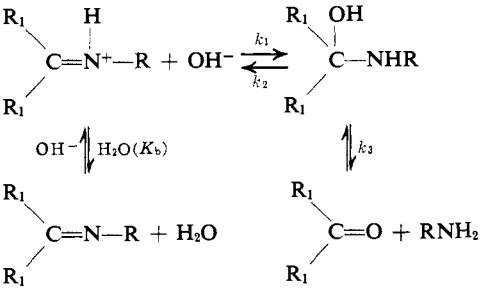
The second-order rate constants given in Table IV may be related to the bimolecular attack of solvent hydroxide at the carbonyl carbon of the amide bond.[22]

Oxygen tracer studies have shown that for the $[Co(en)_2(glyNH_2)]^{3+}$ ion this results in the incorporation of one oxygen atom from the solvent, and that the chelate ring remains intact during hydrolysis.[9] Results in glycine (Tables II and III) and dimethylamine (Table IId) buffers show that general-base-catalyzed addition of water and/or nucleophilic addition of the amine buffer to the carbonyl center is unimportant compared to the hydroxide path under the conditions. It is of interest to note that similar rates occur for the two Co(III) systems, and that glycinamide hydrolyzes some ten times faster than the methyl-substituted amides, which have similar rates to those for the dipeptide acids and esters. Comparison with the entry for $[Co(en)_2(glyOC_3H_7)]^{3+}$ (Table IV) demonstrates that amide bond hydrolysis in these systems is some 10^5–10^6 times slower than ester hydrolysis. A similar rate difference occurs in the uncoordinated molecules, $glyOC_2H_5$ (k = 0.6 M^{-1} sec^{-1} at 25°, μ = 0.1)[23] and $glyglyO^-$ ($k = 2.8 \times 10^{-6}$ sec^{-1} in 0.5 M NaOH, 25°).[20] However, the direct activation of the carbonyl group by the metal results in a $\sim 10^6$-fold enhancement in rate compared to the uncoordinated molecule. Monodentate amides and esters show only small rate enhancements. Thus, the second-order rate constants for ester hydrolysis in $[Co(NH_3)_5(NH_2CH_2CO_2C_2H_5)]^{3+}$ and $NH_2CH_2CO_2C_2H_5$ are 50 M^{-1} sec^{-1}[15] and 0.6 M^{-1} sec^{-1} (μ = 0.1),[23] respectively, and asparagine coordinated to Pt(II) through the NH_2 and CO_2^- groups hydrolyzes at a rate only twice that of the free ligand.[24]



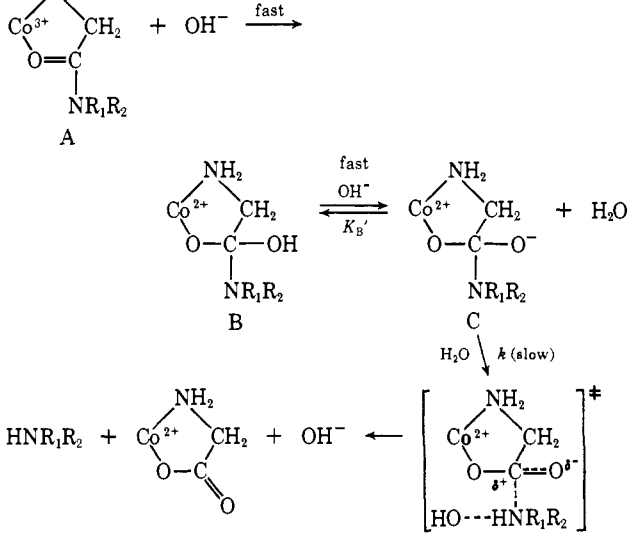


This mechanism is identical with that proposed for the hydrolysis of Schiff's base adducts in alkaline solution, which has been interpreted in terms of the



rate-determining addition of hydroxide to the protonated imine center.[25,26] For benzhydrylidenemethylamine (R_1 = C_6H_5, R = CH_3) the second-order rate constant $k = 7 \times 10^2$ M^{-1} sec^{-1} (25°, μ = 0.5)[26] is $\sim 10^2$ times larger than that obtained here for chelated glycine methylamide. The difference may reflect the relative polarizing ability of a proton on the imine nitrogen and $Co(N)_4^{3+}$ attached to the acyl function. Also, the imine is a poorer base ($K_b = 1.7 \times 10^{-7}$)[26] than the chelated deprotonated amide ($K_b = 10^{-3}$), suggesting that the amide carbon atom will be less susceptible to nucleophilic attack. A more interesting comparison is the effect of $Co(en)_2^{3+}$ and H^+ on the carbonyl oxygen. The second-order rate constants for acid-catalyzed hydrolyses of aliphatic amides are about 10^{-5} M^{-1} sec^{-1} at 25°.[27] The product of this value and the acid ionization constant $K_a = 10^{1.78}$ for glycinamide[28] ($glyglyOH$, $K_a = 10^{3.1}$)[29] gives a first-order rate of $10^{-3.22}$ sec^{-1} for water attack on the protonated amide at 25°. Strong evidence exists for protonation on oxygen in amides and peptides.[30] The comparable

(22) For a closely related reaction, evidence exists for the formation of an intermediate amine–alcohol complex: D. A. Buckingham, J. Dekkers, and A. M. Sargeson, unpublished work. For this reason the mechanism proposed here is of the sequential addition–elimination type, but at this time we do not wish to speculate if k_1 or k_3 is rate determining. However, the kinetic dissimilarity between the dimethylamide and other amide complexes at high pH implies that deprotonation at the alcohol function in the intermediate is not important, and eliminates a mechanism of the type shown below



with rapid irreversible addition of hydroxide followed by water-catalyzed loss of amine in the intermediate alcoholate ion. A similar mechanism involving fast reversible attack of OH^- at the carbonyl center (k_1, forward; k_2, reverse) gives the derived rate law $v_{[Co]} = kk_1[Co][H_2O][OH^-]^2/(k_2K_B' + k_1K_B'[OH^-] + k_1[OH^-]^2)$, where [Co] = [A] + [B] + [C], which is also inconsistent with the data at low pH.

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rate for the water path in $[Co(en)_2(glyNH_2)]^{3+}$ is unknown, but if it is taken as 10^{11} times smaller than k', as found for chelated glycine esters in $[Co(en)_2(glyOC_3H_7)]^{3+}$,[7] then a first-order rate constant of $\sim 10^{-10}$ sec^{-1} is obtained. This value is in agreement with an upper limit of 4.6×10^{-8} sec^{-1} calculated from the failure to observe chromatographically any hydrolysis of $[Co(en)_2(glyNH_2)]^{3+}$ in 1 *M* acid over 5 days, assuming a 2% detection limit. It is clear that the rate of water attack on the protonated amide is some 10^6 times faster than the similar reaction at the $Co(en)_2^{3+}$ activated amide.[31] From electrostatic considerations alone, a similar or even larger rate difference might be expected for the hydroxide path.

Some comment is possible concerning the reaction paths for peptide hydrolysis promoted by the $[CoN_4(H_2O)(OH)]^{2+}$ ions.[2,3] For the trien system some β_2-$[Co(trien)(glyglyOEt)]^{3+}$ is formed during peptide hydrolysis,[4] and the rates obtained in the present study imply that hydrolysis in this ion occurs *via* the same mechanism as that for the $[Co(en)_2(glyNH_2)]^{3+}$ ion. Oxygen tracer studies for the latter species at pH 9 require hydrolysis to proceed without opening of the chelate ring,[9] and the same result is therefore inferred for the trien system. Also, while these results require the chelated intermediate to be on the reaction path to products, this ion can be formed, at least in part, from $[Co(trien)(OH)(glyglyOR)]^{2+}$ produced *via* initial monodentate coordination of the dipeptide esters. Related studies with β_2-$[Co(trien)(NH_3)OH]^{2+}$ [32] imply that exchange between solvent water and bound hydroxide is fast at 25°, pH 7–8, and a similar result is therefore likely in the $[Co(trien)(OH)(glyglyOR)]^{2+}$ ion. The chelated ester could thus be formed *via* competition between solvent H_2O and carbonyl oxygen during hydroxide exchange. Hydrolysis *via* the chelated peptide then only requires water exchange to be fast relative to intramolecular hydrolysis by coordinated hydroxide in the $[Co(trien)(OH)(glyglyOR)]^{2+}$ ion. Related studies have demonstrated that hydrolysis by coordinated ^-OH in $[Co(en)_2(OH)(glyNHR)]^{2+}$ is rapid compared to intermolecular hydrolysis in $[Co(en)_2(glyNHR)]^{3+}$,[9] but since the rate of OH^- exchange is not known for the trien complex no decision can be made concerning the paths for decay of the hydroxo–peptide species.

Hydroxide exchange in the corresponding $[Co(en)_2(OH)NH_3]^{2+}$ ion is slow (no detectable exchange in 3 days at pH 9.5, 30°[33]), and since peptide hydrolysis promoted by the bis(en) system may occur *via* the $[Co(en)_2(OH)(glyNHR)]^{2+}$ ion, the inference is that hydrolysis may occur *via* an intramolecular process in this ion.

Biological Implications. In view of the recent interest generated by the X-ray results of Lipscomb and coworkers for the Zn^{2+}-activated carboxypeptidase *A*-glycyltyrosine complex,[34,35] it is of interest to briefly compare the activation afforded by metal ions with the enzyme rate enhancements. At substrate concentrations where zero-order kinetics are observed ($>10^{-2}$ *M*) the rate of hydrolysis of the peptide bond in benzoyl-glycyl-L-phenylalanine by [Zn(CPDA)] is $\sim 7 \times 10^4$ *M* min^{-1} at pH 7.5 and 25°.[36] Similarly, at 40° and pH ~9, 50% hydrolysis of L-leucinamide and L-leucylglycine by Mn^{2+}-activated leucine aminopeptidase requires 50 and *ca.* 30 min, respectively.[37] The similar Co(III)-promoted hydrolyses would require ~8 and 200 hr for 50% hydrolysis at pH 9 and 7.5, respectively, assuming rates similar to those found here for the glycine residues. Although the mechanistic implications of the kinetic results for the enzyme reactions remain uncertain, it is clear that the rates are some $10–10^4$ times faster than those afforded by Co(III) activation. It is unlikely that divalent metal ions such as Zn^{2+} and Mn^{2+} would be more effective at polarizing the carbonyl group of a peptide bond than Co(III), and unpublished results[7] from this laboratory demonstrate that nitrogen bases and carboxylate oxygen in general are less efficient than hydroxide as nucleophiles or general bases in the lysis of chelated carbonyl derivatives. Thus, although direct activation of the carbonyl center by metal ions can result in a large rate enhancement for hydrolysis ($\sim 10^6$), additional activation in the enzyme system seems necessary. This may arise from neighboring group participation, and we will shortly report results for a cobalt(III) model where close to enzymic activity is observed without direct activation of the carbonyl center by chelation.[9]

Acknowledgment. We wish to acknowledge support in the form of a National Institutes of Health Postdoctoral Fellowship (C. E. D.), No. 1 FO2 AM 37797-01.

(31) A similar value is arrived at from consideration of the acid dissociation constants of $(NH_3)_5Co^{3+}$–OH_2 (pK_a = 6.2) and H^+–OH_2 (pK_a = 1.7). The hydrated proton is $10^{7.9}$ times more polarizing than the $(NH_3)_5Co^{3+}$ moiety from these equilibrium data.

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