data recorded below 1000 K have been used in the model calculations.

The thermodynamics of the Nd-Ga-Cl and Nd-Al-Cl[5] complexes with the same stoichiometry are similar. It is interesting to note that the difference in the enthalpies or entropies for the formation of the Nd-Al-Cl and Nd-Ga-Cl complexes per 0.5 M'_2Cl_6 molecule (M' = Al, Ga) is nearly constant in the sequence $NdM'Cl_6$-NdM'_3Cl_{12}-NdM'_4Cl_{15}. The importance of retaining the coordination number of the central transition-metal ion M in $MAl_2Cl_8(g)$ as in the solid transition-metal chloride has been pointed out in the literature.[5,16] In the proposed structure for $LnAl_3Cl_{12}$ (Ln = Nd, Sm), three $AlCl_4$ tetrahedra are bound by a face to the lanthanide ion,[5,16] thus giving Nd^{3+} a coordination number of 9 as in $NdCl_3(s)$. The complex $LnAl_4Cl_{15}(g)$ would then consist of two $AlCl_4$ groups and one Al_2Cl_7 group, whereas the lower molecular weight complexes can be thought of as being formed by gradually removing $AlCl_3$ groups as the temperature is increased. This agrees with the constant difference found for the enthalpy and entropy of formation for the Nd-Ga-Cl and Nd-Al-Cl[5] complexes and suggests similar structures of the gas complexes, in which the same type and number of bonds are formed. Stepwise formation of vapor complexes between transition-metal dichlorides and $M'Cl_3$ (M' = Fe, Al, Ga, In) has been discussed by Dienstbach and Emmenegger.[17,18] Over most of the studied temperature range, where $NdGa_3Cl_{12}(g)$ and $NdGa_4Cl_{15}(g)$ are the dominating complex species, a constant molar absorption coefficient would be expected. In these two complexes the coordination number of Nd^{3+} is probably the same. At high temperatures, where the lower molecular weight species $NdGa_2Cl_9(g)$ and $NdGaCl_6(g)$ are abundant, the coordination number of Nd^{3+} is lower and an increasing molar absorption coefficient is hence conceivable.

Although it is not conclusive with respect to structure, the empirical bond energy model of Hastie[2] reproduces our value for $\Delta H°_1$ if Nd^{3+} is coordinated by four chloride ions (where two bridge to Ga) and corrections for the change in cation-cation repulsive potential energy are applied.

Acknowledgment. We gratefully acknowledge the assistance of Siv.ing. B. Knapstad and Dr. F. Demiray.

Registry No. $NdGaCl_6$, 87585-73-7; $NdGa_2Cl_9$, 87566-85-6; $NdGa_3Cl_{12}$, 87566-86-7; $NdGa_4Cl_{15}$, 79747-67-4; $NdCl_3$, 10024-93-8; $GaCl_3$, 13450-90-3.

(16) G. N. Papatheodorou and G. H. Kucera, *Inorg. Chem.*, **18**, 385 (1979).

(17) F. Dienstbach and F. P. Emmenegger, *Inorg. Chem.*, **16**, 2957 (1977).
(18) F. Dienstbach and F. P. Emmenegger, *J. Inorg. Nucl. Chem.*, **40**, 1299 (1978).

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Cobalt(III) Complex of Glycylglycyl-L-histidine: Preparation, Characterization, and Conformation

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A diamminecobalt(III) complex of glycylglycyl-L-histidine has been prepared in which the NH_2 group, two deprotonated peptide nitrogens, and the imidazole group are coordinated. The circular dichroism, UV-visible, and ^1H and ^{13}C NMR spectra of the complex are presented. The six-membered diamine chelate ring is exclusively in a puckered conformation with the carboxylate group in an axial orientation. The pK_a values at 298 K for the complex are as follows: coordinated imidazole NH, 9.81 ± 0.3; carboxylate, 4.06 ± 0.03 (with ^2H, 3.82).

Introduction

There has been intense interest in recent years in the tripeptide glycylglycyl-L-histidine as a model for the copper(II) binding site of albumin, the transport protein for copper(II) in blood. The N-terminal portion, Asp-Ala-His, of albumin binds copper(II) strongly in a square-planar complex involving the α-amino group of the aspartic acid residue, an imidazole nitrogen of the histidine, and two intervening deprotonated peptide nitrogens.[1-3] As Gly-Gly-His contains the same potential metal binding sites, there have been extensive studies of the interaction of this tripeptide with labile metal ions, principally copper(II) and nickel(II).[4-14] At physiological pH, the predominant species in solution is believed to be $[M(H_{-2}GGHis)]^-$ with the above mode of coordination.[15] A crystal structure of the copper(II) complex of the methylamide of Gly-Gly-L-His has confirmed this structure.[16] Compared to the copper(II) complexes of simple tripeptides, coordination of the imidazole group leads to large differences in the stability, in the pH response, in the electronic spectrum, and in the kinetic behavior of the copper(II) complex.[7]

In the present study, the coordination of Gly-Gly-L-His to the inert metal ion cobalt(III) is examined. The synthesis of $[Co(NH_3)_2(H_{-2}GGHis)]$ (Figure 1) is reported and its structure characterized by various spectroscopic techniques. The X-ray structures of a number of complexes of histidine peptides have the six-membered chelate ring formed by coordination of the imidazole nitrogen and the adjacent peptide nitrogen in a conformation with the terminal carboxylate group

(1) Shearer, W. T.; Bradshaw, R. A.; Gurd, F. R. N. *J. Biol. Chem.* **1967**, *242*, 5451.
(2) Bradshaw, R. A.; Shearer, W. T.; Gurd, F. R. N. *J. Biol. Chem.* **1968**, *243*, 3817.
(3) Peters, T.; Blumenstock, F. A. *J. Biol. Chem.* **1967**, *242*, 1574.
(4) Bryce, G. F.; Gurd, F. R. N. *J. Biol. Chem.* **1966**, *241*, 122.
(5) Yokoyama, A.; Aiba, H.; Tanaka, H. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 112.
(6) Aiba, H.; Yokoyama, A.; Tanaka, H. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1437.
(7) Cooper, J. C.; Wong, L. F.; Venezky, D. L.; Margerum, D. W. *J. Am. Chem. Soc.* **1974**, *96*, 7560.
(8) Lau, S.; Sarkar, B. *Can. J. Chem.* **1975**, *53*, 710.
(9) Kruck, T. P. A.; Sarkar, B. *Inorg. Chem.* **1975**, *14*, 2383.
(10) Wong, L. F.; Cooper, J. C.; Margerum, D. W. *J. Am. Chem. Soc.* **1976**, *98*, 7268.
(11) Agarwal, R. P.; Perrin, D. D. *J. Chem. Soc., Dalton Trans.* **1977**, 53.
(12) Sakurai, T.; Nakahara, A. *Inorg. Chim. Acta* **1979**, *34*, L245.
(13) Sakurai, T.; Nakahara, A. *Inorg. Chem.* **1980**, *19*, 847.
(14) Lau, S.; Sarkar, B. *J. Chem. Soc., Dalton Trans.* **1981**, 491.
(15) Abbreviations: GGHis, glycylglycyl-L-histidine; GGGG, tetraglycine.
(16) Camerman, N.; Camerman, A.; Sarkar, B. *Can. J. Chem.* **1976**, *54*, 1309.

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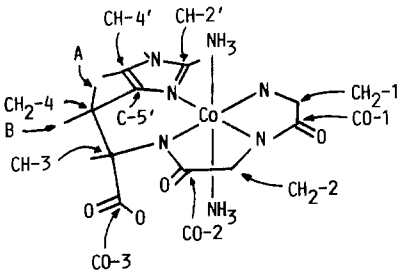

Figure 1. Structure of $[Co(NH_3)_2(H_{-2}GGHis)]$.

in an axial orientation.[16-18] Here the conformer populations for this chelate ring in solution have been determined by an analysis of the ^1H NMR spectrum. The proposed mechanism by which peptides unwrap from metals in acid involves the protonation of coordinated peptide groups.[19] The inertness of the cobalt(III) complex has enabled the acid dissociation constants to be determined for these groups, for the free carboxylate group, and for the uncoordinated imidazole nitrogen.

Experimental Section

Materials. Glycylglycyl-L-histidine was purchased from Vega Biochemicals and used without further purification. All other reagents were of AnalaR grade. The pK_a values of the free carboxylate group and the coordinated imidazole group in the complex were determined at 298 K by titration with standard perchloric acid and CO_2-free sodium hydroxide, respectively, by using a TPS 1852 digital pH meter (±0.01 pH unit) and a thermostated cell with a combination-glass electrode and a nitrogen bubbler. The pK_a values of the coordinated peptide carbonyl groups were investigated by observing the changes in the proton chemical shifts as a function of pD. The pH values for solutions with pH >1.0 were measured with the above pH meter and a Wilmad combination-glass microelectrode. For pH <1.0, H_0 values were estimated. Conversion of pH to pD utilized the relationship pD = pH + 0.40.[20]

Preparation of $[Co(NH_3)_2(H_{-2}GGHis)]\cdot 2.5H_2O$. The peroxo dimer $[(NH_3)_5Co(O_2)Co(NH_3)_5](NO_3)_4\cdot 2H_2O$[21] (1.00 g, 1.76×10^{-3} mol) was added with constant stirring to a solution of Gly-Gly-L-His (0.95 g, 3.52×10^{-3} mol) in aqueous ammonia (10 mL) at pH 9 kept at or below 278 K. Stirring was continued for 3 h before the solution was transferred to a refrigerator for approximately 14 h. The solution was filtered prior to being chromatographed on a Bio-Gel P2 column (50–100 mesh, 3 × 800 cm) with dilute aqueous ammonia (pH 9) as eluant. Four fractions separated on the column: fraction 1 (a broad orange band) was eluted rapidly and appeared to be two bands very close together; fraction 2 (orange) was the desired compound; fraction 3 (orange-pink) was present in very low yield; fraction 4 (pink) adhered to the top of the column. Fraction 2 was frozen on collection, freeze-dried, and rechromatographed on a column of Sephadex DEAE-A25 (2.5 × 25 cm) with water as eluant. A small amount of orange compound adhered to the top of the column, while the desired compound was eluted with the solvent front. Freeze-drying yielded 220 mg of product. Its analysis and spectroscopic properties are consistent with the formula $[Co(NH_3)_2(H_{-2}GGHis)]\cdot 2.5H_2O$. Anal. Calcd for $C_{10}H_{23}CoN_7O_{6.5}$: C, 29.7; H, 5.7; N, 24.3. Found: C, 29.8; H, 5.6; N, 24.5. A pure compound has not been obtained from fraction 1.

Spectroscopic Studies. A Cary 17 spectrophotometer and a Jobin Yvon Mark III dichrograph were used to measure the UV–visible and circular dichroism (CD) spectra, respectively. The ^{13}C and ^1H NMR spectra were recorded on a JEOL FX100 FT instrument. Where greater dispersion was required in the ^1H NMR spectra, a Bruker CXP300 FT instrument was used. Spectra were recorded with the compounds dissolved in D_2O with sodium 3-(trimethylsilyl)-propane-1-sulfonate and dioxane (δ 67.4) as internal references for the ^1H and ^{13}C NMR, respectively. At low pH values, the former reference's chemical shift relative to a Me_4Si capillary is pH dependent. Corrections were made for these shifts.[22] The pH values of the solutions for the NMR studies were adjusted by addition of either concentrated perchloric acid or sodium deuteroxide solution.

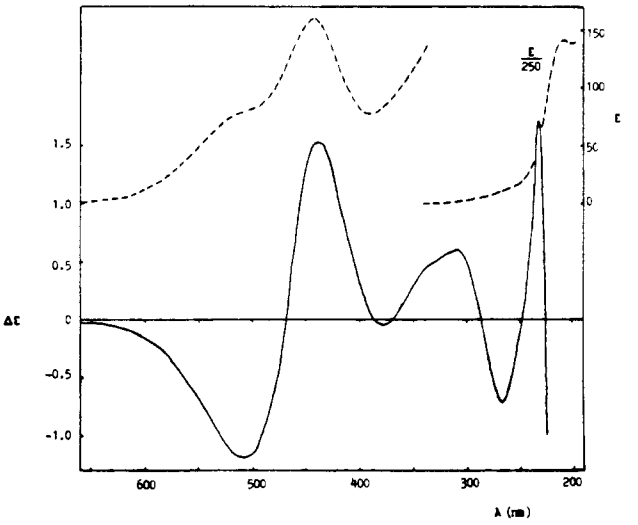

Figure 2. Absorption (---) and circular dichroism (—) spectra of an aqueous solution of $[Co(NH_3)_2(H_{-2}GGHis)]$.

Table I. ^1H Chemical Shifts[a] of Gly-Gly-L-His and $[Co(NH_3)_2(H_{-2}GGHis)]$

pH	CH_2-1	CH_2-2	CH-3	CH_2-4	CH-4'	CH-2'
			Free Peptide			
9.5	3.38	3.91, 3.92	4.43	2.97, 3.10	6.89	7.65
7.4	3.76	3.95	4.45	3.00, 3.14	6.98	7.87
4.5	3.89	3.98	4.53	3.11, 3.27	7.26	8.58
1.0	3.90	4.01	4.8[b]	3.22, 3.37	7.33	8.62
			Complex			
7.0[c]	3.69	4.04	4.63	2.71, 3.49	7.25	8.02
2.5[d]	3.70	4.06	*e*	2.78, 3.54	7.29	8.06

[a] In ppm from DSS. [b] Partially obscured by HDO peak. [c] NH_2 4.99, 5.12; NH_3 2.45, 2.93. [d] NH_2 4.98, 5.13; NH_3 2.48, 2.71. [e] Obscured by HDO peak.

Table II. Proton–Proton Coupling Constants[a] for Gly-Gly-L-His and $[Co(NH_3)_2(H_{-2}GGHis)]$

pH	^3J(CH-3,CH_2-4)	^2J(CH_2-4)	^4J(CH-4,CH-4')	^4J(CH-2',CH-4')
		Free Peptide		
9.5[b]	4.67, 8.18[c]	−14.89	<0.2,[c] 0.8	1.1
7.4	4.88, 8.24[c]	−15.05	<0.2,[c] 0.8	1.2
4.5	5.10, 8.00[c]	−15.47	0.5,[c] 0.8	1.4
1.0	5.16, 8.61[c]	−15.47	0.6,[c] 0.9	1.4
		Complex		
7.0	2.93, 4.88[c]	−15.87	1.2,[c] <0.2	1.2
2.5	2.34, 4.27[c]	−16.00	<1.0,[c] <0.2	1.1

[a] In Hz. [b] $^2J(CH_2\text{-}2) = -17.08$. [c] J value for the more shielded CH_2-4 proton.

Results

UV–Visible and Circular Dichroism Spectra. The UV–visible and CD spectra of $[Co(NH_3)_2(H_{-2}GGHis)]$ are shown in Figure 2. The absorption spectrum shows in the visible a maximum at 442 nm (ϵ 160) with a shoulder at approximately 520 nm. The UV region has a strong absorption at

(17) Blount, J. F.; Fraser, K. A.; Freeman, H. C.; Szymanski, J. T.; Wang, C. H. *Acta Crystallogr.* **1967**, *22*, 396.
(18) deMeester, P.; Hodgson, D. J. *Acta Crystallogr., Sect. B* **1977**, *B33*, 3505.
(19) See, for example: Raycheba, J. M. T.; Margerum, D. W. *Inorg. Chem.* **1980**, *19*, 497 and ref 10.
(20) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Anal. Chem.* **1968**, *40*, 700.
(21) Davies, R.; Mori, M.; Sykes, A. G.; Weil, J. A. *Inorg. Synth.* **1971**, *12*, 197.
(22) Corrections to δ were of the following order: pH 1.0, 0; pH 0.5, +0.010; pH 0, +0.026.

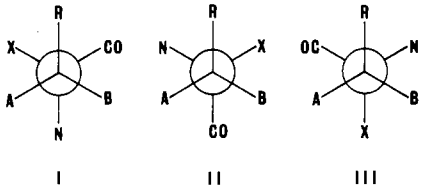

Figure 3. Side-group rotamers.

Table III. Side-Group Rotamer Populations for Gly-Gly-L-His and [Co(NH3)2(H-2GGHis)]

pH	J_{AX}, Hz	J_{BX}, Hz	n_I	n_{II}	n_{III}
		Free Peptide			
9.5	8.18	4.67	0.2	0.6	0.2
	4.67	8.18	0.5	0.1	0.4
7.4	8.24	4.88	0.2	0.6	0.2
	4.88	8.24	0.5	0.2	0.3
4.5	8.00	5.10	0.25	0.55	0.2
	5.10	8.00	0.5	0.2	0.3
1.0	8.61	5.16	0.3	0.6	0.1
	5.16	8.61	0.6	0.2	0.2
		Complex			
7.0	2.93	4.88	0	0	1.0
2.5[a]	2.34	4.27	−0.1	0	1.1

[a] Data taken as first order from the 300-MHz spectrum; simulation not possible as the CH-3 quartet was obscured by the HDO peak.

209 nm (ϵ 3.53 × 10^4). The visible CD spectrum has a negative band at 510 nm ($\Delta\epsilon$ −1.19) followed by a positive band at 437 nm ($\Delta\epsilon$ 1.53) and a small negative band at 378 nm ($\Delta\epsilon$ −0.05). The UV region has Cotton effects at 309 nm ($\Delta\epsilon$ 0.60), 267 nm ($\Delta\epsilon$ −0.72), and 232 nm ($\Delta\epsilon$ 1.71).

^{1}H NMR Spectra. Chemical shift and coupling constant data are presented in Tables I and II. Assignments were made on the basis of chemical shift changes with pH, with proton decoupling experiments, and by reference to previous assignments for peptides, histidine, and peptide complexes.[23-25] In cobalt(III) complexes with asymmetric tripeptides, the nonequivalent central glycine methylene protons usually show an AB pattern.[23] In the present Gly-Gly-L-His complex these protons are degenerate.

Coupling is observed between the two C–H protons of the imidazole ring in both the free peptide and the complex with $^5J \approx 1$ Hz. The CH-4′ proton also couples to the CH-4 methylene protons, the size of the coupling to each proton varying with pH and with coordination. The vicinal coupling constants between CH-3 and CH_2-4 for the free peptide and the complex were analyzed by the method of Feeney[26] to give the populations of the rotamers (Figure 3) of the side group. These are given in Table III.

^{13}C NMR Spectra. Chemical shift data for Gly-Gly-L-His and for the cobalt(III) complex are presented in Table IV. Assignments were based on changes in chemical shift with pH (Table V) and on the data available for other tripeptides.[23] The imidazole ring carbons have been assigned by Reynolds,[27] and the assignments of C-5′ and CH-2′ in the complex were confirmed by an off-resonance decoupling experiment.

Acid Dissociation Constants. The pK_a for the dissociation of the NH proton of the coordinated imidazole was determined to be 9.81 ± 0.03 at 298 K. Three protonation equilibria for the neutral complex were observed. The free carboxylate group had a pK_a of 4.06 ± 0.03 determined by pH titration at 298 K. This assignment of the site of protonation was confirmed by the pH dependence of the ^{1}H NMR spectrum (Figure 4). The pK_a value for the deuteration of the carboxylate group was 3.82. Values for the peptide CO groups, CO-1 and CO-2, could not be determined due to the large degree of overlap in their deuteration regions, but the deuteration took place between pD 0 and 1.

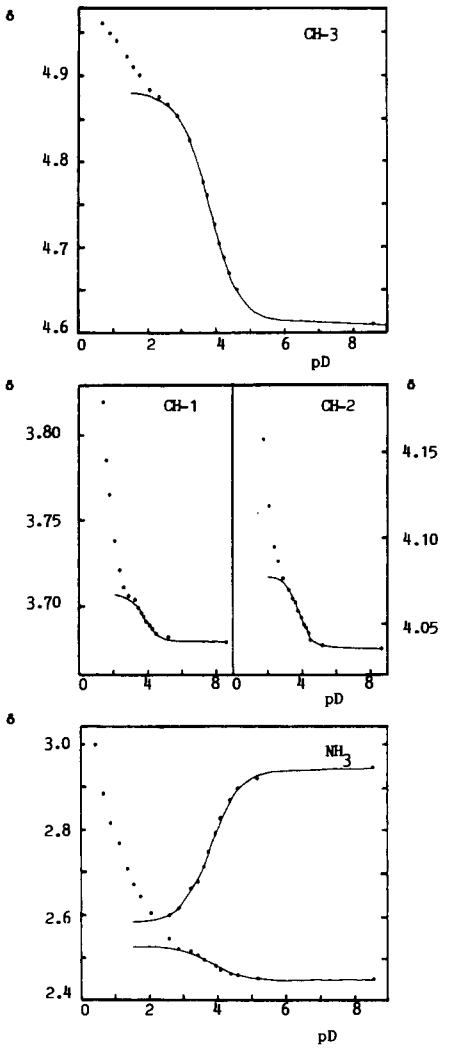

Figure 4. Effect of pD on the ^{1}H chemical shifts for [Co(NH3)2(H-2GGHis)]. The lines are calculated for a pK_a(^{2}H) of 3.82 with the following chemical shifts for the undeuterated and deuterated species (ppm): CH-3, 4.61, 4.88; CH-1, 3.679, 3.707; CH-2, 4.035, 4.077; NH_3 (upper), 2.94, 2.58; NH_3 (lower), 2.45, 2.52.

Discussion

The UV–visible, CD, and ^{13}C and ^{1}H NMR spectra and the elemental analysis of the complex are all consistent with the structure given in Figure 1 (see below). The complex is a nonelectrolyte in neutral aqueous solution and moves with the solvent front down cation- and anion-exchange columns. The protons of the terminal NH_2 group are not exchanged rapidly by deuterium when the complex is dissolved in D_2O, consistent with the NH_2 group being coordinated.

The absorption maximum for the d–d transitions with $^1A_{1g} \rightarrow {}^1T_{1g}$ parentage is observed at 442 nm, intermediate between the absorption maxima of diamminecobalt(III) tripeptides with the terminal carboxylate group completing the quadridentate coordination (λ_{max} 456 nm)[23] and diamminecobalt(III) tetrapeptides where the fourth donor group is a deprotonated peptide nitrogen (λ_{max} 433 nm).[28] A similar relationship exists

(23) Evans, E. J.; Grice, J. E.; Hawkins, C. J.; Heard, M. R. *Inorg. Chem.* **1980**, *19*, 3496.
(24) Tanokura, M.; Tasumi, M.; Miyazawa, T. *Biopolymers* **1976**, *15*, 393.
(25) Hruby, V. J. In "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Winstein, B., Ed.; Marcel Dekker: New York, 1974; pp 1–188.
(26) Feeney, J. *J. Magn. Reson.* **1976**, *21*, 473.
(27) Reynolds, W. F.; Peat, I. R.; Freedman, M. H.; Lyerla, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 328.

Table IV. ^{13}C NMR Chemical Shifts[a] of Gly-Gly-L-His and $[Co(NH_3)_2(H_{-2}GGHis)]$

pH	CH_2-1	CO-1	CH_2-2	CO-2	CH-3	CO-3	CH_2-4	C-5′	CH-4′	CH-2′
					Free Peptide					
9.5	44.47	176.62	43.12	171.36	55.82	178.50	29.84	133.68	118.65	136.61
7.4	41.92	170.13	43.15	171.10	55.64	178.03	29.37	132.72	118.38	135.88
4.5	41.28	168.58	43.15	171.18	54.88	176.92	28.09	130.32	117.62	133.98
1.0	41.25	168.55	42.95	171.71	52.54	173.93	26.97	129.35	118.06	134.27
					Complex					
7.0	48.21	178.85	50.23	181.63	56.40	180.10	29.40	136.08	117.30	138.33
2.5	47.98	178.96	50.08	182.42	54.30	177.74	28.73	135.15	117.68	138.66

[a] In ppm from Me_4Si.

Table V. ^{13}C NMR Chemical Shift Differences[a] on Protonation and Chelation

reaction[b]	CH_2-1	CO-1	CH_2-2	CO-2	CH-3	CO-3	CH_2-4	C-5′	CH-4′	CH-2′
$NH_2 \rightarrow NH_3^+$ [c]	−2.55	−6.49	+0.03	−0.26	−0.18	−0.47	−0.47	−0.96	−0.27	−0.73
$Im \rightarrow ImH^+$ [d]	−0.64	−1.55	0	+0.08	−0.76	−1.11	−1.28	−2.40	−0.76	−1.90
$CO_2^- \rightarrow CO_2H$ [e]	−0.03	−0.03	−0.20	+0.53	−2.34	−2.99	−1.12	−0.97	+0.44	+0.29
$L^- \rightarrow CoL$ [f]	+3.47	+2.23	+7.11	+10.27	+0.58	+1.60	−0.44	+2.40	−1.35	+1.72
$CoL \rightarrow CoLH^+$ [g]	+0.23	+0.11	−0.15	+0.79	−2.10	−2.36	−0.67	−0.93	+0.38	+0.33

[a] In ppm. [b] Predominant reaction based on the following pK_a values for Gly-Gly-L-His: NH_3^+, 7.97; ImH^+, 6.82; CO_2H, 2.78.[24] [c] δ(pH 7.4) − δ(pH 9.5). [d] δ(pH 4.5) − δ(pH 7.4). [e] δ(pH 1.0) − δ(pH 4.5). [f] δ(complex, pH 7.0) − δ(pH 9.5). [g] δ(complex, pH 2.5) − δ(complex, pH 7.0).

for the copper(II) complexes: Gly-Gly-L-His, 525 nm; tripeptides, 550 nm; tetrapeptides, 510 nm.[13] The $^1A_{1g} \rightarrow {}^1T_{2g}$ absorption, which normally occurs at about 350 nm, is not observed in the absorption spectrum, but the small negative Cotton effect at 378 nm and the positive shoulder at about 340 nm in the CD spectrum would result from this cubic absorption band.

The CD spectra of the diamminecobalt(III) complexes of C-terminal substituted tripeptides normally show negative Cotton effects under the $^1A_{1g} \rightarrow {}^1T_{1g}$ absorption band, and the total rotational strength of the d–d bands is negative.[29] For the diamminecobalt(III) complex of Gly-Gly-L-His, the Cotton effect corresponding to the absorption maximum is positive. This difference in CD between the carboxylato-coordinated tripeptides and the imidazole-coordinated tripeptide is also found for the copper(II) and nickel(II) complexes. The former ligands yield a total negative rotational strength for the d–d transitions, whereas the Gly-Gly-L-His complex shows a low-energy negative Cotton effect followed by a larger positive Cotton effect.[13] The above two types of tripeptide cobalt(III) complexes have similar patterns of Cotton effects in the UV region, but the bands for the Gly-Gly-L-His complex are 20–40 nm to higher wavelength than the other tripeptide complexes.

The ^{13}C resonances in the spectra of Gly-Gly-L-His in its anionic, zwitterionic, and cationic forms have been assigned largely on the basis of a comparison of the changes of chemical shift on protonation with those found for other tripeptides. Protonation at the terminal NH_2 group causes large shifts in the positions of the adjacent methylene (CH_2-1) and CO-1 group resonances.[23] For the tripeptides Gly-Gly-L-Ala, Gly-Gly-L-Leu, and Gly-Gly-L-Phe in the anionic form, CH_2-1 and CO-1 resonate at δ 44.6 and 177.1 and shift by −3.3 and −8.7 ppm, respectively, on protonation.[23] For Gly-Gly-L-His the peaks at δ 44.47 and 176.62 are assigned to CH_2-1 and CO-1. They shift by −2.55 and −6.49 ppm on protonation of the NH_2 group. For the four tripeptides the peaks at about δ 43.1 and 171.3 do not shift significantly on protonation of the NH_2 or CO_2^- groups. These have been assigned to CH_2-2 and CO-2, respectively. For Gly-Gly-L-His the resonances at δ 55.82 and 178.50 in the anion have only a small shift on protonation of the NH_2 but undergo major shifts to δ 52.54 and 173.93 on protonation of the carboxylate group. These resonances are assigned to CH-3 and CO-3. The remaining nonaromatic peak at δ 29.84 in the anion is the CH_2-4 resonance. The imidazole-ring carbon resonances show shifts on protonation of the ring (pK_a 6.82).[24] C-4′ is furthest removed from the site of protonation and hence shows the smallest shift on protonation (−0.76 ppm). C-5′ is a singlet and C-2′ a doublet in the "off-resonance" decoupled spectrum, and they are assigned to the resonances in the anion at δ 133.68 and 136.61, respectively. Protonation of the imidazole ring (from the zwitterion) causes these resonances to shift by 2.40 and 1.90 ppm to higher shielding. In histidine the corresponding shifts are 4.8 and 2.4 ppm, respectively.[27] A summary of the shifts of the ^{13}C resonances on protonation of the NH_2, imidazole, and CO_2^- groups is given in Table V.

Coordination of the above Ala, Leu, and Phe tripeptide anions as quadridentates to cobalt(III) causes the backbone ^{13}C resonances to shift to lower shielding: CH_2-1, 3.1; CO-1, 1.7; CH_2-2, 8.7; CO-2, 7.9–8.7; CH-3, 4.2–5.4; CO-3, 8.8–9.3 ppm.[23] On the basis of these shifts, CH_2-1, CO-1, and CH_2-2 are assigned to the resonances at δ 48.21, 178.85, and 50.23, respectively, in the spectrum of $[Co(NH_3)(H_{-2}GGHis)]$. Consequently the resonance at δ 56.40 is assigned to CH-3. This resonance shows a relatively minor shift (+0.58 ppm) for the Gly-Gly-L-His complex compared to that found for the tripeptides that coordinate via the carboxylate group. The His side-group resonance assignments for the complex for CH_2-4, (δ 29.40) and CH-4′ (δ 117.30) are unambiguous, and from an "off-resonance" decoupled spectrum the peaks at δ 136.08 and 138.33 are assigned to C-5′ (s) and CH-2 (d), respectively. On coordination of the imidazole ring to cobalt(III), the resonances of the adjacent carbon atoms, C-5′ and CH-2′, move 2.40 and 1.72 ppm to lower shielding, respectively, whereas the CH-4′ resonance moves 1.35 ppm to higher shielding. With the pulse conditions used in the data accumulation, one CO resonance (δ 180.10) is much smaller in intensity than the other two. When the complex's carboxylate group is protonated, the small CO peak is at δ 177.74. This is consistent with the small peak being assigned to the free CO-3. With either assignment, δ CO-3 does not shift markedly in comparison with the Ala, Leu, and Phe peptides on coordination of the tripeptide, providing strong evidence for its noninvolvement in coordination for Gly-Gly-L-His.

Confirmation of the assignment of the CO-2 and CO-3 resonances can be obtained from the shifts observed on pro-

(28) Hawkins, C. J.; Kelso, M. T. *Inorg. Chem.* **1982**, *21*, 3681.
(29) Evans, E. J.; Hawkins, C. J.; Rodgers, J.; Snow, M. R. *Inorg. Chem.* **1983**, *22*, 34.

tonation of the uncoordinated carboxylate group in the complex. For the free peptide this protonation causes shifts in excess of 0.5 ppm in the following resonances: CO-2, +0.53; CH-3, −2.34; CO-3, −2.99; CH_2-4, −1.12; C-5′, −0.97 ppm. With the assignments given in Table IV similar shifts are observed on protonation of the complex: CO-2, +0.79; CH-3, −2.10; CO-3, −2.36; CH_2-4, −0.67; C-5′, −0.93 ppm. The alternative assignment of CO-2 and CO-3 would yield shifts of +2.32 and −3.89 ppm, respectively, on protonation.

The ^1H NMR spectra of the free tripeptide in its anionic, zwitterionic, and cationic forms show the same trends that have been observed for other tripeptides.[23] Protonation of the NH_2 group causes a shift in excess of 0.1 ppm in only one aliphatic resonance: CH_2-1 shifts by +0.38 ppm. Protonation of the carboxylate group causes significant shifts only for CH-3, and CH_2-4: about +0.3, and +0.1 ppm, respectively. Protonation of the imidazole ring shifts CH-2′ and CH-4′ 0.71 and 0.28 ppm to lower shielding, respectively. The CH_2-2 protons in the anionic form of the peptide are nondegenerate giving rise to an AB pattern with $\Delta\delta_{AB}$ 0.01. This separation is not observable in the other forms of the peptide including the complex for which with other tripeptides an AB pattern is normally observed.[23] Further, for other tripeptide complexes, ^5J coupling is generally observed between the C-2 protons and the protons on C-1 and C-3 indicative of electronic delocalization around the peptide backbone.[23] This coupling is not observed in the present complex suggesting a smaller degree of electronic delocalization.

The vicinal coupling constants for CH-3 and CH_2-4 protons have been analyzed for the free peptide in its various forms and for the complex in D_2O and at pH 2.5 by using the method of Feeney.[26] The results in Table III show that for the free peptide irrespective of the assignment of the A and B CH_2-4 protons the predominant rotamer is either I or II with only slight variations in the populations for the different protonated species. For the complex, rotamer II is not possible for the chelate. Rotamer I has the carboxylate group equatorial, and rotamer III has the carboxylate group axial (Figure 1). When A is assigned to the most deshielded resonance (δ 3.49), the analysis concludes that rotamer III is exclusively populated. If the alternative assignment is used, the impossible solution $n_I = 0.2$, $n_{II} = 0.3$, and $n_{III} = 0.5$ is obtained. The former assignment was therefore assumed to be correct for the complex. Protonation of the carboxylate group appears not to alter significantly the rotamer populations. Simulation of the ^1H NMR spectrum of the protonated complex was not possible as part of the spectrum was obscured by the solvent peak, and the vicinal coupling constants were taken as first order from the 300-MHz spectrum. The conclusion that the carboxylate group has a preference for the axial conformation is consistent with the findings from crystal structure investigations of complexes of histidine peptides.[16-18] The same conclusion was reached from the ^1H NMR spectrum of the nickel(II) complex of Gly-Gly-L-His.[12,13] The axial carboxylate group in the diamminecobalt(III) complex causes the two NH_3 groups to have a chemical shift difference of 0.48. Protonation of the carboxylate shifts the more deshielded resonance 0.22 ppm to higher shielding with little change in the position of the other NH_3 resonance. The NH_3 adjacent to the carboxylate is hence assigned to the more deshielded resonance.

Coupling is observed between the protons attached to C-2′ and C-4′ for all forms of the peptide, with ^4J values between 1.1 and 1.4 Hz being observed. The proton attached to C-4′ also couples to the CH_2-4 protons resulting in a complicated multiplet for this proton. The extent of this coupling for the two CH_2-4 protons depends on the form of the peptide. For the free peptide the less shielded proton has the larger coupling to CH-4′, but in the complex the opposite is true. In the complex, CH-4-B, which lies approximately in the plane of the imidazole ring, couples to CH-4′ with ^4J = 1.22 Hz whereas no coupling was detected for CH-4-A. This result is opposite to that expected on the basis of other coupling across one double and three single bonds, for example allylic coupling.[30]

The acid dissociation constant of the coordinated imidazole ring in the peptide complex, 9.81, is only 0.21 logarithmic units lower than the value reported for pentaammine(imidazole)cobalt(III)[31] but is 0.88 logarithmic units lower than the value for the copper(II) complex of Gly-Gly-L-His.[6] The latter result derives from the greater polarizing power of cobalt(III) compared to that of copper(II).

These studies have shown unambiguously that the protonation of the Gly-Gly-L-His complex that occurs at about pH 4 takes place at the free carboxylate. This is clearly shown in Figure 4 where the protonation causes a large shift in the resonances adjacent to CO-3 and only a minor shift in CH-2. On the other hand protonation at CO-2 and CO-1 takes place in that order at pH <1 resulting in much larger shifts in their adjacent CH proton resonances than in the CH-3 resonance. This conclusion differs from the conclusion of Margerum and his co-workers who for the copper(II) complex have proposed that the protonation of CO-2 has a pK_a value of 4.2.[10] This "outside" protonation is claimed to be stabilized by hydrogen bonding with the free carboxylate group from the histidine residue.[10] For the axial carboxylate, which seems to be generally favored, this type of hydrogen bonding would appear to be most unlikely from an inspection of Dreiding models.

The acid dissociation constant for the carboxylate group that is attached to an α-CH adjacent to a coordinated peptide nitrogen in the zero-charged complex [$Co(NH_3)_2(H_{-2}GGHis)$] is 4.06 ± 0.03 at 298 K. The pK_a value for a similar carboxylate group in the negatively charged complex [$Co(NH_3)_2(H_{-3}GGGG)]^-$ has been found to be 4.40 ± 0.08 at 278 K.[28] The decreased basicity of the carboxylate in the less negatively charged species is expected. The difference in the pK_a values for the protonation and deuteration (0.24 logarithmic unit) is interesting. Similar differences have been obtained for other cobalt(III) peptide complexes studied in this laboratory.

The C-2′ proton in histidine and its peptides is known to undergo deuteration in D_2O.[32,33] In insulin, zinc binding to histidine B10 causes the rate of this exchange to be decreased markedly.[33] In the present study, [$Co(NH_3)_2(H_{-2}GGHis)$] in D_2O at pH 8 for over 3 weeks at 298 K showed no observable deuteration at C-2′.

Registry No. [$Co(NH_3)_2(H_{-2}GGHis)$], 87696-34-2; [$(NH_3)_5Co(O_2)Co(NH_3)_5](NO_3)_4$, 16632-71-6.

(30) Jackman, L. M.; Sternhill, S. "Applications of NMR Spectroscopy in Organic Chemistry", 2nd ed.; Pergamon Press: Oxford, 1969; p 316.
(31) Harrowfield, J. M.; Norris, V.; Sargeson, A. M. *J. Am. Chem. Soc.* **1976**, *98*, 7282.
(32) Bradbury, J. H.; Chapman, B. E.; Crompton, M. W.; Norton, R. S. *J. Chem. Soc., Perkin Trans. 2* **1980**, 693. Bradbury, J. H.; Crompton, M. W.; Teh, J. S. *Eur. J. Biochem.* **1977**, *81*, 411.
(33) Bradbury, J. H.; Ramesh, V.; Dodson, G. *J. Mol. Biol.* **1981**, *150*, 609.