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Metal Peptide Complexes: Preparations and ^1H and ^{13}C NMR Spectra of Cobalt(III) Tripeptide Complexes

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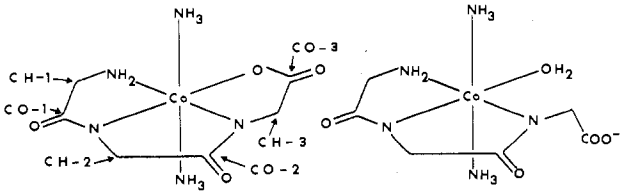
Received March 6, 1980

The preparations of cobalt(III) complexes of a series of tripeptides are reported in which the peptides are coordinated as quadridentate chelates through the terminal NH_2, two peptide N^- and terminal CO_2^- groups. The ^1H and ^{13}C NMR spectra are given for the free peptides in the anionic, zwitterionic, and cationic forms and for the cobalt(III) complexes. The chemical shift data are analyzed in terms of the effects of protonation and coordination, and the coupling constants for the α-CH-β-CH_2 fragments of the leucyl, phenylalanyl, and tyrosyl residues are analyzed in terms of the possible rotamers for the side groups. In $[Co(NH_3)_2$(gly-gly-L-phe)], the phenyl group adopts a conformation that results in an adjacent NH_3 ligand resonating at the very unusual position of δ 0.77.

Introduction

As part of an investigation into the reactions of metal peptide complexes, a series of diammine(tripeptide)cobalt(III) complexes has been prepared with the peptide coordinated as a quadridentate chelate (structure **1**). Although the preparations of a number of cobalt(III) mono(dipeptide),[1-3] bis(dipeptide),[3-9] bis(tripeptide),[10] and mono(tetrapeptide)[1] complexes have been reported previously, there is only one report of cobalt(III) complexes with a peptide coordinated as a quadridentate chelate.[7] The methods of preparation reported for $[Co(gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3.5H_2O$ and $Na[Co(gly\text{-}gly\text{-}gly)(gly)]\cdot 6H_2O$[7] did not yield the desired products in this laboratory, and the postulated configurations for the complexes with the three chelate rings noncoplanar are thought to be most unlikely as coordination involves planar peptide groups.



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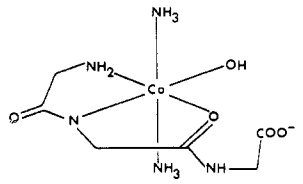

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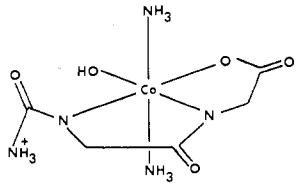

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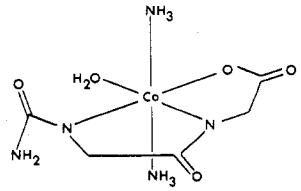

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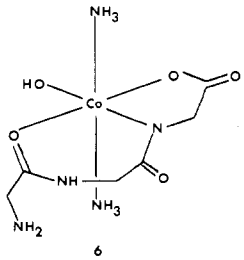

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Wu and Busch have prepared $[Co(dien)(gly\text{-}gly\text{-}glyOEt)]^{2+}$ in which the tetrapeptide is terdentate and have published the ^1H NMR spectrum of this complex.[1] Other NMR studies of cobalt(III) peptide complexes have been concerned with the protonation of bis(dipeptide) complexes,[4,8,11] the activation of peptide esters,[2,12] and the absolute configurations of bis(dipeptide)cobalt(III) complexes.[5,13] No ^{13}C NMR spectra of cobalt(III) peptide complexes have been reported previously, and this is also the first report of ^1H NMR spectra of cobalt(III) tripeptide complexes, although the ^1H NMR spectra of a number of more labile systems with metals such as Cu^{2+}, Ni^{2+}, and Pd^{2+} are in the literature.[14-18]

Experimental Section

Materials. Triglycine was purchased from Calbiochem, and the optically active tripeptides were purchased from ICN Pharmaceuticals Inc. and were used without further purification. All other reagents were of AnalaR grade.

Preparation of Diammine(tripeptide)cobalt(III). $[(H_3N)_5Co(O_2)Co(NH_3)_5](NO_3)_4\cdot 2H_2O$[19] (1.0 g, 0.0017 mol) was added with constant stirring to a solution of tripeptide (0.0034 mol) in aqueous ammonia (10 mL) at pH 9 kept at below 5 °C. Stirring was continued for 1.5 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 7-8) as eluant. Three fractions separated on the column: fraction 1 (pink) was eluted rapidly; fraction

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Table I. Analytical Data for $[Co(NH_3)_2(peptide)]\cdot xH_2O$

peptide	x	formula	% calcd C	% calcd H	% calcd N	% found C	% found H	% found N
gly-gly-gly	2.5	$C_6H_{19}N_5O_{6.5}Co$	22.2	5.9	21.6	22.3	5.5	21.8
gly-gly-L-ala	3.0	$C_7H_{22}N_5O_7Co$	24.2	6.4	20.2	23.2	5.6	23.7
gly-gly-L-leu	3.0	$C_{10}H_{28}N_5O_7Co$	30.9	7.2	18.0	30.8	6.5	17.5
gly-gly-L-phe	3.0	$C_{13}H_{26}N_5O_7Co$	36.9	6.2	16.5	36.9	5.6	16.0
L-ala-gly-gly	2.5	$C_7H_{21}N_5O_{6.5}Co$	24.9	6.2	20.7	24.9	5.9	20.5
L-phe-gly-gly	3.0	$C_{13}H_{26}N_5O_7Co$	36.9	6.2	16.5	36.7	5.5	17.0
L-tyr-gly-gly	3.5	$C_{13}H_{27}N_5O_{8.5}Co$	34.8	6.0	15.7	34.8	5.7	16.0

2 (orange) was the desired compound; fraction 3 (pink) adhered to the top of the column. The desired orange band was frozen on collection and freeze-dried. The compound was rechromatographed twice to ensure purity, giving a yield of approximately 50%. Carbon, hydrogen, and nitrogen analyses (Table I), performed by the departmental analyst, showed the complexes had the formula $[Co(NH_3)_2(peptide)]\cdot xH_2O$ except for the gly-gly-L-ala complex for which satisfactory analyses could not be obtained although the ^1H and ^{13}C NMR spectra showed no sign of impurities, and the visible absorption spectrum was consistent with the above formulation ($x = 3$). Complexes were prepared from the following peptides: gly-gly-gly ($x = 2.5$), gly-gly-L-ala, gly-gly-L-leu ($x = 3$), gly-gly-L-phe ($x = 3$), L-ala-gly-gly ($x = 2.5$), L-phe-gly-gly ($x = 3$), and L-tyr-gly-gly ($x = 3.5$).

NMR Spectra. The ^1H and ^{13}C NMR spectra were recorded on a JEOL FX100 FT instrument using D_2O as solvent and sodium 3-(trimethylsilyl)propane-1-sulfonate and dioxane (67.4 ppm), respectively, as internal references. For the spectra at pH 1, the pH of the D_2O was lowered by the addition of concentrated perchloric acid, and at pH 9.5 the pH was raised by the addition of sodium deuterioxide solution.

Results

^1H NMR Spectra. Chemical shift and coupling constant data are presented in Tables II and III, respectively, for the free peptides in the zwitterionic (D_2O), cationic (pH 1), and anionic (pH 9.5) forms and for the peptide complexes. Assignments were made by noting the systematic changes in the spectra caused by the incorporation of substituents in the N- and C-terminal amino acids and also caused by the change in pH and by coordination and by reference to previous assignments for peptides.[18,20-23] Shifts due to protonation and coordination are presented in Table IV. Nonequivalence of the central glycine methylene protons has been noted previously for some peptides.[20-24] Here AB patterns for these protons have been observed for the free peptides with substituents in the N-terminal residue and for the complexes irrespective of whether the substitution is in the N- or C-terminal residue. In addition, in the complex of gly-gly-L-phe the N-terminal glycine methylene protons show an AB pattern with $\Delta\delta$ 0.07 compared to $\Delta\delta$ 0.12 for the central methylene protons in that complex.

Coupling between the α-C protons for the three residues in the complexes was observed generally to broaden these resonances. For example, in $[Co(NH_3)_2(gly\text{-}gly\text{-}L\text{-}leu)]$ the coupling constants (^5J) were determined to be 1.2 and 1.0 Hz between the methine proton (CH-3) in the C-terminal residue and the two protons of the central methylene group (Figure 1).

Coupling between α-CH and the two protons of the β-CH_2 of the side groups in tyrosine, phenylalanine, and leucine have been used previously to determine the mole fractions of the rotamers shown in Figure 2 for amino acids and peptides.[18,25-28]

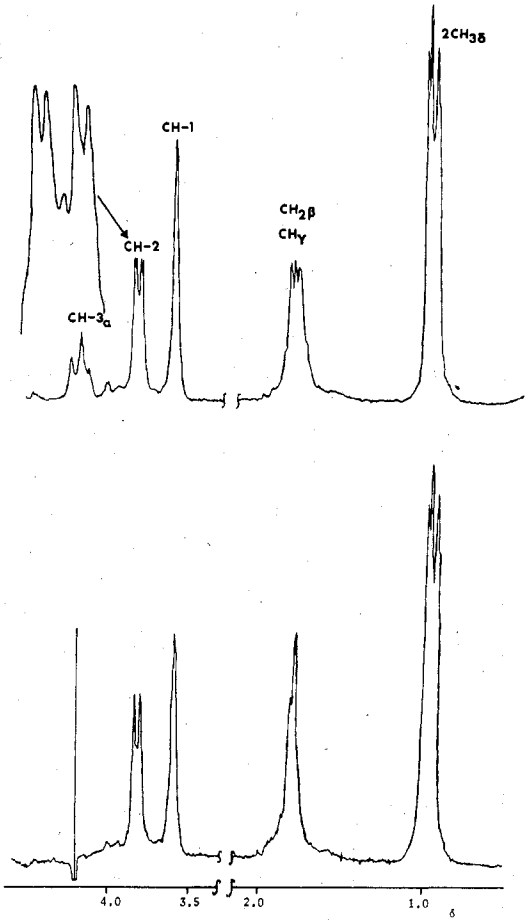

Figure 1. ^1H NMR spectrum of $[Co(NH_3)_2(gly\text{-}gly\text{-}L\text{-}leu)]$ (upper) and the spectrum with CH-3 irradiated (lower) in D_2O.

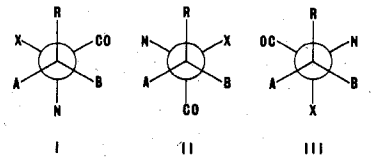

Figure 2. Side-group rotamers where A, B, and X are protons.

The method of Feeney,[25] which has been used by Kozlowski for Pd(II) and Ni(II) peptide complexes,[18] has been applied to the free peptides and the cobalt(III) complexes. The results are presented in Table V. The mole fractions of the rotamers for Ni(II) and Pd(II) complexes of L-tyr-gly-gly have been recalculated from the coupling constant data of Kozlowski[18] and have been included in Table V for comparison with the cobalt(III) data.

^{13}C NMR Spectra. Chemical shift data for the free peptides in the anionic, zwitterionic, and cationic forms and for the cobalt(III) complexes are presented in Table VI, and the shifts

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Table II. ^{1}H Chemical Shifts[a] of Peptides and $[Co(NH_3)_2(peptide)]$

peptide	CH-1	CH-2	CH-3	other
		Free Peptide at pH 9.5: Anion		
gly-gly-gly	3.52	4.10	3.84	
gly-gly-L-ala	3.39	3.96	4.17	1.33[b]
gly-gly-L-leu	3.38	3.96	4.22	0.87,[b] 0.90,[b] 1.59[c,d]
gly-gly-L-phe	3.30	3.86	4.47	2.96,[c] 3.18,[c] 7.31[e]
L-ala-gly-gly	3.62	3.98	3.77	1.31[b]
L-phe-gly-gly	3.72	3.84	3.69	2.95,[c] 7.33[e]
L-tyr-gly-gly	3.64	3.86	3.72	2.82,[c] 6.86[e]
		Free Peptide in D_2O: Zwitterion		
gly-gly-gly	3.88	4.00	3.75	
gly-gly-L-ala	3.90	4.12	4.28	1.37[b]
gly-gly-L-leu	3.89	4.03	4.22	0.88,[b] 0.90,[b] 1.58[c,d]
gly-gly-L-phe	3.83	3.91	4.47	2.95,[c] 3.18,[c] 7.31[e]
L-ala-gly-gly	4.16	4.01	3.77	1.50[b]
L-phe-gly-gly	4.30	3.92[f]	3.78	3.23,[c] 7.39[e]
L-tyr-gly-gly	4.22	3.92[f]	3.74	3.13,[c] 7.03[e]
		Free Peptide at pH 1: Cation		
gly-gly-gly	3.90	4.00	4.03	
gly-gly-L-ala	3.90	4.03	4.41	1.43[b]
gly-gly-L-leu	3.90	4.04	4.41	0.89,[b] 0.93,[b] 1.67[c,d]
gly-gly-L-phe	3.85	3.94	4.71	3.05,[c] 3.23,[c] 7.42[e]
L-ala-gly-gly	4.18	4.03, 4.08	3.97	1.57[b]
L-phe-gly-gly	4.32	3.90, 4.02	4.00	3.24,[c] 7.40[e]
L-tyr-gly-gly	4.27	3.94, 4.03	4.02	3.16,[c] 7.05[e]
		Complex in D_2O		
gly-gly-gly	3.62	3.83	4.08	5.28,[g] 2.50[h]
gly-gly-L-ala	3.61	3.82, 3.90	4.25	5.27,[g] 2.43,[h] 1.56[b]
gly-gly-L-leu	3.61	3.73, 3.97	4.22	5.24,[g] 2.38,[h] 0.93,[b] 0.96,[b] 1.84[c,d]
gly-gly-L-phe	3.39, 3.46	3.77, 3.89	4.56	5.02,[g] 0.77,[h] 2.37,[h] 3.27,[c] 3.85,[c] 7.45[e]
L-ala-gly-gly	3.72	3.85[f]	4.10	5.86,[g] 4.89,[g] 2.40,[h] 1.45[b]
L-phe-gly-gly	4.00	3.86[f]	4.00	5.90,[g] 5.0,[g] 1.47,[h] 2.34,[h] 3.22,[c] 7.40[e]
L-tyr-gly-gly	4.00	3.85[f]	4.00	5.91,[g] 5.06,[g] 1.45,[h] 2.35,[h] 3.08,[c] 3.20,[c] 7.07[e]

[a] In ppm from DSS. [b] CH_3. [c] CH_2. [d] CH. [e] Aromatic. [f] AB but outer lines obscured. [g] NH_2. [h] NH_3.

Table III. Proton-Proton Coupling Constants for the Peptides and $[Co(NH_3)_2(peptide)]$

peptide	form	coupling constants, Hz
gly-gly-gly	complex	$^3J(NH_2, CH_2)$ 7.0; $^5J(CH_2\text{-}1, CH_2\text{-}2$ and $CH_2\text{-}2, CH_2\text{-}3) \sim 1.2$
gly-gly-L-ala	anion	$^3J(\alpha\text{-}CH, CH_3)$ 7.1
	zwitterion	$^3J(\alpha\text{-}CH, CH_3)$ 7.2
	cation	$^3J(\alpha\text{-}CH, CH_3)$ 7.3
	complex	$^3J(\alpha\text{-}CH, CH_3)$ 7.1; $^2J(CH_2\text{-}2)$ 17.5; $^5J(CH_2\text{-}1, CH_2\text{-}2$ and $CH_2\text{-}2, CH\text{-}3) \sim 1.0$
gly-gly-L-leu	anion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.1; $^3J(\gamma\text{-}CH, \delta\text{-}CH_3)$ 5.9, 6.3
	zwitterion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.1; $^3J(\gamma\text{-}CH, \delta\text{-}CH_3)$ 5.5, 5.5
	cation	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.1; $^3J(\gamma\text{-}CH, \delta\text{-}CH_3)$ 6.0, 6.1
	complex	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 4.7, 6.2; $^3J(\gamma\text{-}CH, \delta\text{-}CH_3)$ 5.9, 6.0; $^3J(NH_2, CH_2)$ 7.0; $^2J(CH_2\text{-}2)$ 19.3; $^5J(CH_2\text{-}2, CH\text{-}3)$ 1.0, 1.2; $^5J(CH_2\text{-}1, CH_2\text{-}2) \sim 1.1$
gly-gly-L-phe	anion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 4.9, 8.1
	zwitterion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 5.0, 7.9
	cation	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 5.9, 8.7
	complex	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 5.9, 2.5; $^2J(CH_2\text{-}1)$ 17.3; $^2J(CH_2\text{-}2)$ 18.2; $^5J(CH_2\text{-}2, CH\text{-}3$ and $CH_2\text{-}1, CH_2\text{-}2) \sim 1.0$
L-ala-gly-gly	anion	$^3J(\alpha\text{-}CH, CH_3)$ 7.1
	zwitterion	$^3J(\alpha\text{-}CH, CH_3)$ 7.5
	cation	$^3J(\alpha\text{-}CH, CH_3)$ 7.1
	complex	$^3J(\alpha\text{-}CH, CH_3)$ 7.1; $^3J(NH_2, CH\text{-}1)$ 9.9, 6.5; $^2J(NH_2) \sim 10.0$; $^5J(CH\text{-}1, CH_2\text{-}2$ and $CH_2\text{-}2, CH_2\text{-}3)$ 1.0
L-phe-gly-gly	anion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 6.6, 7.6
	zwitterion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.3; $^2J(CH_2\text{-}2)$ 17.0
	cation	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.3; $^2J(CH_2\text{-}2)$ 17.0
	complex	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 5.4; $^5J(CH\text{-}1, CH_2\text{-}2$ and $CH_2\text{-}2, CH_2\text{-}3) \sim 1.0$
L-tyr-gly-gly	anion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.1
	zwitterion	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.4; $^2J(CH_2\text{-}2)$ 17.2
	cation	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 7.3; $^2J(CH_2\text{-}2)$ 17.1
	complex	$^3J(\alpha\text{-}CH, \beta\text{-}CH_2)$ 4.9, 4.4; $^5J(CH\text{-}1, CH_2\text{-}2$ and $CH_2\text{-}2, CH_2\text{-}3)$ 1.2

in positions of the resonances on protonation of the terminal NH_2 and CO_2^- groups and on coordination to Co(III) are given in Table VII. The assignments of the resonances were based on the work of Christl and Roberts,[29] on undecoupled spectra, and on the shifts in positions of resonances caused by substitution, protonation, and coordination.

Discussion

Preparations. In their early work on cobalt(III) tripeptide complexes, Manyak and co-workers reported the preparations of $[Co(gly\text{-}gly\text{-}gly)(NH_3)_2]\cdot 3.5H_2O$ and $Na[Co(gly\text{-}gly\text{-}$

(29) Christl, M.; Roberts, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 4565.

Table IV. ^{1}H Chemical Shift Differences[a] on Protonation and Chelation

peptide	CH-1	CH-2	CH-3	other
		Protonation of Peptide Anion at NH_2		
gly-gly-gly	+0.36	−0.10	−0.09	
gly-gly-L-ala	+0.51	+0.16	+0.11	+0.04[b]
gly-gly-L-leu	+0.51	+0.07	0.00	+0.01,[b] 0.00,[b] −0.01[c,d]
gly-gly-L-phe	+0.53	+0.05	0.00	−0.01,[c] 0.00,[c] −0.01[e]
L-ala-gly-gly	+0.54	+0.03	0.00	+0.19[b]
L-phe-gly-gly	+0.58	+0.08	+0.09	+0.28,[c] +0.06[e]
L-tyr-gly-gly	+0.58	+0.06	+0.02	+0.31,[c] +0.17[e]
		Protonation of Peptide Zwitterion at CO_2^-		
gly-gly-gly	+0.02	0.00	+0.28	
gly-gly-L-ala	0.00	−0.09	+0.13	+0.06[b]
gly-gly-L-leu	+0.01	+0.01	+0.19	+0.01,[b] +0.03,[b] +0.09[c,d]
gly-gly-L-phe	+0.02	+0.03	+0.24	+0.10,[c] +0.05,[c] +0.12[e]
L-ala-gly-gly	+0.02	+0.02, +0.07	+0.20	+0.07[b]
L-phe-gly-gly	+0.02	−0.02, +0.11	+0.28	+0.03,[c] +0.02[e]
L-tyr-gly-gly	+0.05	+0.02, +0.11	+0.28	+0.03,[c] +0.02[e]
		Chelation of Peptide Anion		
gly-gly-gly	+0.10	−0.27	+0.24	
gly-gly-L-ala	+0.22	−0.14, −0.06	+0.08	+0.23[b]
gly-gly-L-leu	+0.23	−0.23, +0.01	0.00	+0.06,[b] +0.25[c,d]
gly-gly-L-phe	+0.09, +0.14	−0.09, +0.03	+0.09	+0.31 (0.89),[c,f] +0.67 (0.09),[c,f] 0.14[e]
L-ala-gly-gly	+0.10	−0.13	+0.33	+0.14[b]
L-phe-gly-gly	+0.28	+0.02	+0.31	+0.27,[c] +0.07[e]
L-tyr-gly-gly	+0.36	−0.01	+0.28	+0.26,[c] +0.21[e]

[a] In ppm. [b] CH_3. [c] CH_2. [d] CH. [e] Aromatic. [f] Alternative assignment.

Table V. Side-Group Rotamer Populations Based on the Method of Feeney[a] for the Peptides and $[Co(NH_3)_2(peptide)]$

peptide	form	J_{AX}, Hz	J_{BX}, Hz	n_I	n_{II}	n_{III}
gly-gly-L-leu	*b*	7.1	7.1	0.40	0.42	0.18
	complex	4.7	6.2	0.22	0.17	0.60
		6.2	4.7	0.11	0.36	0.53
gly-gly-L-phe	anion	4.9	8.1	0.44	0.17	0.39
		8.1	4.9	0.19	0.56	0.25
	zwitterion	5.0	7.9	0.42	0.18	0.40
		7.9	5.0	0.20	0.54	0.26
	cation	5.9	8.7	0.54	0.27	0.19
		8.7	5.9	0.32	0.62	0.06
	complex	5.9	2.5	−0.14	0.36	0.78
		2.5	5.9	0.12	−0.06	0.94
L-phe-gly-gly	anion	7.6	6.6	0.36	0.49	0.15
		6.6	7.6	0.44	0.36	0.20
	c	7.3	7.3	0.43	0.44	0.13
	complex	5.4	5.4	0.16	0.26	0.58
L-tyr-gly-gly	anion	7.1	7.1	0.40	0.42	0.18
	zwitterion	7.4	7.4	0.44	0.45	0.10
	cation	7.3	7.3	0.43	0.44	0.13
	complex	4.9	4.4	0.03	0.22	0.75
		4.4	4.9	0.07	0.16	0.77
	Ni(II) complex[d]	5.0	5.0	0.10	0.22	0.68
	Pd(II) complex[e]	9.0	3.3	0.04	0.69	0.27
		3.3	9.0	0.48	−0.02	0.54
	Pd(II) complex[f]	9.6	2.4	−0.04	0.77	0.27
		2.4	9.6	0.52	−0.13	0.61
	Pd(II) complex[g]	9.7	3.3	0.07	0.76	0.17
		3.3	9.7	0.56	−0.03	0.47

[a] Reference 25. [b] Anion, zwitterion, and cation. [c] Zwitterion and cation. [d] Data from ref 18 at pH 10. [e] Data from ref 18 at pH 2.85. [f] Data from ref 18 at pH 9.71. [g] Data from ref 18 at pH 13.35.

gly)(gly)]·$6H_2O$ and postulated structures for the complexes in which the terminal NH_2, the two peptide N donors, and the CO_2^- group were coordinated in a cis α configuration. Subsequent to that work, X-ray structural studies have shown that the peptide group retains its rigid planar structure within 6° upon coordination, and, when a metal is coordinated to three consecutive donor groups of a peptide chain with the central donor a peptide N, the three donor atoms lie in the same coordination plane.[30] The structures postulated by Manyak and co-workers[7] are therefore not possible. Attempts in this laboratory to repeat the preparations of the above complexes using the methods described were unsuccessful. Freeman and Robinson have also reported the failure of the method for the diammine complex using (±)-ala-gly-gly.[31]

The preparation of $[Co(NH_3)_2(gly\text{-}gly\text{-}gly)]$ was attempted by a number of different methods. The reaction of the peptide with an equimolar quantity of pentaammineaquacobalt(III) in water in the presence of 3 mol of sodium hydroxide, Manyak's method,[7] yielded no peptide complex after stirring at room temperature for 1 h, but on stirring of the solution

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(31) Freeman, H. C.; Robinson, G. *J. Chem. Soc.* **1965**, 3194.

Table VI. ^{13}C Chemical Shifts[a] of Peptides and $[Co(NH_3)_2(peptide)]$

peptide	CH-1	CO-1	CH-2	CO-2	CH-3	CO-3	other
				Free Peptide at pH 9.5: Anion			
gly-gly-gly	44.67	177.30	43.12	172.09	44.00	177.30	
gly-gly-L-ala	44.61	177.15	43.09	171.21	51.75	180.78	18.26[b]
gly-gly-L-leu	44.64	177.15	43.15	171.48	54.71	180.75	21.56,[b] 23.26,[b] 25.42,[d] 41.51[c]
gly-gly-L-phe	44.58	177.01	43.09	171.30	56.87	178.58	38.35,[c] 127.66,[h] 129.38,[g] 130.14,[f] 138.31[e]
L-ala-gly-gly	50.76	179.29	43.03	171.92	44.03	177.33	20.22[b]
L-phe-gly-gly	57.07	178.23	43.15	171.74	43.97	177.12	41.31,[c] 127.83,[h] 129.59,[g] 130.14,[f] 137.93[e]
L-tyr-gly-gly	57.28	178.56	43.18	171.86	44.00	177.21	40.46,[c] 118.09,[g] 126.05,[e] 131.40,[f] 161.09[h]
				Free Peptide in D_2O: Zwitterion			
gly-gly-gly	41.32	168.61	43.20	171.64	44.03	177.28	
gly-gly-L-ala	41.30	168.48	43.11	170.82	51.77	180.65	18.19[b]
gly-gly-L-leu	41.53	168.42	43.05	171.00	54.81	180.65	21.64,[b] 23.22,[b] 25.39,[d] 41.24[c]
gly-gly-L-phe	41.22	168.32	43.06	170.80	57.07	178.53	38.41,[c] 127.63,[h] 129.38,[g] 130.14,[f] 138.39[e]
L-ala-gly-gly	49.96	172.23	43.23	171.47	43.99	177.70	17.14[b]
L-phe-gly-gly	55.41	170.54	43.15	171.30	43.74	176.68	37.59,[c] 128.86,[h] 130.03,[i] 130.20,[i] 134.62[e]
L-tyr-gly-gly	55.51	170.71	43.17	171.23	43.99	177.14	36.79,[c] 116.71,[g] 126.36,[e] 131.63,[f] 156.02[h]
				Free Peptide at pH 1: Cation			
gly-gly-gly	41.37	168.58	43.12	172.47	41.86	173.96	
gly-gly-L-ala	41.28	168.46	43.01	171.30	50.32	178.47	17.41[b]
gly-gly-L-leu	41.28	168.40	42.92	171.80	52.34	177.24	21.42,[b] 22.97,[b] 25.22,[d] 40.20[c]
gly-gly-L-phe	41.28	168.35	42.89	171.51	54.85	175.45	37.42,[c] 128.01,[h] 129.56,[g] 130.11,[f] 137.31[e]
L-ala-gly-gly	50.00	172.21	43.15	172.09	42.54	174.99	17.15[b]
L-phe-gly-gly	55.35	170.51	43.03	171.94	41.84	173.87	37.56,[c] 128.86,[h] 130.03,[i] 130.26,[i] 134.59[e]
L-tyr-gly-gly	55.44	170.60	43.03	171.97	41.84	173.87	36.75,[c] 116.72,[g] 126.28,[e] 131.67,[f] 156.00[h]
				Complex in D_2O			
gly-gly-gly	47.85	178.93	51.05	178.99	48.70	186.94	
gly-gly-L-ala	47.80	178.85	51.78	179.26	55.99	190.08	19.19[b]
gly-gly-L-leu	47.74	178.85	51.90	179.37	59.03	189.55	22.12,[b] 23.93,[b] 25.51,[d] 43.06[c]
gly-gly-L-phe	47.63	178.73	51.78	179.96	62.25	188.09	36.57,[c] 128.54,[h] 130.17,[g] 130.52,[f] 138.48[e]
L-ala-gly-gly	55.23	180.78	51.55	178.67	48.68	186.98	19.78[b]
L-phe-gly-gly	59.97	179.08	51.61	178.61	48.62	186.92	38.44,[c] 128.77,[h] 130.23,[i] 130.52,[i] 136.41[e]
L-tyr-gly-gly	59.97	179.20	51.55	178.61	48.62	186.92	37.45,[c] 117.19,[g] 127.60,[e] 131.99,[f] 156.50[h]

[a] In ppm from Me_4Si. [b] CH_3. [c] CH_2. [d] CH. [e] C1 phenyl. [f] C2,6 phenyl. [g] C3,5 phenyl. [h] C4 phenyl. [i] C2,6 or C3,5 phenyl.

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

peptide	CH-1	CO-1	CH-2	CO-2	CH-3	CO-3	other
				Protonation of Peptide Anion at NH_2			
gly-gly-gly	−3.35	−8.69	+0.08	−0.45	+0.03	−0.02	
gly-gly-L-ala	−3.31	−8.67	+0.02	−0.39	+0.02	−0.13	−0.07[b]
gly-gly-L-leu	−3.11	−8.73	−0.10	−0.48	+0.10	+0.08	+0.08,[b] −0.04,[b] −0.03,[d] −0.27[c]
gly-gly-L-phe	−3.36	−8.69	−0.03	−0.50	+0.20	−0.05	+0.06,[c] −0.03,[h] 0,[f,g] +0.08[e]
L-ala-gly-gly	−0.80	−7.06	+0.20	−0.45	−0.04	−0.13	−3.08[b]
L-phe-gly-gly	−1.66	−7.69	0	−0.44	−0.23	−0.44	−3.72,[c] +1.03,[h] −3.31[e]
L-tyr-gly-gly	−1.77	−7.85	−0.01	−0.63	−0.01	−0.07	−3.67,[c] −1.38,[g] +0.31,[e] +0.23,[f] −5.07[h]
				Protonation of Peptide Zwitterion at CO_2^-			
gly-gly-gly	+0.05	−0.03	−0.08	+0.83	−2.17	−3.32	
gly-gly-L-ala	−0.02	−0.02	−0.10	+0.48	−1.45	−2.18	−0.78[b]
gly-gly-L-leu	−0.25	−0.02	−0.13	+0.80	−2.47	−3.41	−0.22,[b] −0.25,[b] −0.17,[d] −1.04[c]
gly-gly-L-phe	+0.06	+0.03	−0.17	+0.71	−2.22	−3.08	−0.99,[c] +0.38,[h] +0.18,[g] −0.03,[f] −1.08[e]
L-ala-gly-gly	+0.04	−0.02	−0.08	+0.62	−1.45	−2.21	+0.01[b]
L-phe-gly-gly	−0.06	−0.03	−0.12	+0.64	−1.90	−2.81	−0.03,[c,e] 0[h]
L-tyr-gly-gly	−0.07	−0.11	−0.14	+0.74	−2.15	−3.27	−0.04,[c] −0.01,[g] −0.08,[e] +0.04,[f] −0.02[h]
				Chelation of Peptide Anion			
gly-gly-gly	+3.18	+1.63	+7.93	+6.90	+4.70	+9.64	
gly-gly-L-ala	+3.19	+1.70	+8.69	+8.05	+4.24	+9.30	+0.93[b]
gly-gly-L-leu	+3.10	+1.70	+8.75	+7.89	+4.32	+8.80	+0.56,[b] +0.67,[b] +0.09,[d] +1.55[c]
gly-gly-L-phe	+3.05	+1.72	+8.69	+8.66	+5.38	+9.51	−1.78,[c] +0.88,[h] +0.79,[g] +0.38,[f] +0.17[e]
L-ala-gly-gly	+4.47	+1.49	+8.52	+6.75	+4.65	+9.65	−0.44[b]
L-phe-gly-gly	+2.90	+0.85	+8.46	+6.87	+4.65	+9.80	−2.87,[c] +0.94,[h] −1.52[e]
L-tyr-gly-gly	+2.69	+0.64	+8.37	+6.75	+4.62	+9.71	−3.01,[c] −0.90,[g] +1.55,[e] +0.59,[f] −4.59[h]

[a] In ppm. [b] CH_3. [c] CH_2. [d] CH. [e] C1 phenyl. [f] C2,6 phenyl. [g] C3,5 phenyl. [h] C4 phenyl.

for over 15 h, a small yield of $[Co(H_2O)_2(gly\text{-}gly\text{-}gly)]\cdot 2H_2O$ was obtained. Browning's method[3] for triammine(peptide)-cobalt(III) based on the peroxide oxidation of a cobalt(II) solution containing the peptide and ammonia gave 11 fractions when the reaction product was chromatographed on a CM-Sephadex C-25 column. The main products were $[Co(H_2O)_2(gly\text{-}gly\text{-}gly)]$ and $[Co(NH_3)_2(gly\text{-}gly\text{-}gly)]$, but the yields were poor. A poor yield of the diammine was also obtained from the reaction of the peptide with $[Co(NH_3)_6]^{3+}$ in the presence of charcoal in water at 60 °C. The "peroxo dimer" method reported here was the most successful method, giving over 50% yield of the desired product.

Structure of the Complexes. On the basis of the C, H, and N analyses and the fact that the complexes are nonelectrolytes in neutral aqueous solution, the most probable structures for the complexes are **1**–**6**. The visible absorption spectra for the complexes of the various peptides have maxima for the first spin-allowed d–d transition at 456 nm, compared with 489 nm for $[Co(NH_3)_3(gly\text{-}gly)]^+$,[32] in which the peptide is coordinated through the NH_2, N^-, and CO_2^- groups. Considering the spectrochemical parameters for the various donors,[30] this result is consistent with structures **1** and **2** and not with the other structures. The protons of the terminal NH_2 group are not exchanged rapidly by deuterium when the complex is dissolved in D_2O, consistent with a coordinated NH_2 group as found in structures **1**–**3** but not in **4**–**6**. The shifts of the ^1H and ^{13}C resonances of the peptide CH_2 group of the C-terminal residue and of the ^{13}C resonance of the CO_2^- group (Tables IV and VII and below) confirm that the carboxylate group is coordinated and that the complex has structure **1**. Indeed the ^1H and ^{13}C shifts for the three residues confirm structure **1**. Finally, the absorption maximum is independent of pH between pH 5 and 9 consistent with the lack of water coordinated to Co(III).

^{13}C NMR Spectra. The ^{13}C NMR spectra of peptides have been analyzed by Christl and Roberts in terms of the effects of substituents and of pH changes.[29] For the peptides in common between that study and the present, the results are generally in good agreement.

For gly-gly-gly the effect of protonation at NH_2 is, as expected, markedly more pronounced in the N-terminal residue than in the other two residues with the carbonyl and methylene groups shifted to higher shielding by 8.7 and 3.35 ppm, respectively. The reasons for these shifts have been discussed previously.[29,32,33] The very large shift of the carbonyl has been associated with the sp^2 hybridization of the carbonyl carbon. The other CH_2 resonances are practically unaltered by the protonation, but CO-2 is shifted to higher shielding by 0.45 ppm.

Substitution in the C-terminal residue (at CH-3) does not alter the size of the shifts for the various ^{13}C resonances on protonation of the NH_2 group. However, substitution in the N-terminal residue (at CH-1) attenuates the effects on CH-1 (-1.3 ± 0.5 ppm) and CO-1 (-7.7 ± 0.6 ppm). The side-group β-C resonance undergoes a relatively large shift (-3.6 ± 0.5 ppm) when substituted at CH-1, significantly larger than the α-C resonance. The aromatic resonances for L-phe-gly-gly and L-tyr-gly-gly also experience relatively large shifts, especially C-4 (+1.0 and −5.1 ppm, respectively) for both peptides, C-1 (−3.3 ppm) for the former and C-3,5 (−1.4 ppm) for the latter peptide.

The effects of protonation at CO_2^- for gly-gly-gly are largest at CO-3 (−3.3 ppm) and CH-3 (−2.2 ppm). The only other resonance to be significantly affected is CO-2 which experiences a shift to lower shielding by 0.8 ppm. Possible reasons for the changes have been discussed previously.[29,34,35] Substitutions at CH-3 and CH-1 have relatively minor effects on these shifts. The side-group resonances for the CH-1 substituted peptides are not significantly affected by protonation at CO_2^-, but, for the CH-3 substituted peptides, β-C, and C-1 for the phenylalanine peptide undergo large shifts to higher shielding, and C-4 for the phenyl group is shifted by 0.4 ppm to lower shielding.

Chelation of the tripeptide anion to Co(III) shifts the backbone ^{13}C resonances to lower shielding: CH-1, ~3; CO-1, ~1.5; CH-2, ~8.5; CO-2, ~7; CH-3, ~4.5; CO-3, ~9.5 ppm. Coordination of an amino acid zwitterion via the carboxylate to Co(III) causes the carboxylate to shift to lower shielding by about 4 ppm, but the α-C shifts to higher shielding (gly, 0.2; L-ala, 0.6 ppm).[36] Chelation of an amino acid anion, on the other hand, results in both α-C and CO_2^- resonances shifting to lower shielding. (For gly: α-C, 0.7; CO, 3.6 ppm. For L-ala: α-C, 2.6; CO, 1.6 ppm. For L-leu: α-C, 1.0; CO, 2.3 ppm.[36]) The peptide resonances generally undergo larger shifts on chelation. Two factors that would contribute to this are the deprotonation of the peptide nitrogens on coordination and the strain associated with the quadridentate chelation.

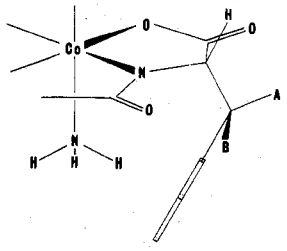

Figure 3. Preferred orientation of side group in $[Co(NH_3)_2(gly\text{-}gly\text{-}L\text{-}phe)]$.

Chelation also has marked effects on β-C resonances: gly-gly-L-ala, +0.93; gly-gly-L-leu, +1.55; gly-gly-L-phe, −1.78; L-ala-gly-gly, −0.44; L-phe-gly-gly, −2.87; L-tyr-gly-gly, −3.01 ppm. The two methyl resonances in gly-gly-L-leu shift to lower shielding on chelation of the peptide by 0.56 and 0.67 ppm. The aromatic resonances C1, C2, C3, and C4 show an interesting variation: gly-gly-L-phe, +0.17, +0.38 +0.79, +0.88; L-phe-gly-gly, −1.52, ..., ..., +0.94; L-tyr-gly-gly, +1.55, +0.59, −0.90, −4.59 ppm. At least part of these changes for the side group resonances must be due to the increased electronic delocalization in the peptide backbone on the chelation, to the positioning of the side group above the chelate ring, and to the proposed hydrogen-bonding interaction between a coordinated NH_3 and the aromatic rings (see below).

^1H NMR Spectra. Protonation of the NH_2 group in the tripeptide anions caused the CH-1 resonance to shift approximately 0.5 ppm to lower shielding. The only other major shifts were experienced by the β-C protons for substituents at CH-1 ($+0.25 \pm 0.06$ ppm). Protonation of the CO_2^- group had its only marked effect on CH-3, causing a shift of 0.2 ± 0.1 ppm to lower shielding. Other shifts were of the order of 0.1 ppm or less. Chelation generally caused CH-1, CH-3, and the substituent resonances to move to lower shielding and CH-2 to move to higher shielding although CH-2 for L-phe-gly-gly and L-tyr-gly-gly did not shift significantly. The β-C protons showed relatively large shifts consistent with the large shifts observed for the β-^{13}C resonances.

A chemical shift difference ($\Delta\delta_{AB}$) for the central methylene protons is not observed for the free tripeptides with C-terminal substituents. In the Co(III) complexes it is observed to be 0.08, 0.24, and 0.12 for gly-gly-L-ala, gly-gly-L-leu, and gly-gly-L-phe, respectively. The shifts for the L-leu and L-phe peptides are related to the populations of the rotamers in Figure 2. As far as $\Delta\delta_{AB}$ for the central CH_2 group is concerned, rotamer I for the C-terminal substituted peptides would have a similar effect as the methyl group in the L-ala-peptide (0.08 ppm). The larger values of $\Delta\delta_{AB}$ for the L-leu and L-phe complexes arise from contributions from rotamers II and III because in these rotamers the side group is closer to the central methylene and is in a position to shield A and B differently. The unique observation of an AB pattern for the N-terminal CH_2 group in $[Co(NH_3)_2(gly\text{-}gly\text{-}L\text{-}phe)]$ and the large difference in the chemical shifts of the two NH_3 groups arise from a large

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population of rotamer III in this complex. The unusual observation of an NH_3 resonance with δ less than 1 compared to the normal position of about 2.4 ppm in these peptide complexes must result from a high population of rotamer III in which the phenyl is shielding the NH_3. This rotamer could be stabilized by a hydrogen bond between the NH_3 and the phenyl π system (Figure 3). For the L-phe-gly-gly and L-tyr-gly-gly complexes the population of rotamer III appears to be less as the NH_3 resonance is at about 1.45 ppm compared to 0.77 ppm for gly-gly-L-phe.

The vicinal coupling constants for α-CH and β-CH_2 for the side groups have been analyzed previously in terms of the rotamer distribution for free peptides and some Ni(II) and Pd(II) complexes[18,27,28] by using the Feeney[25] or Pachler[26] methods. The Feeney method has been applied here. For the free peptides, irrespective of the assignment of the A and B protons, rotamer III is little populated ($n_{III} \leq 0.2$) for gly-gly-L-leu, L-phe-gly-gly, and L-tyr-gly-gly in the anionic, zwitterionic, and cationic forms and for gly-gly-L-phe in the cationic form. Both rotamers I and II are significantly populated in these cases but the calculated relative populations vary with substituent, with pH, and with the assignment (Table V). For the anionic and zwitterionic forms of gly-gly-L-phe, n_{III} is of the order of 0.25–0.40. Irrespective of the assignment of the β-CH_2 protons, n_{III} is markedly higher in the Co(III) complex of this peptide. For one assignment with $J_{AX} = 2.5$ Hz and $J_{BX} = 5.9$ Hz, n_{III} was calculated to be 0.94 which is consistent with the observed marked shielding of one NH_3 (Table II). This assignment is shown in Figure 3. One proton, B, is approximately 0.6 ppm less shielded than the other. Part of this difference could result from differences in the long-range anisotropic shielding by the peptide and carboxylate groups, but the major part of the difference is probably derived from the orientation of the two protons with respect to the aromatic plane. The optimum orientation of the phenyl group to maximize hydrogen bonding with the NH_3 group has H_B closer to the plane of the phenyl than H_A, H_B therefore experiencing greater deshielding by the phenyl group. For the other peptides, the n_{III} values for the Co(III) complexes are also markedly higher than for the free peptides. For gly-gly-L-leu and L-phe-gly-gly, n_{III} is calculated to be about 0.6, and for L-tyr-gly-gly n_{III} is about 0.8. Again these results for the peptides with the aromatic groups are consistent with the appearance of one NH_3 resonance at an unusually shielded position (δ 1.5). For the Ni(II) complex of L-tyr-gly-gly, n_{III} is calculated to be about 0.7. For the Pd(II) complex at different pH values, only one assignment has $n_{III} \geq 0.5$. It has been proposed for these square-planar complexes that rotamer III is stabilized by an interaction of the aromatic ring with the d orbitals of the metal.[18,28] Rotamer III has been found by X-ray analysis to exist for a C-terminal tyrosine in the solid state for a polymeric complex with the formula $[Cu_2(gly\text{-}L\text{-}leu\text{-}L\text{-}tyr)_2]\cdot 8H_2O\cdot Et_2O$.[37]

The above rotamer populations calculated by the Feeney[25] method should be considered as only approximate because there are a number of unsatisfactory aspects of the calculations. The method is based on set coupling constants J_{AX} and J_{BX} for the three rotamers. No allowance is made for changes in dihedral angle, for example, between the $H_x(\alpha\text{-C})(\beta\text{-C})$ and $H_A(\beta\text{-C})(\alpha\text{-C})$ planes, or electronegativity on changing from one peptide to another or on changing from free peptide to complex. The dihedral angles might not vary significantly for the free peptides, but in the complexes the rather rigid structure of the chelate and the presence of apical ligands in the octahedral complexes introduce nonbonded and other interactions that would require a change in the dihedral angles and hence in the individual coupling constants for the rotamers. Possibly, as a consequence of this, negative values were obtained for populations of one rotamer for some of the metal complexes. Without a knowledge of the dihedral angles appropriate to the three rotamers a more rigorous treatment is not possible.

The observation of ^5J coupling between the CH-2 protons and the protons on CH-1 and CH-3 is indicative of the electronic delocalization around the peptide backbone in the complex. The values of about 1 Hz are typical of ^5J values for olefins.[38]

Acknowledgment. The authors gratefully acknowledge financial support form the Australian Research Grants Committee.

Registry No. $Co(NH_3)_2$(gly-gly-gly), 74808-50-7; $Co(NH_3)_2$(gly-gly-L-ala), 74808-51-8; $Co(NH_3)_2$(gly-gly-L-leu), 74808-52-9; $Co(NH_3)_2$(gly-gly-L-phe), 74808-53-0; $Co(NH_3)_2$(L-ala-gly-gly), 74808-54-1; $Co(NH_3)_2$(L-phe-gly-gly), 74808-55-2; $Co(NH_3)_2$(L-tyr-gly-gly), 74808-56-3; gly-gly-gly, 556-33-2; gly-gly-L-ala, 16422-05-2; gly-gly-L-leu, 2576-67-2; gly-gly-L-phe, 14656-09-8; L-ala-gly-gly, 19729-30-7; L-phe-gly-gly, 6234-26-0; L-tyr-gly-gly, 17343-07-6; $[(H_3N)_5Co(O_2)Co(NH_3)_5](NO_3)_4$, 16632-71-6.

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