Optically Active Co-ordination Compounds. Part XXX.[1] Mono-dipeptide Complexes of Cobalt(III)

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Chloride and perchlorate salts of the cations $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$ and $[Co(dien)(\alpha_1\alpha_2)]^+$, where $\alpha_1\alpha_2H_2$ is a dipeptide, and dien = diethylenetriamine, have been prepared and characterised. A general structure for these complexes is proposed, in which the dipeptide ligand is terdentate and planar, having lost the amide proton. The protonation of $[Co(dien)(glygly)]^+$ and $[Co(NH_3)_3(glygly)]^+$ has been studied in detail by electronic absorption and ^1H n.m.r. spectroscopy; complexes in which the oxygen atom of the amide group is protonated have been isolated from solutions in concentrated perchloric acid. In hot concentrated hydrochloric or hydrobromic acid $[Co(NH_3)_3(glygly)]ClO_4$ gives crystalline $[Co(NH_3)_3(glyglyH_2)X]X_2$ (X = halide) containing a bidentate peptide ligand. Concentrated aqueous solutions of this product deposit crystalline $[Co(NH_3)_3(glyglyH)X]X$, in which the peptide remains bidentate. Dilute aqueous solutions of $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2$ undergo aquation as well as deprotonation; on the addition of base ring closure occurs to give $[Co(NH_3)_3(glygly)]^+$. $[Co(NH_3)_3(glygly)]ClO_4$ can be recovered from dilute aqueous solutions of $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2$ after ageing and addition of sodium perchlorate. The disproportionation of $[Co(dien)(\alpha_1\alpha_2)]^+$ and $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$ in alkaline solution and the base-catalysed exchange at the methylene group of the C-terminal amino-acid are reported, together with some preliminary observations on the activation of the peptide towards condensation with aldehydes at the C-terminal residue.

PREVIOUS investigation of the complexes of cobalt(III) with the dipeptides as ligands has been limited to the anionic bis-dipeptide series, $[Co(\alpha_1\alpha_2)_2]^-$.† The preparation and properties of the ion $[Co(glygly)_2]^-$ have been studied[2] and an *X*-ray structural analysis of the ammonium salt has been carried out.[3] Optically active dipeptides give analogous complexes.[4-6] In acid solutions protonation of the amide oxygen of both the peptide ligands occurs giving cationic complexes of the type $[Co(\alpha_1\alpha_2H)_2]^+$ which have been studied by proton magnetic resonance,[7,8] circular dichroism,[7] and *X*-ray crystallography.[8,9]

We now report the preparation of cationic mono-dipeptide complexes of the types $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$ and $[Co(dien)(\alpha_1\alpha_2)]^+$. These complexes have been characterised by elemental microanalysis, electrophoresis, conductivity, electronic spectra, and (where appropriate) circular dichroism spectra. Their reactions in acidic and alkaline solution have been examined and some observations on the activation of the dipeptide towards base-catalysed proton exchange and condensation with aldehydes are reported.

RESULTS AND DISCUSSION

Preparation and Characterisation of Complexes.—Two possible routes for the preparation of mono-dipeptide–ammine complexes were investigated. Firstly by analogy with the work of Manyak *et al.*[10,11] on tripeptide complexes, $[Co(NH_3)_5Cl]Cl_3$ was treated with glycylglycine in the presence of ammonium hydroxide. Low yields of $[Co(NH_3)_3(glygly)]^+$ were obtained both as the chloride and perchlorate salts. Starting from $[Co(NH_3)_6]Cl_3$, or using sodium hydroxide or potassium hydroxide in place of ammonia, yielded $[Co(glygly)_2]^-$ but no mono-dipeptide complex. Reaction of $[Co(dien)Cl_3]$ with glycylglycine and sodium hydroxide, and subsequent chromatography on Sephadex G10, gave among other products $[Co(dien)(glygly)]^+$ which was identified from the electronic spectrum but was not isolated.

The mono-dipeptide complexes of both series were most conveniently prepared by the oxidation, with hydrogen peroxide, of solutions containing cobalt(II) chloride, peptide, and either an excess of ammonia or the stoicheiometric amount of diethylenetriamine. In a few cases, particularly with glycylglycine as ligand, slow evaporation of the reaction mixture gave brick-red crystals or precipitates of the required product as its chloride salt: usually only gums or glasses were obtained. The addition of an excess of sodium perchlorate to the reaction mixture followed by slow evaporation of the solution permitted isolation of the solid perchlorate salts of the following cations: $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$ ($\alpha_1\alpha_2$ = glygly, gly-L-ala, or L-alagly) and $[Co(dien)(glygly)]^+$. Aqueous solutions containing the perchlorates of $[Co(NH_3)_3(L\text{-ala-}L\text{-ala})]^+$, $[Co(dien)(gly\text{-}L\text{-ala})]^+$, and $[Co(dien)(L\text{-ala-gly})]^+$ failed to crystallise, but were purified by chromatography on Sephadex G10 until no further change of spectroscopic properties was achieved.

The cationic nature of these complexes was established by paper electrophoresis (in 0·05M-KCl the orange-red

† $\alpha_1\alpha_2$ Represents the dianion of the dipeptide $\alpha_1\alpha_2H_2$, where α_1 is the N-terminal component; gly = glycine, ala = alanine, threo = threonine.

[1] Part XXIX, R. D. Gillard, J. R. Lyons, and C. Thorpe, *J.C.S. Dalton*, 1972, 1584.
[2] R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Co-ordination Chem. Rev.*, 1966, **1**, 263.
[3] R. D. Gillard, E. D. McKenzie, R. Mason, and G. B. Robertson, *Nature*, 1966, **209**, 1347.
[4] R. D. Gillard, P. M. Harrison, and E. D. McKenzie, *J. Chem. Soc.* (*A*), 1967, 618.
[5] M. S. Michailidis and R. B. Martin, *J. Amer. Chem. Soc.*, 1969, **91**, 4683.
[6] N. C. Payne, Ph.D. Thesis, University of Sheffield, 1967.
[7] R. D. Gillard, P. R. Mitchell, N. C. Payne, and D. A. Phipps, in preparation.
[8] M. T. Barnet, H. C. Freeman, D. A. Buckingham, I-Nan Hsu, and D. Van der Helm, *Chem. Comm.*, 1970, 367.
[9] E. D. McKenzie, *J. Chem. Soc.* (*A*), 1969, 1655.
[10] A. R. Manyak, C. B. Murphy, and A. E. Martell, *Arch. Biochem. Biophys.*, 1955, **59**, 373; but see ref. 11.
[11] H. C. Freeman and G. Robinson, *J. Chem. Soc.*, 1965, 3194.

band moved towards the cathode in all cases). Conductivity measurements on the complexes isolated as solids indicated 1 : 1 electrolytes (see Table 1).

TABLE 1

Properties of cations of the type $[CoL_3(\alpha_1\alpha_2)]^+$

L_3	$\alpha_1\alpha_2$	Absorption spectrum $\lambda_{max}(\varepsilon_{max})$	Circular dichroism spectrum $\lambda_{max}/nm(\Delta\varepsilon_{max})$	Conductivity [a] Ω^{-1}
$(NH_3)_3$	glygly	489 (217)		90·5
		338 (118)		
$(NH_3)_3$	gly-L-ala	486 (205)	504 (−1·74)	85·0
		345 (88)	348 (−0·48)	
$(NH_3)_3$	L-alagly	485 (205)	508 (−0·55)	
		345 (96)	420 (+0·05)	98·5
			346 (−0·23)	
$(NH_3)_3$	L-ala-L-ala	485 (—)	504 (−2·20 [b])	
		345 (—)	346 (−0·72 [b])	
dien	glygly	482 (284)		90·6
		340 (97)		
dien	L-alagly	482 (—)	482 (−0·46 [b])	
		345 (—)	348 (−0·20 [b])	
dien	gly-L-ala	480 (—)	486 (−0·150 [b])	
		345 (—)	346 (−0·35 [b])	

[a] In H_2O (*ca.*10^{-3}M). [b] Calculated using $\varepsilon_{max.}$ measured for the glygly analogue.

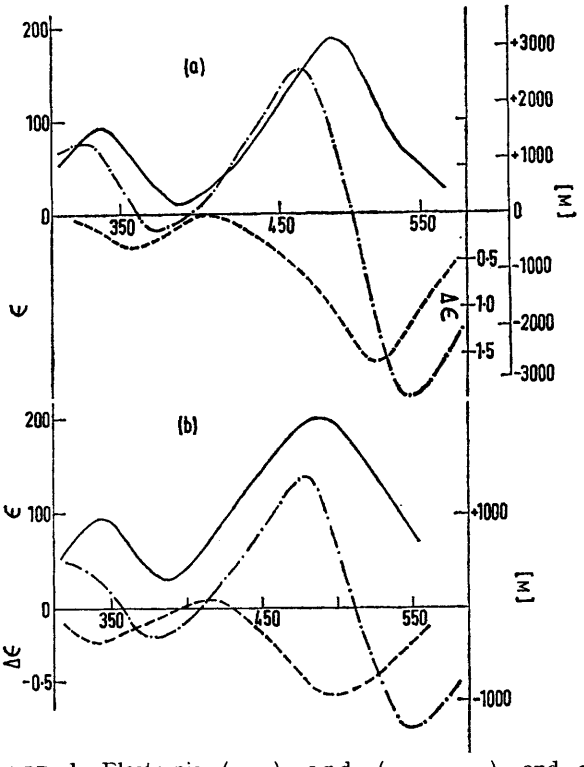


FIGURE 1 Electronic (——), o.r.d. (— · — · —), and c.d. (– – – –) spectra of (a) $[Co(NH_3)_3(gly\text{-}L\text{-}ala)]^+$ and (b) $[Co(NH_3)_3(L\text{-}ala\text{-}gly)]^+$

$[Co(NH_3)_3(glygly)]Cl$ was shown by Evans method [12] to be diamagnetic, confirming the presence of cobalt(III).

† The term protonation is here taken to refer to addition of either H^+ or D^+.

[12] D. F. Evans, *J. Chem. Soc.*, 1959, 2003.
[13] H. Dobie and W. O. Kermack, *Biochem. J.*, 1955, **59**, 246.
[14] D. Rogers, M. D. Shami, D. M. Williams, personal communication.

The electronic, i.r., and p.m.r. spectra and, for the complexes isolated as solids, elemental microanalysis were in accord with the formulation $[CoL_3(\alpha_1\alpha_2)]X,nH_2O$ (L = NH_3 or ½ dien). Table 1 and Figure 1 contain data on the electronic absorption spectra and optical activity of these complex ions. The spectroscopic properties of the glycylglycine complexes are discussed in detail later.

The formulation of the mono-dipeptide complexes as unipositive cations implies that in every case the amide nitrogen is ionised, *viz.* $NH_2{\cdot}CHR'{\cdot}CO{\cdot}\bar{N}{\cdot}CHR''{\cdot}CO_2^-$, as in the bis-dipeptidatocobaltate(III) [3-6] and mono-dipeptidatocopper(II) complexes.[13] The trigonal planar nature of the deprotonated nitrogen atom dominates the stereochemistry of the peptide ligand, in which the chelate rings are co-planar according to the preliminary results of the *X*-ray structural analysis of $[Co(NH_3)_3(gly\text{-}L\text{-}ala)]ClO_4$.[14] The absence of ring puckering is typical of aminoacidate complexes [15] which generally do not exhibit this phenomenon unlike the 1,2-diamine analogues.[16-18]

TABLE 2

Electronic spectra of the glycylglycine complexes in acidic aqueous solution: sh = shoulder

Complex	$\lambda_{max.}$/nm ($\varepsilon_{max.}$)	Conditions
$[Co(NH_3)_3(glygly)]ClO_4$	489 (217), 338 (118)	H_2O
,,	489 (204), 341 (128)	1N-H_2SO_4
,,	488 (201), 342 (134)	4N-H_2SO_4
,,	487 (197), 342 (140)	6N-H_2SO_4
,,	488 (191), 342 (126)	9N-H_2SO_4
,,	495 (179), 338 (117)	18N-H_2SO_4
,,	499 (178), 339 (118)	24N-H_2SO_4
,,	500 (176), 330 (118)	36N-H_2SO_4
$[Co(dien)(glygly)]Cl$	482 (284), 340 (71)	H_2O
,,	478 (278), 343 (82)	1N-H_2SO_4
,,	476 (273), 335 (97)	18N-H_2SO_4
,,	480 (245), 325 (~200)	36N-H_2SO_4
$[Co(NH_3)_3(glyglyH_2)Cl]Cl_2$	524 (97), sh350	H_2O (freshly prepd.)
,,	489 (194), 344 (86)	H_2O (1 week at ~25°)
,,	527 (82), 362 (—)	9N-H_2SO_4
,,	529 (—), 365 (—)	24N-H_2SO_4
,,	528 (87), 364 (80)	11N-HCl
,,	525 (—), 357 (—)	11N-HCl *
$[Co(NH_3)_3(glyglyH)Cl]Cl$	524 (103) sh354 (76)	H_2O
,,	527 (92) sh362 (85)	11N-HCl
$[Co(NH_3)_3(glyglyH_2)Br]Br_2$	535 (—)	H_2O (freshly prepd.)
,,	535 (ca. 70)	8N-HBr *

* Prepared *in situ* from $[Co(NH_3)_3(glygly)]ClO_4$ in hydrohalic acid.

Protonation † *of* $[Co(NH_3)_3(glygly)]^+$ *in the Absence of Co-ordinating Anions.*—The glycylglycine complex is described in detail as an example of the behaviour of the mono-dipeptide species in acid solution. In sulphuric acid solution the protonation of $[Co(NH_3)_3$-

[15] H. C. Freeman, *Adv. Protein Chem.*, 1967, **22**, 257.
[16] R. D. Gillard and N. C. Payne, *J. Chem. Soc.* (*A*), 1969, 1197.
[17] E. J. Corey and J. C. Bailar, *J. Amer. Chem. Soc.*, 1959, **81**, 2620.
[18] J. R. Gollogly and C. J. Hawkins, *Inorg. Chem.*, 1970, **9**, 576.

$(glygly)]^+$ can be detected by changes in the electronic spectrum. Of the data summarised in Table 2 and Figure 2 the most informative are those on the lowest energy *d–d* band, which moves first to slightly shorter wavelength and then to longer wavelength as the pH is decreased. These shifts are reversible. The first shift (489 to 487 nm) is smaller but comparable with that observed for $[Co(glygly)_2]^-$ (520 to 500 nm);[9] however the bis-dipeptide complex decomposes under the strongly acidic conditions required to show the second shift. The changes reflect successive addition of two protons (the second of which may be incomplete) and are

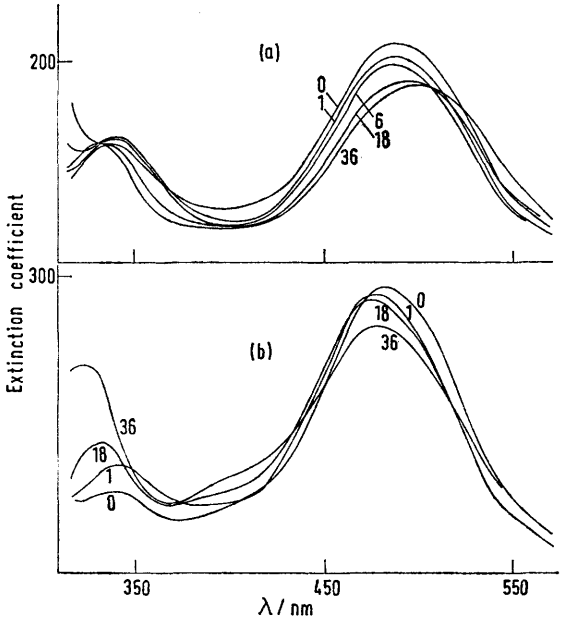


FIGURE 2 Electronic spectra of (a) $[Co(NH_3)_3(glygly)]^+$ in H_2SO_4 (0, 1, 6, 18, 36N); (b) $[Co(dien)(glygly)]^+$ in H_2SO_4 (0, 1, 18, 36N)

discussed in detail below. On standing at room temperature for 24 h these acidic solutions are stable but heating solutions in conc. H_2SO_4 on a water-bath overnight results in destruction of the complex (there is an irreversible shift of the lowest energy *d–d* band to still longer wavelength).

A mono-protonated glycylglycine complex, $[Co(NH_3)_3(glyglyH)](ClO_4)_2$, has been prepared by repeated crystallisation of a solution of $[Co(NH_3)_3(glygly)]ClO_4,H_2O$ in 60% perchloric acid. The i.r. spectra of the starting complex and of the product are essentially similar except in the carbonyl stretching vibration region where the differences are consistent with the presence in the product of peptide protonated at the amide oxygen. The spectrum of the product shows a sharp band at 1715 cm^{-1} absent from the spectrum of $[Co(NH_3)_3(glygly)]ClO_4,H_2O$ but no absorption corresponding to the peak at 1620 cm^{-1} in the spectrum of the starting material. A similar shift (1605 to 1700 cm^{-1}) has been reported[8] on protonation of $[Co(glygly)_2]^-$ to give $[Co(glyglyH)_2]^+$.

The p.m.r. spectrum of $[Co(NH_3)_3(glygly)]^+$ freshly dissolved in neutral or slightly acid (pD *ca.* 6) D_2O shows broad peaks due to N–H protons of the ammine ligands and of glycylglycine at *ca.* τ 5·95 and 6·8, a singlet due to the C-terminal methylene group at τ 5·87 and a triplet ($J \simeq 6$ Hz) due to the N-terminal methylene group at τ 6·57. In neutral solution exchange of the N–H protons occurs readily giving a simplified spectrum with sharp singlets at τ 5·87 and 6·57.

The positions of both resonances are dependent on pH (slight quantitative differences were observed between solutions acidified with sulphuric acid and with hydrochloric acid). Between pD 2 and 0 the N-terminal CH_2 resonance shifts from τ 6·57 to 6·02 and that of the C-terminal CH_2 from τ 5·87 to *ca.* 5·65. The difference in magnitude of the shifts is evidence that protonation occurs at the amide oxygen and not at the peptide nitrogen: the size of the change in the resonance position is a measure of the distance of the methylene group from the site of protonation. The analogous shifts in the p.m.r. spectrum[7] of $[Co(glygly)_2]^-$ have been interpreted using the same argument and the conclusion reinforced by *X*-ray crystallographic study of the solid.[8] The pK * of protonation at the amide oxygen of $[Co(NH_3)_3(glygly)]^+$ is 0·43 ± 0·03, which is only slightly lower than for $[Co(glygly)_2]^-$, 0·6 ± 0·05, despite the difference in charge.

On increasing the acid concentration still further (pD < 0), the N-terminal CH_2 resonance is unaffected; but the C-terminal CH_2 resonance is shifted to τ 5·48 in conc. hydrochloric acid and to *ca.* τ 5·41 in 18M-sulphuric acid (though the shift may not be complete even in the latter solvent). We attribute this change, which is unknown for $[Co(glyglyH)_2]^+$, to the protonation of the carboxy-group. Alternative structures are shown as (III) and (IV) in the Scheme {which summarises all of the reactions of $[Co(NH_3)_3(glygly)]^+$ in acid solution}. For the following reasons we believe that the protonated carboxy-group remains co-ordinated to the metal [*viz.* (III)] and that no significant degree of ring opening and corresponding aquation at the cobalt takes place.

(i) The steady change in the resonance position of the C-terminal CH_2 group as the pD is lowered and the observation of only one resonance due to this whatever the acid concentration indicate that either ring opening is rapid and reversible or that (IV) is present in only very low concentration. The rapid ring-closure required by the former alternative is unlikely as carboxylates are not good donors in strongly acidic media.

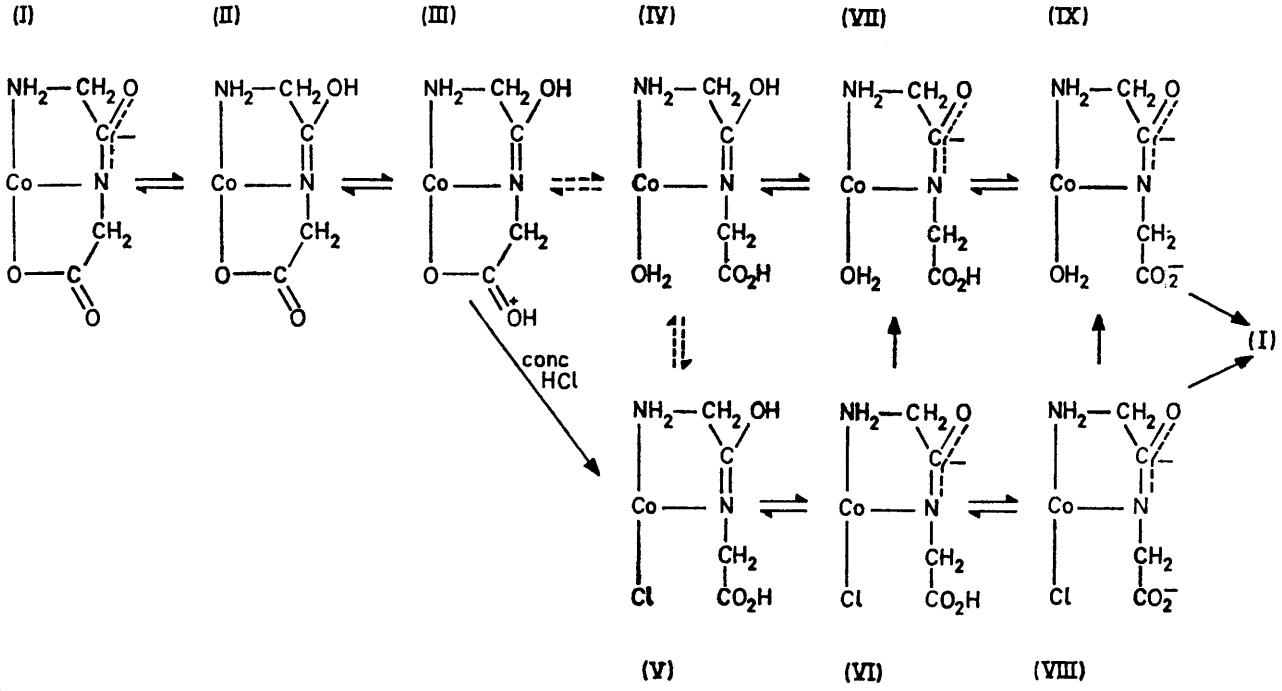
(ii) When ring opening does occur, on treating $[Co(NH_3)_3(glygly)]^+$ with hot concentrated hydrochloric acid (see later), there is an upfield shift of the resonance of the C-terminal CH_2 to τ 5·67. An upfield shift of similar magnitude would be expected if the aquo-complex (IV) were formed in perchloric acid.

* Values of pK quoted are as measured for D_2O solutions on a Radiometer pH 4 pH-meter, and are not corrected for the effect of D_2O solutions on the response of a glass electrode,[19] or for the primary isotope effect of the ionisation.[19]

[19] P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.

(iii) The absorption maximum in the electronic spectrum shifts to lower energy during this second protonation, from 487 to 500 nm for $[Co(NH_3)_3(glygly)]^+$ {and similarly from 476 to 480 nm for [Co(dien)-(glygly)]$^+$}. We know of no reports of electronic spectra similar protonation has been observed [21] for the amino-acid complexes $[Co(gly)_3]$ and $[Co(en)_2(gly)]^+$.

It has been suggested that the acid-catalysed hydrolyses of carboxylato-penta-amminecobalt(III) complexes [22] and monodentate amino-acid complexes,[23] such



SCHEME $[Co(NH_3)_3(glygly)]^+$ In acid solution; the ammine ligands and the charge carried by the complexes are omitted for clarity. There is no evidence for the reactions marked ⇌ and they are probably unimportant

of complexes of protonated carboxylates, but it would be expected that considerable weakening of the cobalt–oxygen bond would occur, whichever of the carboxylate oxygen atoms were protonated. The observed shift is consistent with this but not with ring opening and aquation {which by analogy with the difference between $[Co(NH_3)_5(acetate)]^{2+}$ and $[Co(NH_3)_5H_2O]^{3+}$ (503 and 485 nm respectively),[20] should give a shift to higher energy}.

(iv) Finally it is possible to prepare solutions of an aquo bidentate-glycylglycinato-complex (VII) (see below). Acidification with concentrated sulphuric acid gives a large down-field shift of the N-terminal CH_2 group resonance and a small shift for the C-terminal CH_2 group, as expected for the protonation of the amide oxygen. This acidified solution, which presumably contains the protonated complex (IV), has a different p.m.r. spectrum to that of (I) in very strong acid.

This formation of a protonated co-ordinated carboxy-group of a dipeptide is surprising. No other examples have been reported, and under comparable conditions the bisdipeptide complexes are unstable. However as (*O*-glycinato)penta-amminecobalt(III), involve protonation of the donor oxygen:

$$[(NH_3)_5Co{-}O{-}\underset{\underset{O}{\|}}{C}{-}R]^{2+} + H^- \rightleftharpoons [(NH_3)_5Co{-}O{-}\overset{\overset{H}{|}}{\underset{\underset{O}{\|}}{C}}{-}R]^{3+} \xrightarrow{H_2O} [(NH_3)_5CoOH_2]^{3+} + RCO_2H$$

Furthermore evidence for the existence of protonated $[Co(NH_3)_5(carboxylato)]^{2+}$ species has been obtained in studies of the electroreduction of cobalt(III) complexes; at high acid concentration ($[H^+] > 0{\cdot}15$M for formato, $[H^+] > 0{\cdot}05$M, for acetato, butyrato, or valerato complexes) protonation of the complex occurs prior to electron transfer.[24]

However these protonated $[Co(NH_3)_5(carboxylato)]^{2+}$ complexes are very unstable, whereas solutions of complexes of the type $[CoL_3(glygly)]^+$ in strong acid seem

[20] C. K. Jørgensen, 'Absorption Spectra and Chemical Bonding in Complexes,' Pergamon, Oxford, 1962, p. 293; the spectrum of the acetato-complex was measured in this laboratory.

[21] R. D. Gillard and P. R. Mitchell, unpublished results.

[22] F. Monacelli, F. Basolo, and R. G. Pearson, *J. Inorg. Nuclear Chem.*, 1962, **24**, 1241.

[23] K. Ogino, T. Murakami, and K. Saito, *Bull. Chem. Soc. Japan*, 1968, **41**, 1615; T. Murakami, K. Ogino, H. Kobayashi, H. Yamazaki, and K. Saito, *Bull. Chem. Soc. Japan*, 1971, **44**, 120.

[24] A. A. Vlček, 'Advances in the Chemistry of Coordination Compounds,' ed. S. Kirschner, Macmillan, New York, 1961, p. 590—603.

quite stable. This is despite the fact that a considerable proportion of the co-ordinated carboxylate group of the complex is protonated and it would appear more likely that, in this case, the unco-ordinated oxygen is protonated.

The changes in the electronic and p.m.r. spectra of $[Co(dien)(glygly)]^+$ in acidic solution in the absence of co-ordinating anions are similar to those for $[Co(NH_3)_3(glygly)]^+$ and occur with comparable pK values: $0{\cdot}5 \pm 0{\cdot}03$ and *ca.* -1. Accurate determination of the second pK in either complex is complicated by incomplete protonation and difficulty of measurement of meaningful pH in strongly acidic solutions. However the associated shift in the electronic spectrum (see Table 2) requires a higher acid concentration for the diethylenetriamine complex, which therefore probably has the lower pK.

Crystallisation of a solution of $[Co(dien)(glygly)]Cl,2H_2O$ in 60% perchloric acid at room temperature gave an orange solid, which analysed as $[Co(dien)(glygly)]ClO_4,HClO_4,H_2O$. Protonation at the amide group is confirmed by the appearance in the i.r. spectrum of the product of a band at 1710 cm^{-1} and the absence of a band at ~1620 cm^{-1} (see above).

Reactions of $[Co(NH_3)_3(glygly)]^+$ *in Hydrochloric Acid.* Solutions of $[Co(NH_3)_3(glygly)]^+$ in concentrated hydrochloric acid showed the same changes of electronic and p.m.r. spectra as were observed in solutions in sulphuric acid. However the lower hydrogen-ion concentration meant that the second protonation does not occur completely. If the solution in concentrated hydrochloric acid is heated for 5 min on a steam-bath and allowed to stand overnight at 4°, purple crystals, which analysed as $Co(NH_3)_3(glygly)Cl,2HCl,H_2O$ are deposited. The electronic spectrum of a more dilute solution of $[Co(NH_3)_3(glygly)]^+$ in concentrated hydrochloric acid at *ca.* 30° showed a smooth change from $\lambda_{max.}$ 492 and 335 nm to $\lambda_{max.}$ 525 and 327 nm (after 24 h) with isosbestic points occurring at 398 and 552 nm. The final spectrum is closely similar to that of a solution of the purple crystalline product dissolved in concentrated hydrochloric acid: the spectrum of the product dissolved in water is different.

On warming a solution of $[Co(NH_3)_3(glygly)]^+$ in HCl/D_2O (8—10M), the peak due to the C-terminal CH_2 at τ 5·48 slowly disappears and is replaced by one at τ 5·67 due to a free CH_2CO_2H group. No change occurs in the resonance of the N-terminal CH_2 so the environment of this group in the product must closely resemble that in the protonated complex from which it is formed: in particular, the amide oxygen must still be protonated. For all the complexes studied in this work, those with a chelated $N{=}C(OH){\cdot}CH_2{\cdot}NH_2$ group show the resonance of the methylene protons at τ 6·0—6·1 whereas those with a chelated $\dot{N}{\cdot}CO{\cdot}CH_2{\cdot}NH_2$ group show the resonance at around τ 6·6. This is also true of the bis-dipeptide cobalt(III) complexes.[7]

The complex isolated from the reaction in concentrated hydrochloric acid when dissolved in acid (either HCl/D_2O or H_2SO_4/D_2O) has a p.m.r. spectrum identical to that observed on warming a solution of $[Co(NH_3)_3(glygly)]^+$. The purple colour of these latter solutions is sometimes masked by the intense blue of cobalt(II) chloro-species, which however account for less than 5% of the total complex present, as judged by the electronic spectrum.

A similar reaction in hydrobromic acid gave the bromo-analogue, $Co(NH_3)_3(glygly)Br,2HBr,H_2O$. No corresponding product could be obtained if $[Co(dien)(glygly)]^+$ was treated with concentrated hydrochloric or hydrobromic acids.

The product of treatment of $[Co(NH_3)_3(glygly)]^+$ with concentrated hydrochloric acid was assigned the structure (V), *viz.* $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2,H_2O$. The evidence consists of the p.m.r. spectrum and the following. The differences in the position of the lowest energy *d–d* band in the electronic spectra of $[Co(NH_3)_3(glygly)]Cl$ (489 nm), $Co(NH_3)_3(glygly)Cl,2HCl,H_2O$ (528 * nm) and $Co(NH_3)_3(glygly)Br,2HBr,H_2O$ (535 * nm) parallel those[20] between $[Co(NH_3)_5(acetate)]^{2+}$ (503 nm), $[Co(NH_3)_5Cl]^{2+}$ (535 nm), and $[Co(NH_3)_5Br]^{2+}$ (549 nm). The i.r. spectrum of $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2,H_2O$ is characterised by two sharp bands at 1680 and 1725 cm^{-1}, which are absent from the spectrum of $[Co(NH_3)(glygly)]ClO_4,H_2O$. The bromo-analogue $[Co(NH_3)_3(glyglyH_2)Br]Br_2,H_2O$ also has bands at 1680 and 1725 cm^{-1}. These absorptions are associated with free protonated carboxy-group (generally observed in the range 1700—1750 cm^{-1}, *e.g.* in quinquedentate ethylenediaminetetra-acetic acid complexes[25]) and with the protonated amide group (see above).

The mechanism of formation of (V) from (I) is envisaged in terms of species (I)—(V). The failure of $[Co(dien)(glygly)]^+$ to undergo a similar series of reactions, even on prolonged treatment with concentrated halogen acids, is surprising and the explanation obscure.

An analogous reaction is shown by the cobalt(III) ethylenediaminetetra-acetate complex which will add HCl with ring opening:[26]

$$[Co(edta)]^- \underset{H_2O}{\overset{HCl}{\rightleftharpoons}} [Co(edtaH)Cl]^-$$

Comparable reactions are known for amino-acidate complexes, for example in the platinum(II)–glycine system:[27]

$$trans\text{-}[Pt(gly)_2] \underset{H_2O}{\overset{HCl}{\rightleftharpoons}} trans\text{-}[Pt(glyH)_2Cl_2]$$

Similarly compounds of the type $[M^{III}(gly)_2(glyH)Cl]$, $[M^{III}(gly)(glyH)_2Cl_2]$, and $[M^{III}(glyH)_3Cl_3]$ have been

* In concentrated hydrohalic acid to minimize hydrolysis.

[25] K. Nakamoto, 'Infrared spectra of Inorganic and Co-ordination Compounds,' Wiley, New York, 1963, p. 206; and references therein.

[26] F. P. Dwyer and F. L. Garvan, *J. Amer. Chem. Soc.*, 1958, **80**, 4480.

[27] F. W. Pinkard, E. Sharratt, W. Wardlaw, and E. J. Cox, *J. Chem. Soc.*, 1934, 1012.

obtained from $[M(gly)_3]$ and HCl for M = cobalt [28] and chromium,[29] although the structures are unknown.

Reactions of $[Co(NH_3)_3(glyglyH_2)Cl]^{2+}$.—Aqueous solutions of $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2$ are strongly acidic: a 0·08M-solution has a pH of 0·7. (This is to be expected in view of the ready ionisation of the protonated amide group). On standing at room temperature or at 33° saturated aqueous solutions of $[Co(NH_3)_3(glyglyH_2)Cl]Cl_2$ deposited reddish crystals which analysed as $Co(NH_3)_3(glygly)Cl,HCl,H_2O$, *i.e.* only one additional mole of HCl is present. This complex was assigned structure (VI), *viz.* $[Co(NH_3)_3(glyglyH)Cl]Cl,H_2O$, by arguments parallel to those used for (V). The ready interconversion of (V) and (VI) by adjustment of pH and the large change in the position of the N-terminal methylene resonance [τ 6·02 for (V) and τ 6·63 for (VI)] compared with that for the C-terminal methylene [τ 5·73 for (V) and τ 5·76 for (VI)] are points of similarity with the protonation of the amide carbonyl oxygen in (I) to give (II). The i.r. spectrum of complex (VI) shows a sharp absorption at 1700 cm^{-1} due to the free protonated carboxy-group and a peak at 1615 cm^{-1} due to the unprotonated amide carbonyl group. The pK of the protonation (VI) ⟶ (V) has not been measured accurately, owing to concomitant aquation to (VII) and (IV) respectively, but is *ca.* 0·5.

The electronic spectrum of dilute aqueous solutions of (V) (10^{-2}—10^{-3}M) set aside at room temperature slowly changed and after 24 h was closely similar to that of (I) in neutral solution. The rate and completeness of this process were diminished by raising the concentration of complex and increased by removal of chloride ions with silver nitrate. In experiments where the concentration (10^{-1}M; $\lambda_{max.}$ 492 nm) was sufficient to allow measurement of the p.m.r. spectrum, this was unchanged, *i.e.* not that of (I). Nevertheless after addition of sodium perchlorate, a red crystalline solid could be obtained from such solutions and was identified by analysis (for C, H, N) and by i.r. and electronic spectra as the perchlorate salt of (I), $[Co(NH_3)_3(glygly)]ClO_4,H_2O$.

The addition of base to strong aqueous solutions (10^{-1}M) of (V) did produce shifts in the p.m.r. spectrum, first corresponding to the complete deprotonation of (V) to (VI), and then a further, smaller shift of the C-terminal methylene, at *ca.* pH 4, corresponding presumably to deprotonation of the carboxylic acid group [*i.e.* giving a solution of (VIII)]. When the solution was set aside or warmed its pH slowly fell * and the peaks due to $[Co(NH_3)_3(glygly)]^+$, (I), appeared. As the pH fell, this reaction became slower; if sufficient base was added the solution was reconverted almost completely to a solution of (I).

We believe that the major product in aged strong solutions of (V), in the absence of base, is the aquo-complex (VII). This is consistent with the large shift in the electronic spectrum (from $\lambda_{max.}$ 524 to 490 nm on ageing) and with the absence of change in the p.m.r. spectrum compared with that of a freshly prepared aqueous solution of (V) (the environment of the protons on the peptide will be essentially unaffected by substitution at the cobalt). Attempts to isolate crystalline salts of any of the aquo-complexes, (IV), (VII), or (IX) have been unsuccessful. The isolation of $[Co(NH_3)_3(glygly)]ClO_4,H_2O$ from solutions of (VII), to which sodium perchlorate had been added, is presumably the result of the low solubility of the perchlorate salt of (I) compared to the perchlorate salts of (VII) and (IX).

The suggested reaction path is summarised in the Scheme, and relevant data on the electronic and p.m.r. spectra of the various complexes are given in Table 2 and Figure 3.

Aqueous solutions of the bromo-analogue of (V), *viz.* $[Co(NH_3)_3(glyglyH_2)Br]Br_2,H_2O$, when set aside at room temperature rapidly deposited crystalline $[Co(NH_3)_3(glyglyH)Br]Br,H_2O$, *i.e.* the analogue of (VI). This product was identified by analysis (for C, H, N) and by its i.r. spectrum which showed peaks at 1700 (sharp) and 1610 cm^{-1} [*cf.* the data for (VI) given above]. In very dilute aqueous solution the absorption spectrum of $[Co(NH_3)_3(glyglyH_2)Br]Br,H_2O$ changes from $\lambda_{max.}$ 535 to 489 nm. This process occurs at a faster rate than for the corresponding chloro complex (V).

Reactions of $[CoL_3(\alpha_1\alpha_2)]^+$ *in Basic Solution.*—Neutral aqueous solutions of the complexes are stable at room temperature, as shown by the absence of change in their visible and optical rotatory dispersion spectra over several days. When set aside in alkaline solution ($[OH^-]$ = 1N) or when boiled in the presence of activated charcoal the complexes of both series decompose:

$$[Co(NH_3)_3(\alpha_1\alpha_1)]^+ \longrightarrow [Co(\alpha_1\alpha_2)_2]^- + [Co(NH_3)_6]^{3+}$$

$$[Co(dien)(\alpha_1\alpha_2)]^+ \longrightarrow [Co(\alpha_1\alpha_2)_2]^- + [Co(dien)_2]^{3+}$$

However whereas $[Co(NH_3)_6]^{3+}$ decomposes rapidly in alkaline solution, so that $[Co(\alpha_1\alpha_2)_2]^-$ is the major decomposition product of $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$, both $[Co(dien)_2]^{3+}$ and $[Co(\alpha_1\alpha_2)_2]^-$ are stable, although $[Co(\alpha_1\alpha_2)_2]^-$ will decompose on prolonged heating on a steam-bath with 1M-base, under which conditions $[Co(dien)_2]^{3+}$ is almost unaffected.

Further insight into the behaviour of the mono-dipeptide complexes in basic solution was gained from their p.m.r. spectra in alkaline deuterium oxide. Figure 4 shows the spectrum of $[Co(NH_3)_3(glygly)]^+$. At pD > 11 the methylene protons of the C-terminal amino-acid residue exchange with the deuterons of heavy water with a half-life of *ca.* 1 h at 33°. Some

* The reason for this fall in pH is the one outstanding anomaly of this back reaction: however calculation of the amount of proton released indicates that about 0·5% of the complex undergoes some side-reaction.

[28] V. P. Ogoleva, *Trudy Dagestan, Sel'skokhoz, Inst.*, 1955, **7**, 130; *Referat. Zhur. Khim.*, 1956, Abstr. No. 15836; (*Chem. Abs.*, 1958, 52, 6046g: National Lending Library for Science and Technology, Great Britain, Translation No. RTS 6777, 1971).

[29] L. M. Volshtein, *Izvest. Sektora Platiny i Drug Blagorod. Metal*, 1952, **27**, 33 (*Chem. Abs.*, 1956, **50**, 16515d).

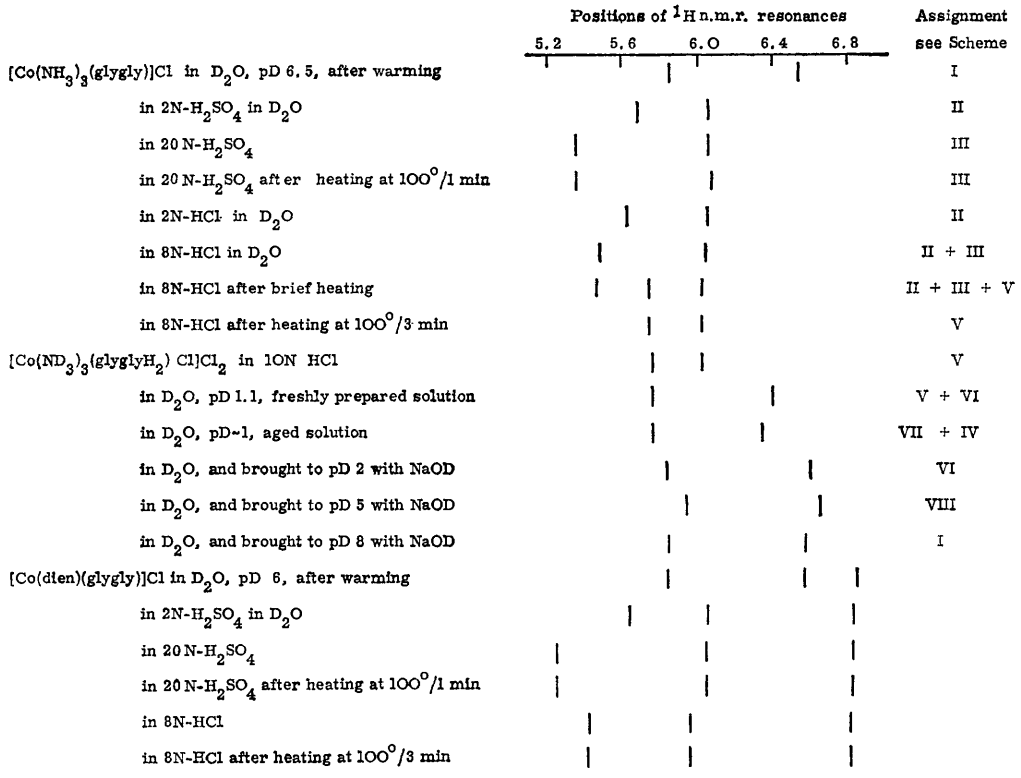


FIGURE 3 Diagrammatic p.m.r. spectra of $[Co(NH_3)_3(glygly)]^+$ derivatives and of $[Co(dien)(glygly)]^+$ showing the changes in line position on protonation and ring opening in acid solution

broadening of the p.m.r. peaks was observed during this time because of partial decomposition of the complex (a brown precipitate, presumably of cobaltic hydroxide, was formed). Proton exchange, with decomposition of the complex, was also observed in $[Co(NH_3)_3(L\text{-alagly})]^+$. With $[Co(dien)(glygly)]^+$ exchange was similarly restricted to the C-terminal methylene group, but without concomitant decomposition (Figure 4). The exchange

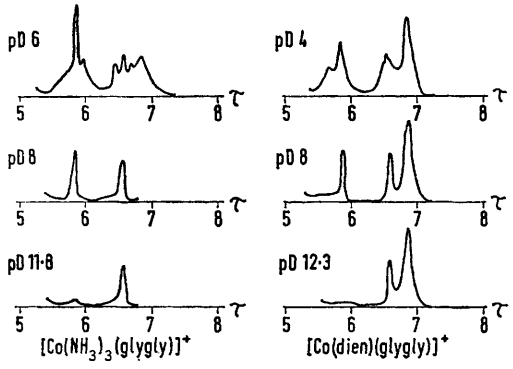


FIGURE 4 P.m.r. spectra of $[Co(NH_3)_3(glygly)]^+$ and $[Co(dien)(glygly)]^+$: exchange studies in alkaline solution

of the C-terminal methylene group of the mono-dipeptide complexes is much more rapid than with the bis-dipeptide complexes [7,30] with which rapid proton exchange requires a pH of 14.

In the case of dipeptides containing an optically active C-terminal residue the exchange is stereoselective. Thus the optical activity of $[Co(dien)(gly\text{-}L\text{-}ala)]^+$ was only reduced to 70% of its initial value during several exchange half-lives. Similar stereoselective exchange occurs in the bis-dipeptide complexes.[7,30]

Additional evidence for the activation of the peptide at the C-terminal residue is provided by some preliminary observations on the base-catalysed condensation of the co-ordinated peptide with aldehydes. For example $[Co(dien)(glygly)]^+$ and acetaldehyde were mixed in aqueous solution at pH *ca.* 11 for 12 h. Chromatography on Sephadex G10 gave a fraction with $\lambda_{max.}$ 490 nm, and a p.m.r. spectrum consistent with its being $[Co(dien)(glythreo)]^+$: a broad weak resonance at τ *ca.* 5·8 (>*CH*–*CH*< protons of threonine); a resonance at τ *ca.* 6·5 (CH_2 of glycine residue); a strong resonance at τ 6·8 (dien protons); and a resonance at τ *ca.* 8·3 (CH_3 of threonine). In a similar experiment starting from $[Co(NH_3)_3(glygly)]^+$ and acetaldehyde, the cobalt species in the reaction mixture were decomposed, the free peptide hydrolysed, and the component amino-acids analysed by paper chromatography. Two ninhydrin-positive spots were found with R_F values identical with those of authentic glycine and threonine run under the same conditions.

The formation of co-ordinated glycyl threonine from co-ordinated glycylglycine and acetaldehyde has already been observed for $[Co(glygly)_2]^-$ although this requires a much higher pH, with a corresponding increase in

[30] R. D. Gillard, P. R. Mitchell, and N. C. Payne, *Chem. Comm.*, 1968, 1150.

formation of polymers of acetaldehyde, compared with $[CoL_3(glygly)]^+$. However, with this latter type of complex simultaneous decomposition occurs, although this is less important with the diethylenetriamine than with the triammine analogue.

Optical Activity of $[CoL_3(\alpha_1\alpha_2)]^+$.—Unlike the bis-dipeptide complexes, the chelate rings forming the immediate environment of the cobalt are symmetrical, ruling out any configurational contribution to the optical activity. Also, as the peptide ligand is planar,[14] no conformational contribution to the optical activity can be invoked, at least for $[Co(NH_3)_3(\alpha_1\alpha_2)]^+$. Therefore the sole source of optical activity in the lower energy *d–d* band of the visible spectrum of the triammine mono-dipeptide complexes, is the 'vicinal effect.'[31] That $\Delta\varepsilon_{max.}$ for $[Co(NH_3)_3$(L-ala-L-ala)$]^+$ is the sum of $\Delta\varepsilon_{max.}$ for $[Co(NH_3)_3$(L-alagly)$]^+$ and $\Delta\varepsilon_{max.}$ for $[Co(NH_3)$(gly-L-ala)$]^+$ is a further demonstration that only the vicinal effect is responsible for the optical activity. The similarity in the magnitudes of $\Delta\varepsilon_{max.}$ for analogous triammine and diethylenetriamine complexes is readily understood as both have the same average ligand-field, charge, and geometry of the peptide chelate rings. Though the puckering in the diethylenetriamine chelate ring must give rise to a conformational contribution to the total circular dichroism it is unlikely that one conformation and hence one sign of contribution would be favoured.

Two points emerge from the circular dichroism results presented in Table 1. Firstly the magnitude of the circular dichroism is large. This must be contrasted with L-amino-acidatotetra-amminecobalt(III) ions which only show very small circular dichroism;[32] even the $[Co(NH_3)_3$(L-asp)$]^+$ complex with terdentate aspartic acid has[33] $\Delta\varepsilon = 0{\cdot}25$ whereas $[Co(NH_3)_3$(gly-L-ala)$]^+$ has $\Delta\varepsilon = 1{\cdot}5$. Finally there is a large difference in the magnitude of $\Delta\varepsilon_{max.}$ for $[CoL_3$(gly-L-ala)$]$ and $[CoL_3$(L-ala-gly)$]$, which is not easy to explain, as bis-dipeptide complexes of these two peptides have similar values of $\Delta\varepsilon_{max.}$.

EXPERIMENTAL

Elemental microanalyses for C, H, and N were carried out by Alfred Bernhardt, West Germany, or by Mr. Powell in this department. Halogen was determined gravimetrically as the silver salt.[34] Perchlorate was determined gravimetrically as the nitron salt.[35] Spectra were recorded on the following instruments: Unicam SP 800B and SP 600 spectrophotometers (electronic); Bendix N.P.L. 'Polarmatic' spectropolarimeter (Optical rotatory dispersion); Roussel-Jouan 'Dichrographe' spectrometer, model B (Circular Dichroism); Perkin-Elmer 137 spectrophotometer (i.r., as Nujol mulls) and Perkin-Elmer model R10 spectrometer (p.m.r. at 60 MHz and 14,092 gauss). pH Measurements were carried out on a Radiometer pH meter type pH 4 using a semi-micro electrode with which it was possible to measure the pH of 0·3—0·5 ml samples.

Glycylglycinatotriamminecobalt(III) *Perchlorate Monohydrate*.—Concentrated aqueous ammonia (20 ml) was added to glycylglycine (5·28 g, 0·04 mol) and cobalt(II) chloride hexahydrate (9·45 g, 0·04 mol) in water (20 ml). 30% Hydrogen peroxide (4 ml) was then added slowly with constant stirring and the solution was gently warmed for 10 min.

Concentrated ammonia (10 ml) was added and warming was continued for a further 15 min. Sodium perchlorate (6·2 g, 0·05 mol) was added, and the solution was set aside overnight at 4°. The finely divided red product was filtered off, washed with ice cold water (2 × 20 ml) and with ethanol, and dried at room temperature (yield: 9·7 g, 0·027 mol, 68%) (Found: C, 13·4; H, 4·7; N, 19·7. $C_4H_{17}ClCoN_5O_8$ requires C, 13·4; H, 4·8; N, 19·6%). The i.r. spectrum showed a broad absorption in the region 3600—3200 cm^{-1} and strong bands at 1620, 1580, 1360, 1200, 1110, 1080, 945, 920, 820, 720, 620, and 545 cm^{-1}. Ion exchange on Deacidite FF-IP (SRA65) anion exchange resin gave the chloride salt.

Glycyl-L-alaninatotriamminecobalt(III) *Perchlorate Monohydrate*.—The above preparation was used with the modification that activated charcoal (1·0 g) was added prior to the addition of the second quantity of ammonia and warming. After filtration the solution was brought to the point of precipitation by addition of ethanol (Found: C, 16·5; H, 5·6; N, 19·2. $C_5H_{19}ClCoN_5O_8$ requires C, 16·2; H, 5·1; N, 18·9%). This formulation is confirmed by *X*-ray crystallographic data.[14] The i.r. spectrum showed a broad absorption in the region 3600—3200 cm^{-1} and strong absorption at 1600, 1080, and 820 cm^{-1}.

L-Alanylglycinatotriamminecobalt(III) *Perchlorate Monohydrate*.—This complex was prepared by the method used for $[Co(NH_3)_3$(gly-L-ala)$]^+$ (Found: C, 16·0; H, 5·4; N, 18·8. $C_5H_{19}ClCoN_5O_8$ requires C, 16·2; H, 5·1; N, 18·9%). The i.r. spectrum shows absorptions in the region 3600 to 3150 (broad) and at 1600, 1360, 1080, and 820 cm^{-1}.

L-Alanyl-L-alaninatotriamminecobalt(III) *Perchlorate*.—This complex was prepared by the method used for $[Co(NH_3)_3$(gly-L-ala)$]^+$. It was not possible to isolate a solid product, but chromatography on Sephadex G10 showed the presence of three components. The two minor bands {corresponding in spectroscopic and chromatographic properties to $[Co(NH_3)_6]^{3+}$ and $[Co$(L-ala-L-ala)$_2]^-$} were rejected and the major red fraction was collected. This showed no tendency to separate into further fractions during either repeated chromatography or electrophoresis and was judged pure.

Glycylglycinatodiethylenetriaminecobalt(III) *Salts*.—A solution of glycylglycine (2·64 g, 0·02 mol) and cobalt(II) chloride hexahydrate (4·77 g, 0·02 mol) in water (10 ml) was treated with diethylenetriamine (2·14 g, 2·5 ml, 0·02 mol) and then 30% hydrogen peroxide (2·5 ml) with continuous stirring. The mixture was heated for 10 min at 40° and then activated charcoal (0·5 g) was added to it; the mixture was then stirred for a further 20 min. The mixture was filtered and sodium perchlorate (3·1 g, 0·025 mol) followed by ethanol was added to the filtrate. After several days at 4° the solution deposited $[Co(dien)(glygly)]ClO_4, 1\frac{1}{2}H_2O$ as a finely divided orange-red powder {Found:

[31] Y. Shimura, *Bull. Chem. Soc. Japan*, 1958, **31**, 315.
[32] T. Yasui, J. Hidaka and Y. Shimura, *Bull. Chem. Soc., Japan*, 1966, **39**, 2417.
[33] J. I. Legg and D. W. Cooke, *J. Amer. Chem. Soc.*, 1967, **89**, 6854.
[34] A. I. Vogel, 'Quantitative Inorganic Analysis,' Longmans, London, 2nd edn., 1951, p. 399.
[35] J. F. Flagg, 'Organic Reagents used in Gravimetric and Volumetric Analyses,' Interscience, New York, 1948, p. 243; and ref. 34, p. 583.

C, 23·0; H, 5·4; N, 16·6. [Co(dien)(glygly)]ClO_4,1½H_2O requires C, 23·0; H, 5·3; N, 16·7%}. The i.r. spectrum showed bands at 3580, 3300, 3260, 3160, 1620, 1595, 1355, 1085, 720, and 625 cm^{-1}. Crystallisation in the absence of added perchlorate ions gave [Co(dien)(glygly)]Cl,2H_2O (Found: C, 26·4; H, 6·4; N, 19·4. $C_8H_{23}ClCoN_5O_5$ requires C, 26·4; H, 6·4; N, 19·3%). Peaks in the i.r. spectrum occurred at 3440, 3360, 3080, 1655, 1615, 1590, 1350, 1085, 720, and 530 cm^{-1}.

L-*Alanylglycinatodiethylenetriaminecobalt*(III) *Perchlorate and Glycyl-*L*-alaninatodiethylenetriaminecobalt*(III) *Perchlorate.*—Solutions of these complexes were prepared by the method used for [Co(dien)(glygly)]ClO_4. Attempted crystallisation gave only brown crystals identified as bis(diethylenetriamine)cobalt(III) perchlorate by analysis (C, H, N), i.r. spectrum, and conductivity. The solutions were therefore purified as described for [$Co(NH_3)_3$-(L-ala-L-ala)]ClO_4.

Reactions of Glycylglycinatotriamminecobalt(III) *Perchlorate Monohydrate with Acids.*—(a) *Hydrochloric acid.* The complex (1·1 g) was dissolved in concentrated hydrochloric acid (10 ml) and heated on a steam-bath for 5 min. The colour of the solution changed from red to purple [excessive heating gave blue ($CoCl_4)^{2-}$]. The solution was left overnight at 4 °C, and then the purple crystals [$Co(NH_3)_3$(glyglyH$_2$)Cl]Cl_2,H_2O were collected, washed with methylated spirits, and dried at 100 °C for 30 min. The i.r. spectrum of the product showed two characteristic bands at 1725 and 1680 cm^{-1} with other bands at 3400—3000 (broad), 1600 (broad), 1410, 1350, 1305, 1255, 1205, 1110, 1050, 890, 845, 655, and 500 cm^{-1} (Yield 0·59 g, 52%) (Found: C, 13·2; H, 5·3; N, 18·9; Cl, 29·0. $C_4H_{19}Cl_3CoN_5O_4$ requires C, 13·1; H, 5·2; N, 19·1; Cl, 29·05%).

(b) *Hydrobromic acid.* The complex reacted with concentrated hydrobromic acid under the same conditions as in (a) above to give [$Co(NH_3)_3$(glyglyH$_2$)Br]Br_2,H_2O as a purple crystalline solid. The i.r. spectrum of the product shows the similar characteristic bands at 1725 and 1680 cm^{-1} with other bands at 3350—3050 (broad), 1600 (broad), 1350, 1310, 1290, 1253, 1225, 1195, 1180, 1100, 1060, 1030, 820, 722, 672, and 570 cm^{-1} (Yield 45·8%) (Found: C, 9·6; H, 3·8; N, 14·1; Br, 49·6. $C_4H_{19}Br_3CoN_5O_4$ requires C, 9·60; H, 3·85; N, 14·0; Br, 48·0%).

(c) *Perchloric acid.* The complex was dissolved in 60% $HClO_4$ and left at room temperature for 24 h. The red precipitate was filtered off, washed with methylated spirits and acetone, and dried at room temperature. *X*-Ray powder photographs, i.r. spectra, and analyses (C, H, N) show this material to be only partially protonated. Slow recrystallisation (3 weeks) from 60% $HClO_4$ gave red needles of the monoprotonated product {Found: C, 11·2; H, 3·7; N, 16·05. Calc. for [$Co(NH_3)_3$(glyglyH)]$(ClO_4)_2$: C, 10·9; H, 3·65; N, 15·9%}. The i.r. spectrum shows bands at 3310, 3250, 1715, 1595, 1325, 1295, 1100(broad), 1050, 985, 935, 870, 720, 620, 590, 580, 555, and 460 cm^{-1}.

Reactions of Chloro(glycylglycine)triamminecobalt(III) *Chloride Monohydrate.*—(*a*) In a dilute aqueous solution of the complex (*e.g.* 0·01M) there is a steady reaction which can be followed from $\lambda_{max.}$ 525 nm (ε_{525} 87) to $\lambda_{max.}$ 489 nm (ε_{489} 196); isosbestic points occur at 548 and 386 nm. When sodium perchlorate was added to this solution and the solution left to evaporate in air for several days red crystals of [$Co(NH_3)_3$(glygly)]ClO_4,H_2O, (I) separated out (Found: C, 13·4; H, 4·6; N, 20·0. Calc. for $C_4H_{17}ClCoN_5O_8$: C, 13·4; H, 4·8; N, 19·6%). The i.r. spectrum shows a broad band with peaks at 1625 and 1580 cm^{-1}; the other i.r. bands are also characteristic of (I), while the two sharp bands at 1725 and 1680 cm^{-1} characteristic of (V) and (VI), have disappeared.

(*b*) When the complex was dissolved in water to give a near saturated solution (*e.g.* 0·0725M) a red precipitate of [$Co(NH_3)_3$(glyglyH)Cl]Cl,H_2O started to settle out after a few minutes (Found: C, 14·75; H, 5·0; N, 21·1; Cl, 18·6. $C_4H_{18}Cl_2CoN_5O_4$ requires C, 14·55; H, 5·45; N, 21·2; Cl, 21·5%). The i.r. spectrum showed a sharp peak at 1700 cm^{-1}, a broad peak with two maxima at 1615 and 1580 cm^{-1}, and other peaks at 3320, 3262, 3180, 1410, 1352, 1323, 1305, 1292, 1264, 1208, 1120, 1066, 964, 874, 830, 760, 655, 580, and 517 cm^{-1}.

Reactions of Bromoglycylglycinetriamminecobalt(III) *Bromide Hydrate.*—When set aside at room temperature for a few minutes concentrated aqueous solutions of [$Co(NH_3)_3$(glyglyH$_2$)Br]Br_2,H_2O deposited crystals which analysed as [$Co(NH_3)_3$(glyglyH)Br]Br,H_2O (Found: C, 11·7; H, 4·3; N, 16·7. $C_4H_9Br_2CoN_5O_3$ requires C, 11·45; H, 4·30; N, 16·7%). The i.r. spectrum of this product showed peaks at 3310, 3260, 3180, 1700, 1610, 1578, 1408, 1293, 1190, 1115, 1060, 810, 720, 648, and 513 cm^{-1}. Dilute aqueous solutions of [$Co(NH_3)_3$(glyglyH$_2$)Br]Br_2,H_2O reacted rapidly at room temperature; the electronic spectrum showed $\lambda_{max.}$ at 489 nm after 12 h.

Reaction of Glycylglycinatodiethylenetriaminecobalt(III) *Chloride Dihydrate in Perchloric Acid.*—The addition of methylated spirit to a saturated solution of [Co(dien)-(glygly)]Cl,2H_2O in 60% perchloric acid at room temperature caused the precipitation of [Co(dien)(glygly)]-ClO_4,$HClO_4$,H_2O as an orange powder, which was washed with methylated spirit and with ether and stored *in vacuo* over silica gel [Found, Sample (1): C, 18·8; H, 4·0; N, 13·3; ClO_4, 39·1. Found, Sample (2): C, 18·8; H, 4·5; N, 13·4. $C_8H_{22}Cl_2CoN_5O_{12}$ requires C, 18·85; H, 4·35; N, 13·75; ClO_4, 39·0%]. The i.r. spectrum showed peaks at 3310, 3260, 3220, 1710, 1595, 1100 (broad), 720, and 620 cm^{-1}. In the absence of added methylated spirit, solutions of the starting complex in perchloric acid failed to crystallise (or deposited only very small quantities of product) even after several days at 4°.

Reactions in Basic Solution.—The experiments described in the Results section are largely self-explanatory. Products were identified, after chromatographic separation on Sephadex G10, by their electronic spectrum and in some cases additionally by their p.m.r. or i.r. spectrum and microanalysis.

The Reaction of Acetaldehyde with $[CoL_3(glygly)]^+$.—A solution of glycylglycinatotriamminecobalt(III) chloride (2·7 g, *ca.* 0·01 mol) and acetaldehyde (2·2 g, 0·05 mol) in water (250 ml) was adjusted to pH 12 with 1N-sodium hydroxide and was then set aside for 12 h. The solution was neutralised with dilute hydrochloric acid, concentrated *in vacuo* to 25 ml, and filtered through a small column of silica gel (to remove aldehyde polymers and precipitates). The eluant was chromatographed on Sephadex G10 and fractions with $\lambda_{max.} \simeq 490$ nm were collected, and pooled. After concentration the solution was rechromatographed and the leading fraction was separated and evaporated to dryness. The p.m.r. spectrum of this product dissolved in deuterium oxide was recorded (see text).

In another experiment the product was dissolved in water (10 ml) and the solution was adjusted to pH 8·5 with

sodium hydroxide (0·1M); and hydrogen sulphide was bubbled through the solution for 15 min. The solution was then adjusted to pH 3·5 with hydrochloric acid (0·1M) and the cobalt sulphide was filtered off. The whole process was then repeated. The resulting solution was evaporated to dryness on a rotatory evaporator and extracted with 2-methoxyethanol (2 × 2 ml). The combined extracts were taken to dryness and redissolved in constant-boiling hydrochloric acid (1 ml). The solution was placed in a glass tube, which was sealed, and heated to 110° for 24 h. The hydrolysate was cautiously evaporated to dryness, taken up in water (1 ml), and the process repeated twice to remove excess of hydrochloric acid. The resulting solution was chromatographed on Whatman No. 1 paper.

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