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Spectroscopic Study of 1:1 Copper(II) Complexes with Schiff Base Ligands Derived from Salicylaldehyde and L-Histidine and Its Analogues[1]

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Four complexes of Cu(II) with Schiff base ligands derived from salicylaldehyde and glycine, ϵ-acetyl-L-lysine, histamine, and L-histidine have been prepared and characterized by elemental analysis, room-temperature electronic absorption and circular dichroism spectroscopy, pH titration, and low-temperature EPR spectroscopy. The room-temperature spectroscopic and titration data confirm earlier studies, which indicated that Cu(sal–his) has its imidazole group coordinated in an axial position, while the other three donor groups reside in the equatorial plane, as they do in Cu(sal–gly). Well-resolved EPR spectra from discrete, nonpolymerized glassy frozen solutions of these complexes were obtained in 25% aqueous dioxane and in the presence of nitrogen-donor ligands (γ-collidine, pyridine, and *N*-methylimidazole). Computer simulation of the glassy EPR spectra indicates that they are magnetically axial, with an extra absorption peak, rather than rhombic. Cu(sal–gly) and Cu(sal–lysAc) form square-planar complexes with γ-collidine in dry dioxane and have much smaller $g_{\parallel}$ and larger $|A_{\parallel}|$ values than the other complexes of this study. All other complexes appear to have some degree of axial solvation. In aqueous dioxane without added nitrogen bases, Cu(sal–his) exhibits EPR parameters very similar to those of Cu(sal–gly) and Cu(sal–lysAc), suggesting that in frozen solution the equatorial plane is composed of the same three donor groups in each case, with water as the probable fourth equatorial ligand, leaving the imidazole group to interact weakly in the axial position. However, when pyridine or *N*-methylimidazole (0.6 and 0.5 M, respectively) are added to each of the four complexes, the glassy EPR parameters of Cu(sal–his) become uniquely different from those of the other three, although the gross appearances of the spectra closely resemble those of the analogous complexes of Cu(sal–hm). These results suggest that the fourth donor group of the sal–his ligand confers unique spectroscopic properties in the metal, which should be given careful consideration in further studies of metal–Schiff base complexes of amino acids and salicylaldehyde or other aldehydes such as pyridoxal or pyruvate.

Introduction

Metal complexes with Schiff base ligands composed of salicylaldehyde and a primary amine have been studied for many years.[3-29] Among these studies, a number have involved Schiff bases in which the primary amine is an α-amino acid.[8-29] Comparative studies have also been made where salicylaldehyde has been replaced by pyruvate,[29-31] (1*R*)-3-(hydroxymethylene)camphor,[31,32] or pyridoxal or pyridoxal phosphate,[3,17,29,34-44] usually with the aim of elucidating the mechanism of action of vitamin B_6 containing enzymes.[45-48]

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Table I. Elemental Analyses

		% C	% H	% N	% Cu	% other
Cu(sal-gly)·1 1/2H_2O	calcd	40.38	3.76	5.23	23.74	
	found	40.31	3.82	5.20	23.7	
Cu(sal-lysAc)·H_2O	calcd	48.45	5.42	7.50	17.09	
	found	49.96	5.21	7.83	17.60	
Cu(sal-his)·1 1/2H_2O	calcd	44.89	4.06	12.08	18.27	0.00 (Na)
	found	45.45	3.65	13.08	19.70	0.07 (Na)
Cu(sal-hm)(OH^-) or Cu(sal-hm^-)·H_2O	calcd	48.89	4.44	14.25	21.55	0.00 (Cl)
	found	47.59	4.19	14.13	24.30	0.68 (Cl)
Cu(sal-hm^-)	calcd	52.08	4.01	15.18	22.96	0.00 (Cl)
	found	50.80	4.04	15.18	22.30	0.55 (Cl)

These enzymes all contain pyridoxal phosphate (PLP) at the active site,[46-50] bound to the enzyme by Schiff base formation between the 4-carboxylate groups of pyridoxal and an enzyme residue containing an amino group (lys-258 in the case of chicken mitochondrial aspartate aminotransferase[50]).

The pyridoxal phosphate dependent enzymes are involved in a wide variety of transformations of amino acids, including α-, β-, and γ-elimination reactions,[46-49] whose common mechanistic feature appears to be the stabilization of carbanionic intermediates in which the Schiff base of PLP acts as a delocalizing electron sink for the extra electron density. Both metal ions[17,18,37-39,43,44] and H^+ [43-45] have been used to promote such transformations in model studies, but the enzymatic reactions appear to involve only H^+ catalysis[45-52] and may even be inhibited by the presence of metal ions.[59] Thus, at present there appears to be no known role for metal complexes of Schiff bases of pyridoxal phosphate at the active sites of the PLP-dependent enzymes.

Many other roles for pyridoxal phosphate and its Schiff bases have been suggested which would indicate that studies of metal complexes of Schiff bases composed of aldehydes related to pyridoxal phosphate and amino acids may provide useful information that would help to elucidate some of those roles. For example, pyridoxine phosphate, PNP (vitamin B_6), is involved in absorption of zinc by the intestines.[53] Pyridoxal phosphate, PLP, is contained in the enzyme glycogen phosphorylase, where it is believed to be involved in the catalytic mechanism,[54] as well as in regulating the phosphorylation state of the enzyme.[55] PLP has also been implicated as having roles in regulation of enzyme degradation,[56,57] lymphoid and immune system response function,[58,59] regulation of the properties of steroid-receptor complexes,[60-64] and blood coagulation.[65] It has also been shown that the metabolism of vitamin B_6 by fast-growing tumors (Morris hepatomas) is vastly different from that by normal tissue[66] and, finally, that pyridoxal phosphate will react with hemoglobin to form Schiff base derivatives of the two β-chain lysines of the diphosphoglycerate binding site.[67]

Many of the model studies of the metal complexes of Schiff base ligands composed of pyridoxal phosphate or salicylaldehyde and amino acids have focused upon the tridentate ligand binding mode of these ligands.[8-41,43,44] Crystal and molecular structural studies of some of these have shown that the phenolic oxygen, imine nitrogen, and carboxylate oxygen are roughly in the equatorial plane.[10,11,15,26,36,37,40] Few studies have considered amino acids with a potential fourth donor atom, of which one of the most obvious is histidine. Recently, Casella and Gullotti[28] have shown one reason for the paucity of reports of Schiff base ligands involving histidine: The imine carbon of the initially formed Schiff base is attacked by the 5-position of the imidazole ring to produce 4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole compounds of both pyridoxal phosphate and salicylaldehyde in water or aqueous methanol, respectively. Only in the presence of a metal ion ($Zn(II)$[28] or $Cu(II)$[29]) could the Schiff base ligand be stabilized. These workers reported circular dichroism results that indicate that the bonding mode of salicylidine- or pyridoxylidine–histidine ligands to Zn^{2+} and Cu^{2+} is "glycine-like", as found in the earlier crystal structures,[10,11,15,26,35,36,40] rather than "histamine-like", where the imidazole nitrogen rather than the carboxylate oxygen is in the plane.[28,29] Our own studies[1] of Schiff base complexes of metal ions, in which the ligand is composed of salicylaldehyde and histidine, glycine, or ϵ-acetyllysine, indicate that, in the case of Cu(II), the coordination mode of the histidine derivative is unique, particularly in the presence of additional Lewis bases. This report summarizes our findings and emphasizes the strong tendency for Cu(II) to bind imidazole.

Experimental Section

Materials. Salicylaldehyde, glycine, ϵ-acetyl-L-lysine, histamine dihydrochloride, and L-histidine hydrochloride hydrate (Aldrich) and copper(II) acetate hydrate (Alfa) were used without additional purification. Spectroscopic dioxane (MCB) was used as received, or reagent grade (Fisher) was dried further by refluxing with sodium for 24 h and distilling under a stream of dry nitrogen. γ-Collidine was purified as reported previously,[69] and pyridine and *N*-methylimidazole (Aldrich) were distilled prior to use and stored over molecular sieves.

Preparation of Complexes. Cu^{II}(sal-gly)·1 1/2H_2O. One equivalent of salcylaldehyde was dissolved in ethanol (2.0 mmol in 10 mL of

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Table II. EPR Parameters for Cu(II) Schiff Bases

complex	solvent	$g_\parallel$[a]	$g_\perp$[a]	$10^3 \times \lvert A_\parallel \rvert$,[b] cm^{-1}	$10^3 \times \lvert A_\perp \rvert$,[b] cm^{-1}	$\lvert A_N \rvert$,[b] cm^{-1}	α^2[c]	β_1^2[c]	β^2[c]
Cu(sal-gly)	3:1 diox-H_2O	2.258	2.054	18.0	...	...	0.82	0.80	0.67
+pyridine[d]	diox	2.247	2.052	17.8	...	...	0.80		
+pyridine	3:1 diox-H_2O	2.252	2.052	18.0	...	...	0.81		
+*N*-MeIm[e]	diox	2.256	2.057	17.1	...	...	0.79		
+*N*-MeIm	3:1 diox-H_2O	2.263	2.062	16.4	...	...	0.78		
+γ-collidine[f]	diox	2.199	2.053	19.1	...	...	0.79		
Cu(sal-lysAc)	3:1 diox-H_2O	2.255	2.050	17.7	...	...	0.81	0.80	0.63
+pyridine[d]	diox	2.243	2.043	17.7	...	...	0.79		
+pyridine	3:1 diox-H_2O	2.249	2.046	17.6	...	...	0.79		
+*N*-MeIm[e]	diox	2.254	2.054	17.5	...	...	0.80		
+*N*-MeIm	3:1 diox-H_2O	2.268	2.056	16.2	...	...	0.78		
+γ-collidine[f]	diox	2.195	2.041	19.0	1.6	1.6	0.78		
Cu(sal-his)	3:1 diox-H_2O	2.256	2.058	17.7	...	1.4	0.81	0.84	0.98
+pyridine[d]	diox	2.256	2.045	17.2	...	...	0.79		
+pyridine	3:1 diox-H_2O	2.260	2.054	17.1	...	1.2	0.80		
+*N*-MeIm[e]	diox	2.257	2.062	17.2	...	...	0.80		
+*N*-MeIm	3:1 diox-H_2O	2.267	2.053	16.6	...	1.5	0.79		
+γ-collidine[f]	diox	...	...	...	...	...			
Cu(sal-hm)	3:1 diox-H_2O	2.250	2.050	18.3	...	1.4	0.82	0.79	0.64
+pyridine[d]	3:1 diox-H_2O	2.252	2.053	17.9	...	...	0.81		
+*N*-MeIm[e]	3:1 diox-H_2O	2.257	2.058	17.3	...	1.4	0.80		
+γ-collidine[f]	diox	...	...	...	...	...			

[a] g values ±0.001. [b] $\lvert A \rvert$ values $\pm 0.3 \times 10^{-3}$ cm^{-1}. [c] $P = 0.036$ cm^{-1}, $\lambda = -828$ cm^{-1}; $T(n)$ and S are calculated from a weighted average of nitrogen and oxygen atoms in the equatorial plane (nitrogen, $T(n) = 0.333$, $S = 0.093$; oxygen, $T(n) = 0.220$, $S = 0.076$).[29] [d] [pyridine] = 0.6 M. [e] [*N*-MeIm] = 0.5 M. [f] [λ-collidine] = 0.6 M.

ethanol) and added to an aqueous solution of glycine (2.0 mmol in 5 mL of water) with stirring. A yellow solution indicated the formation of Schiff base. One equivalent of copper(II) acetate was dissolved in water (2.0 mmol in 5 mL of water) and added to the Schiff base solution with stirring. Two equivalents of NaOH in water (2 M) was added and the solution stirred for 24 h. The precipitate was filtered from solution, washed twice with water, then ethanol, and then ethyl ether, and allowed to dry at least 24 h under vacuum over P_2O_5. The product was dried further under reduced pressure at 40–50 °C for 24 h. Analytical results for this and the other complexes of this study are listed in Table I.

Cu^{II}(sal-lysAc)·H_2O. The procedure was followed as above, with acetyllysine substituted for glycine. The product was precipitated by evaporating the solution to half-volume with gentle heating.

Cu^{II}(sal-hm)·H_2O. The procedure was followed as above with histamine dihydrochloride plus 2 equiv of NaOH substituted for glycine. The stirred solution was heated at 70 °C for 30 min. The brown product precipitated from solution.

Cu^{II}(sal-his)·$1^1/_2H_2O$. The procedure was followed as above, with histidine hydrochloride plus 1 equiv of NaOH substituted for glycine. The stirred solution was heated at 70 °C for 30 min. The green product precipitated from solution.

Physical Measurements. Visible spectra were recorded on a Perkin-Elmer 552 spectrophotometer. The circular dichroism spectrum of Cu(sal-his) was measured on a JASCO J-20 spectrophotometer in the Department of Chemistry, University of California, Berkeley, CA. Glassy EPR spectra (77 K) were recorded on a Varian E-12 spectrometer with a E-101 microwave bridge operating at X band. The microwave frequency was calibrated with use of the spectrum of the Varian weak pitch sample ($g = 2.0027$). EPR tubes and other glassware used for preparing samples in dry dioxane were dried at least 24 h at 150 °C and stored in dry desiccators with P_2O_5 prior to use. Elemental analyses (Table I) were done by the Microanalytical Laboratory at the Department of Chemistry, University of California, Berkeley, CA. Some metal analyses were done in-house by atomic absorption on a Varian Techtron AA6.

Some EPR spectra were computer simulated with use of the program EPRPOW,[70-72] which was kindly made available by Professor N. D. Chasteen.

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Table III. Electronic Spectra in 25% Aqueous Dioxane

	ν_4, 10^3 cm^{-1}	ν_3, 10^3 cm^{-1}	ν_2, 10^3 cm^{-1}	ν_1, 10^3 cm^{-1}
Cu(sal-gly)	42.2	38.0 (35.1 sh)[a]	27.9	15.5
Cu(sal-lysAc)	42.2	37.6 (36.4 sh)[a]	27.9	15.4
Cu(sal-hm)	42.2	37.6 (34.5 sh)[a]	27.8	15.7
Cu(sal-his)	42.0	37.5	26.8	16.4

[a] sh = shoulder.

Results

Properties of Complexes. Cu(sal-gly)·$1^1/_2H_2O$. The glycine derivative is a turquoise amorphous solid, insoluble in methanol, ethanol, acetone, and dioxane. Previous investigators isolated a sesquihydrate[9] and a green pentahydrate[8,9] complex depending upon the temperature of isolation. The possibility of polymerization with bridging via the carboxylate group in all these species is evident from the solid-state structure of the pentahydrate form[15,11] and may account for some variation in color seen from one lot to another.

The glycine derivative in this study did not produce an EPR-detectable signal in dry dioxane. EPR spectra were obtained with the complex in 25% aqueous dioxane and with added (about 5% by volume) pyridine, *N*-methylimidazole, and γ-collidine. Parameters from these spectra and others are reported in Table II. Electronic absorption parameters are reported in Table III.

Cu(sal-lysAc)·H_2O. This new complex is a turquoise amorphous solid, soluble in water, methanol, and ethanol and insoluble in acetone and dioxane.

Cu(sal-his)·$^1/_2H_2O$. The product is a green amorphous powder, sufficiently soluble in wet dioxane to permit reprecipitation. It is very slightly soluble in water, absolute ethanol and methanol, and insoluble in acetone. The circular dichroism spectrum is discussed below.

Cu(sal-hm)(OH) or Cu(sal-hm$^-$)·H_2O. The brown product is very slightly soluble in dioxane, ethanol, and methanol and insoluble in water and acetone. EPR spectra of the complex were obtained in 25% aqueous dioxane only after dissociation was facilitated by the addition of one drop of acid. This

complex is different from complexes reported earlier, Cu(sal-hm)Cl·H_2O[27] and a tetramer,[27] which is explained by a slightly different method of isolation. The solid-state structure of the complex in this study can be formulated as either Cu(sal-hm)(OH) or Cu(sal-hm$^-$)(H_2O); the latter form, containing the deprotonated imidazolate group, is more reasonable considering the base strength of hydroxide ion as compared to imidazolate: the pK_a of the N-H proton of imidazole is ca. 14.2,[73] and this value could be lower with the coordination of the imidazolate group to the metal ion. The poor solubility of the complex in dry dioxane may be accounted for by polymerization whereby the deprotonated imidazolate group engages in bonding to the axial position on a copper in an adjacent metal ion center. In aqueous dioxane, however, a cationic form with protonated imidazolate (no hydroxide) exists over a wide pH range, as discussed below with the pH titration results.

The insolubility of all of the complexes of this study limited their purification. Although most analyses were high in copper (Table I), probably as less soluble Cu(II) salts, the impurities are believed to have had little effect on the EPR results, since all samples were filtered before recording the spectra (see below).

EPR Spectra. Two types of EPR spectra were observed in frozen solutions (77 K) of the compounds in this study: (a) magnetically concentrated polycrystalline spectra similar to those obtained with the solids[23] and (b) glassy-type frozen-solution spectra typical of square-planar or tetragonal Cu(II) compounds having a $d_{x^2-y^2}$ ground state. Development of the latter spectra occurred slowly (if at all) upon addition of 10 mg of powdered sample to 2 mL of anhydrous dioxane with stirring. Elimination of the undissolved finely divided solid often required repeated filtration. Failure to remove all undissolved solid resulted in the superposition of the two types of EPR signals (e.g., Figure 1a,f) with varying intensities of the two overlapping signals from one spectrum to the next.

Reproducible glassy EPR spectra (Figure 1b–e) were often obtained after adding 25% water to each complex stirred in dry dioxane and waiting up to 24 h. Apparently the presence of water is required to achieve depolymerization of the complex structure found in the solid state.[10,11,15,26,35,36,40] We note that many[20,23,33] (but not all[21,29,32]) published EPR spectra of Cu(II) Schiff base complexes of salicylaldehyde (and some related aldehydes) and an amino acid are of the type shown in Figure 1a,f. Thus some g values (and also A values) previously reported are inaccurate or at least not very useful since they describe the EPR properties of Cu(II) complexes of uncertain mixed composition.

All complexes were insoluble in anhydrous dioxane by normal criteria: Cu(sal-gly)·$1^1/_2H_2O$ showed no EPR signal after being stirred in dioxane for 3 days. Cu(sal-lysAc)·H_2O gave only a symmetrical polycrystalline signal after 1 day ($\langle g \rangle$ = 2.20), and Cu(sal-hm$^-$)·H_2O and Cu(sal-his)·$1^1/_2H_2O$ gave axial polycrystalline signals ($g_\parallel$ = 2.17, $g_\perp$ = 2.07 and $g_\parallel$ = 2.14, $g_\perp$ = 2.08, respectively). Addition of water (25% by volume) or amine (5% by volume) improved the solubility and the resolution of the EPR spectra, but the parameters differed depending upon the substances(s) added (Table II). Addition of 25% water to each complex in dry dioxane produced identical EPR spectral parameters for Cu(sal-gly), Cu(sal-lysAc), and Cu(sal-his) ($g_\parallel$ = 2.256 ± 0.002, $g_\perp$ = 2.052 ± 0.002, $|A_\parallel|$ = (17.8 ± 0.2) × 10^{-3} cm^{-1}. The similarity of the parameters of these complexes shows that the coordination

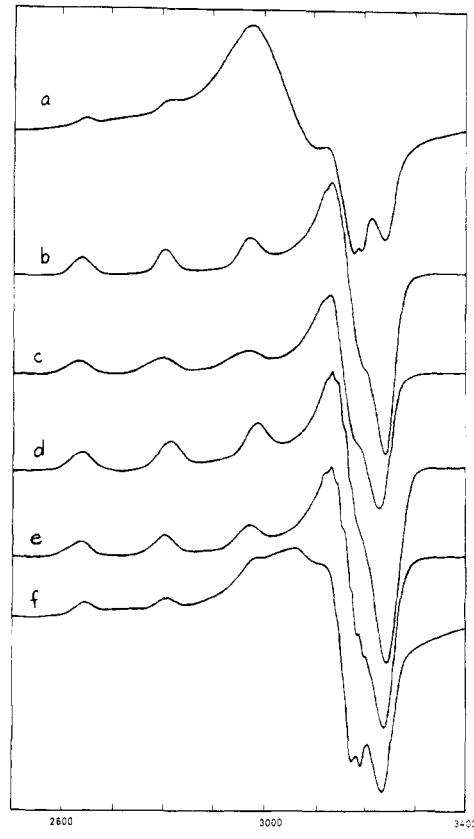

Figure 1. EPR spectra of the four complexes of this study: (a) Cu(sal-lysAc) in 100% dioxane, incompletely filtered; (b) Cu(sal-lysAc) in 25% aqueous dioxane (dioxane:water = 3:1 by volume), filtered; (c) Cu(sal-gly) in 25% aqueous dioxane, filtered; (d) Cu(sal-hm) in 25% aqueous dioxane, filtered; (e) Cu(sal-his) in 25% aqueous dioxane, filtered; (f) Cu(sal-his) in 25% aqueous dioxane, incompletely filtered.

geometry of Cu(sal-his) is "glycine-like" rather than "histamine-like" in frozen solutions in agreement with CD spectral results reported by others with solutions at room temperature.[29]

The perpendicular region of the spectrum of Cu(sal-his) is more like that of Cu(sal-hm) in aqueous dioxane (Figure 1d,e) than of Cu(sal-gly) or Cu(sal-lysAc). Since the unpaired electron of Cu(II) is in the $d_{x^2-y^2}$ orbital, only the nitrogen atoms in the xy plane can give rise to ^{14}N superhyperfine splitting[76-78] (shfs) of the order of magnitude observed here (14–16 G). The perpendicular region of the EPR spectrum of Cu(sal-his) (Figure 1e) suggests that $|A_\perp{}^{Cu}| \simeq |A_\parallel{}^{N}|$, which should lead to a six-line pattern if one nitrogen is in the plane or an eight-line pattern if two are in the plane. Six lines are resolved, but the possibility of there being an additional line on either end cannot be ruled out. Although Cu(sal-hm) should show ^{14}N shfs from two nitrogens in the plane, unfortunately, the EPR spectrum of Cu(sal-hm) (Figure 1d) is not well enough resolved. Attempts to analyze the perpendicular region of these spectra by computer simulation in order to determine the number of nitrogens coupled to the unpaired electron were not successful because of the excessive amount of computer time required to carry out such simulations.

Computer simulations of spectra with Cu(sal-gly) were carried out in order to assure correct axial or rhombic interpretation of the spectra. The spectra may be axial with an extra absorption peak[79] ("overshoot" peak[80]) at ca. 3230 G or

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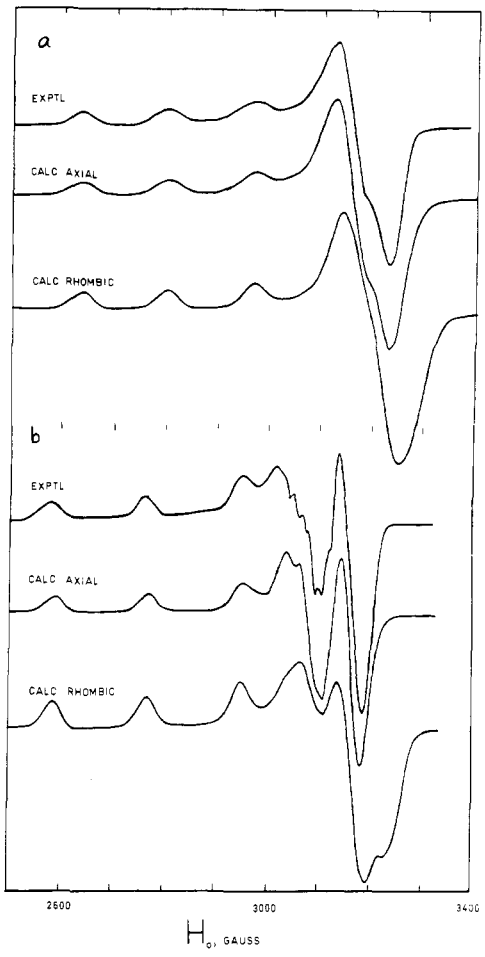

Figure 2. EPR spectra of two complexes of this study and computer simulations of each assuming axial and rhombic symmetry (all A values in 10^{-3} cm^{-1}): (a) Cu(sal–gly) in 25% aqueous dioxane (axial $g_{\parallel}$ = 2.258, $g_{\perp}$ = 2.054, $|A_{\parallel}|$ = 18.0, $|A_{\perp}|$ = 1.18; rhombic g_z = 2.258, g_y = 2.04, g_x = 2.015; $|A_z|$ = 18.0, $|A_y|$ = $|A_x|$ = 1.18); (b) Cu(sal–lysAc) + γ-collidine in dry dioxane (axial g_n = 2.195, $g_{\perp}$ = 2.041, $|A_{\parallel}|$ = 19.0, $|A_{\perp}|$ = 1.60; rhombic g_z = 2.195, g_y = 2.041, g_x = 1.979, $|A_z|$ = 19.0, $|A_y|$ = $|A_x|$ = 1.60). ^{14}N superhyperfine structure was not stimulated for (b) due to the excessive computer time required (more than 45 min/simulation).

rhombic, with $g_3 \approx 2.01$. Figure 2 clearly shows the spectrum is axial with an extra absorption peak, despite the unsymmetrical nature of the sal–gly Schiff base ligand. Computer simulation of the spectrum of Cu(sal–lysAc) with γ-collidine also proved an axial symmetry interpretation is correct. No evidence of ^{14}N shfs on the extra absorption peak was observed in any of the spectra, unlike those reported for galactose oxidase,[80] among other complexes.

When pyridine or N-methylimidazole was added with stirring to each complex in anhydrous dioxane, Cu(sal–gly)·1$^1/_2$H$_2$O, Cu(sal–lysAc)·H$_2$O and Cu(sal–his)·1$^1/_2$H$_2$O became considerably more soluble and gave glassy EPR spectra indicative of tetragonal coordination of Cu(II). Addition of 25% water to each of these samples caused an increase in g_{11} and little change (with pyridine) or a decrease (with N-MeIm) in $|A_{\parallel}|$. Addition of either amine to anhydrous dioxane containing solid Cu(sal–hm$^-$)·H$_2$O did not improve the solubility, and only polycrystalline-type EPR spectra were observed. Upon addition of 25% H$_2$O to the same solution, the solubility increased and a tetragonal glassy EPR spectrum was observed. The values of $g_{\parallel}$ and $|A_{\parallel}|$ of the pyridine complex of Cu(sal–hm) are quite similar to those of the pyridine

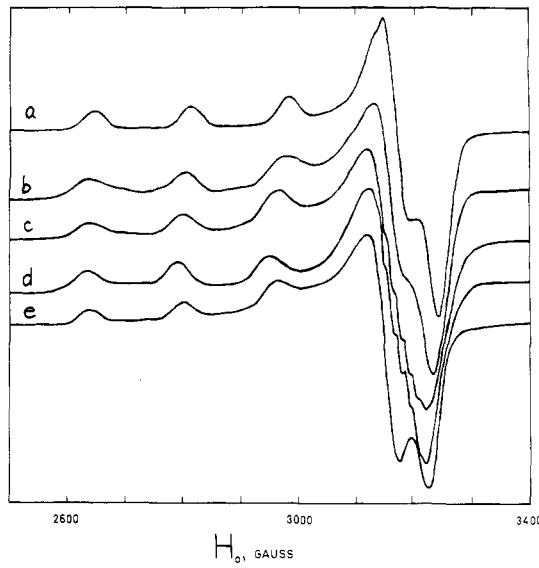

Figure 3. Comparison of the EPR spectra of amine adducts of the Schiff base–Cu complexes: (a) Cu(sal–gly) + 0.6 M pyridine in 25% aqueous dioxane; (b) Cu(sal–hm) + 0.6 M pyridine in 25% aqueous dioxane; (c) Cu(sal–hm) + 0.5 M N-methylimidazole in 25% aqueous dioxane; (d) Cu(sal–his) + 0.5 M N-methylimidazole in 25% aqueous dioxane; (e) Cu(sal–his) + 0.5 M N-methylimidazole in dry (100%) dioxane.

complex of Cu(sal–gly), while the spectra of the pyridine and N-MeIm complexes of Cu(sal–hm) are quite different (Figure 3). The N-MeIm complex has a perpendicular spectrum very similar to that of the N-MeIm complex of Cu(sal–his) with resolved nitrogen superhyperfine splitting (Figure 3).

Previous workers[81] have used 2,6-lutidine to block axial coordination in Ni(II) oligopeptide complexes. The analogous Cu(II) complexes were proposed to be five-coordinate in aqueous solution.[81] We have investigated the coordination of γ-collidine (2,4,6-trimethylpyridine) to the Cu(II) complexes of this study in dry dioxane. No change occurred to the polycrystalline-like spectra observed for Cu(sal–his)·1$^1/_2$H$_2$O or Cu(sal–hm$^-$)·H$_2$O, but Cu(sal–gly)·1$^1/_2$H$_2$O and Cu(sal–lysAc)·H$_2$O gave EPR spectra indicative of square-planar coordination[82,83] (Table II), with much larger $|A_{\parallel}|$ and smaller $g_{\parallel}$ values. Addition of water to the solutions containing γ-collidine produced an additional set of parallel hyperfine peaks and changes in the perpendicular region, which suggests the formation of additional species.

None of the samples in this study that gave polycrystalline-type EPR spectra showed half-field lines; thus, either the copper centers are too far apart or their magnetic axes are not properly aligned to allow strong enough spin coupling for observation of the signal due to S = 1 coupled Cu(II) dimers.

Electronic Absorption Spectra. The frequencies of the absorption maxima obtained with room-temperature aqueous dioxane solutions are listed in Table III. The d–d transitions apparently all occur under the broad envelope labeled ν_1, whose position is indicative of planar or weakly tetragonal Cu(II) complexes.[5,14,23,25,29,84,85] It should be noted that ν_1 for Cu(sal–his) is shifted significantly to higher energy in comparison to the other three complexes, indicating that the average ligand field effect of the donor groups of Cu(sal–his) is a combination of those of Cu(sal–gly) and Cu(sal–hm) or, in other words, implying strongly that the axial imidazole of Cu(sal–his) is

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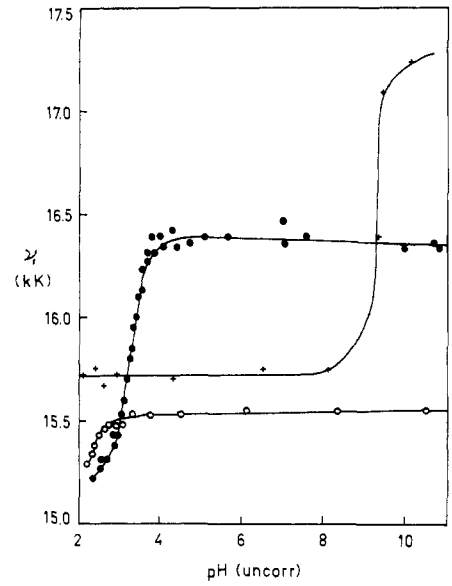

Figure 4. Plot of the pH titration of Cu(sal–hm) (crosses), Cu(sal–his) (closed circles), and Cu(sal–gly) (open circles) in 25% aqueous dioxane. The pH values are uncorrected for the mixed-solvent system.

indeed coordinated. The bands ν_2 and ν_3 have previously been assigned to the ligand-related $\pi_1^* \leftarrow \pi$ and $\pi_2^* \leftarrow \pi$ transitions[13,84,86] and appear in all spectra. Likewise ν_4 must be ligand related. A peak at 27.8×10^3 cm^{-1} (here called ν_2) confirms the presence of the salicylaldimine group in a monoanionic phenolate form[13,86] and is consistent with the dianionic form of Schiff bases of salicylaldehyde and amino acids and their metal complexes previously reported.[13,14,17,23,25,29,86] The near-identical peak locations at $27.8–27.9 \times 10^3$ cm^{-1} for Cu(sal–gly), Cu(sal–lysAc), and Cu(sal–hm) indicate that substitution of imidazole for a carboxylate group has no effect on this absorption.

A significant feature in interpretation of the data is the lower frequency of ν_2 for Cu(sal–his), suggesting a different coordination in this complex. On the basis of CD results and the pH titration curve of Cu(sal–his) in 25% aqueous dioxane discussed below, the only difference in coordination that is consistent with all data is that the histidine imidazole coordinates to an axial position of the Cu(II) at room temperature, thus shifting ν_2 to lower energy through some deformation of the angles in the planar part of the Schiff base ligand and ν_1 to higher energy by increasing the total ligand field.

pH Titration of Cu(sal–his) and Cu(sal–hm). Aqueous dioxane solutions of Cu(sal–his) and Cu(sal–hm), as well as Cu(sal–gly), were titrated with strong acid (concentrated HCl) and base (6 M NaOH) in order to determine the state of protonation of the imidazole groups. The electronic absorption maximum of ν_1 and pH measured with a glass electrode (uncorrected for the mixed solvent) were recorded upon each acid or base addition. The results (Figure 4) indicate that Cu(sal–hm) is in the (protonated) imidazole form over a wide pH range, pH 2–8, and that the apparent pK_a for the imidazole–imidazolate proton dissociation reaction in 25% aqueous dioxane is 9.3. The pK_a has been lowered by ca. 4.9 (from 14.2[73]) by coordination to Cu(II), which is more than the lowering of 2–3 pH units found upon coordination of the histidine imidazole to a metal ion.[74] Deprotonation of the imidazole ring causes a shift in ν_1 from 15.8 to 17.2×10^3 cm^{-1}. Cu(sal–his) does not show an obvious shift in ν_1 in the pH 7–11 range, suggesting that the crystal field splitting is unaffected by deprotonation of the axial imidazole or that the deprotonation of the axial imidazole occurs above pH 11.

At lower pH a large spectral shift occurs for Cu(sal–his) between pH 3.8 and pH 2. The apparent pK_a of the group being titrated is ca. 3.3. In comparison, the apparent pK_a of the carboxyl group of Cu(sal–gly) is ca. 2.4. Thus it would appear that both the imidazole and the carboxyl groups of Cu(sal–his) are titrated in acidic solution. If it is assumed the pK_a of the protonated carboxyl group on Cu(sal–his) is the same as that on Cu(sal–gly), the pK_a of the doubly protonated imidazole group is ca. 3.3–3.4. The large change in the position of ν_1 as this group is titrated confirms its coordination to Cu(II) at neutral pH but in a geometry different from that of Cu(sal–hm).

CD Spectra of Cu(sal–his). The circular dichroism spectrum of a saturated solution of Cu(sal–his) in 25% aqueous dioxane is similar to spectra reported recently for Cu(sal–his) in neat pyridine,[29] a small positive Cotton effect (relative $\epsilon_l - \epsilon_r = +0.15$) in the region 420–535 nm (maximum 447 nm), a negative Cotton effect (relative $\epsilon_l - \epsilon_r = -0.5$) in the region 535–720 nm (maximum 613 nm), and a very small positive Cotton effect (relative $\epsilon_l - \epsilon_r = +0.05$) from 720 nm to beyond 900 nm (broad maximum around 770 nm). Two additional strong maxima appear at 275 nm (relative $\epsilon_l - \epsilon_r = -4.9$) and 380 nm (relative $\epsilon_l - \epsilon_r = -8.7$) in the negative field and a shoulder at 240 nm (relative $\epsilon_l - \epsilon_r = +11.5$) in the positive field. The addition of *N*-methylimidazole to the solution (to 6 M) did not change the major features of the CD spectrum, indicating that the "glycine-like" mode of coordination is unchanged and the imidazole group of Cu(sal–his) remains axial in the presence of nitrogenous ligands at least at room temperature.

Discussion

We have concentrated our attention on the EPR spectra of Cu(sal–his) under a variety of conditions to ascertain how the imidazole group imparts unique behavior to the sal–his ligand compared to the analogous ligand from glycine, ϵ-acetyllysine, and histamine. It is seen that all the complexes in this study gave EPR values in the range $g_\parallel = 2.243–2.268$ and $|A_\parallel| = 16.6–18.0 \times 10^{-3}$ cm^{-1} except for the values obtained for Cu(sal–gly) and Cu(sal–lysAc) in the presence of γ-collidine. These values are reasonable for tetragonal five- or six-coordinated copper(II) complexes normally classified as type 2[75] and agree with values obtained from powder spectra of the glycine-, alanine-, and phenyl-derived Schiff base complexes of salicylaldehyde with Cu(II).[23] The EPR values obtained with Cu(sal–gly) and Cu(sal–lysAc) with 0.6 M γ-collidine of ca. $g_\parallel = 2.2$ and $|A_\parallel| = 19 \times 10^{-3}$ cm^{-1} are reasonable for four-coordinate Cu(II) complexes.[82,83] They correspond to a γ-collidine adduct in which the coordination of γ-collidine at an equatorial site blocks the axial sites for additional coordination. Glassy spectra of the complexes from histidine and histamine in the presence of γ-collidine were not obtained. This sterically hindered ligand was unable to depolymerize the solid-state structure of the last two complexes, possibly due to their common factor, the presence of an imidazole group.

The bonding parameters α^2, β_1^2, β^2, the mixing coefficients of the metal d orbitals involved in the in-plane σ ($d_{x^2-y^2}$) and π (d_{xy}) bonding and out-of-plane π (d_{xz},d_{yz}) bonding, respectively,[79,87–89] have been calculated and appear in Table II. These calculations are appropriate only if the complexes are indeed six-coordinate, which is only approximately true in these aqueous dioxane solutions, due to the expected weak coordination of axial water. Thus, the calculations are included only for qualitative comparison. The values of ΔE_{xy} and ΔE_{xz}, necessary for the calculation of β_1^2 and β^2, respectively, have

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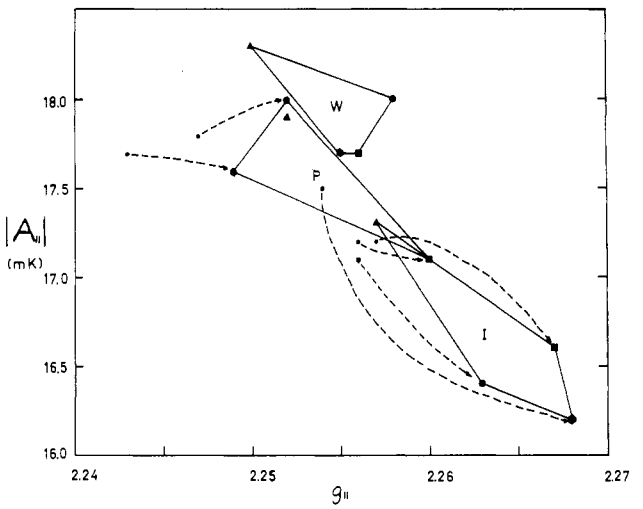

Figure 5. Plot of $|A_{\parallel}|$ vs. $g_{\parallel}$ for the complexes of this study: (circles) Cu(sal–gly); (hexagons) Cu(sal–lysAc); (triangles) Cu(sal–hm); (squares) Cu(sal–his). Enclosed areas represent the following: (W) samples in 25% aqueous dioxane only; (P) samples in 25% aqueous dioxane containing 0.6 M pyridine; (I) samples in 25% aqueous dioxane containing 0.5 M *N*-methylimidazole. The dots with arrows pointing toward the four symbols represent the EPR parameters of the anhydrous samples.

been assumed to lie under the broad maximum ν_1 in the room-temperature visible absorption spectra. In particular, we note that while α^2 and ${\beta_1}^2$ are fairly constant throughout the series of complexes, β^2 is significantly larger for Cu(sal–his) than for Cu(sal–gly), Cu(sal–lysAc), and Cu(sal–hm). The larger β^2 value for Cu(sal–his) indicates much less covalency in the out-of-plane π bonding. This is consistent with the fact that imidazoles are π-donor ligands[90] and that the π-symmetry orbitals of Cu(II) are filled. An alternative assignment of the transition energies is possible for Cu(sal–his) based on the CD spectral results.[29] In this case ΔE_{xy} and ΔE_{xz} were equated with the CD peak maxima at 620 and 450 nm, respectively.[29] ${\beta_1}^2$ is unchanged by this alternative assignment from the value in Table II. The new value of β^2 is 0.76, which is considerably smaller than the value given in Table II but is still significantly larger than the values obtained with the other complexes. In summary, bonding parameters indicate that Cu(sal–his) is unique in having significantly less covalency in its out-of-plane π bonding.

A more general way of expressing the changes in EPR parameters corresponding to five- or six-coordinated Cu(II) complexes is shown in Figure 5 on a $g_{\parallel}$ vs. $|A_{\parallel}|$ plot. The aqueous dioxane data in Figure 5 are contained in three clusters labeled W, P, and I, for those complexes having only water, water and pyridine, or water and *N*-methylimidazole added, respectively. The fairly clean separation of the clusters facilitates our interpretation.

On the basis of structural determination by X-ray crystallography,[10,11,15,26,35,36,40] we expect that in 25% aqueous dioxane Cu(sal–gly) and Cu(sal–lysAc) have the phenolic oxygen, imine nitrogen, and carboxylate oxygen coordinated at three equatorial sites, one water molecule coordinated at the fourth equatorial site, and at least one water molecule occupying an axial site. The $g_{\parallel}$ and $|A_{\parallel}|$ values are both slightly smaller for Cu(sal–lysAc) than for Cu(sal–gly), which suggests that the ϵ-acetamide group is also coordinated in the former complex at 77 K. Cu(sal–hm) in 25% aqueous dioxane has EPR parameters different from those of Cu(sal–gly) and Cu(sal–lysAc) (smaller $g_{\parallel}$ and larger $|A_{\parallel}|$) consistent with the presence of an imidazole nitrogen in place of a carboxylate oxygen in the *xy*-plane of copper.[91] $g_{\parallel}$ and $|A_{\parallel}|$ of Cu(sal–his) in an aqueous dioxane solution are close to values for Cu(sal–gly) and Cu(sal–lysAc) and quite different from values for Cu(sal–hm), which we interpret as a glycine-like coordination of the phenolic oxygen, imine nitrogen, and carboxylate oxygen at three equatorial positions, leaving the imidazole nitrogen to coordinate at an axial position. The parameters with Cu(sal–his) are closer to those of Cu(sal–lysAc) than to those of Cu(sal–gly), again supporting our suspicion of involvement of the ϵ-acetamide group in axial coordination in Cu(sal–lysAc). For Cu(sal–his) such axial coordination adequately explains the shift of ν_1 in the visible spectrum to higher energy (Table III). No such shift is observed for Cu(sal–lysAc), which means that either the acetamide group provides a ligand field identical with that of a water molecule or, more likely, the acetamide group is not coordinated at room temperature.

The major effect of adding pyridine to each of the complexes in solution is expected to be the replacement of the equatorially coordinated water molecule by a pyridine. This appears to be the case for Cu(sal–gly) and Cu(sal–lysAc), where $g_{\parallel}$ decreases slightly upon the addition of pyridine as expected when a nitrogen atom replaces an oxygen atom in the equatorial donor set.[91] Pyridine could also bind at the axial metal sites, but at its relatively low concentration in these studies (0.6 M) and its relatively weak basicity ($pK_a(BH^+) = 5.2$) it is unlikely that it could compete favorably with the much more abundant ($\sim$13 M) and more basic ($pK_a(H_3O^+) \approx 7.0$) water molecules, especially in view of the generally expected weakness of the axial ligand bonds in tetragonal Cu(II). That there is indeed more axial solvation in 25% aqueous dioxane than in dry dioxane is clear from a comparison of the EPR parameters of Cu(sal–gly), Cu(sal–lysAc), and Cu(sal–his) plus pyridine in dry dioxane (dots in Figure 5) to those in aqueous dioxane ("p" triangle in Figure 5).

The EPR spectrum of Cu(sal–his) is strongly affected by the addition of pyridine, but the change in the parameters (increased $g_{\parallel}$ and decreased $|A_{\parallel}|$) is opposite the change observed for Cu(sal–gly) and Cu(sal–lysAc). We expect pyridine to replace water at the fourth equatorial site, which by itself should produce a decrease in $g_{\parallel}$,[91] as noted above. Apparently, the histidine imidazole group, which coordinates only weakly to an axial position in the absence of pyridine, now interacts strongly to produce an overall increase in $g_{\parallel}$ and decrease in $|A_{\parallel}|$. The effect is larger in the presence of the axial imidazole of Cu(sal–his) than in the case of the equatorial imidazole of Cu(sal–hm).

When 0.5 M *N*-methylimidazole is added to Cu(sal–gly), Cu(sal–lysAc), and Cu(sal–his), all three complexes yield similar parameters in dry dioxane (near $g_{\parallel} = 2.256$, $|A_{\parallel}| = 17.2 \times 10^{-3}$ cm^{-1}) and 25% aqueous dioxane solutions (near $g_{\parallel} = 2.265$, $|A_{\parallel}| = 16.5 \times 10^{-3}$ cm^{-1}). In each case the effect of adding *N*-MeIm is a further increase in $g_{\parallel}$ and decrease in $|A_{\parallel}|$, again suggesting that the dominant factor following replacement of the equatorial water by *N*-MeIm is an increase in axial interaction. The effect on Cu(sal–his) as one traverses the series pyridine/dry dioxane → pyridine/aqueous dioxane → *N*-MeIm/dry dioxane is the smallest (and is in the reverse

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(92) The values of $g_{\parallel}$ (2.252) and $|A_{\parallel}|$ (17.9×10^{-3} cm^{-1} reported previously[29] for Cu(sal–his) in pure pyridine are smaller and larger, respectively, than those observed by us with 0.6 M pyridine in aqueous dioxane. However, the parameters cannot be compared directly (for example, in Figure 5) because of (a) the relatively low dielectric constant of pyridine compared to that of 25% aqueous dioxane, (b) the obvious involvement of axial solvation by water in our system, discussed in the preceding paragraph, and (c) the tendency of pure pyridine to crystallize rather than to form true glasses.[93]

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direction for the third step than for the other two), consistent with the fact that Cu(sal-his) already has built in the potential for strong axial ligation once the equatorial plane becomes an N_2O_2 coordination sphere. The large difference between parameters with dry dioxane and aqueous dioxane solutions with *N*-MeIm suggests an important role for axial solvation by water, in addition to *N*-MeIm in the mixed-solvent system. Thus this system most nearly approaches that of a six-coordinate tetragonal geometry.

The EPR spectra of the Cu(sal-his) and Cu(sal-hm) complexes with *N*-MeIm in aqueous dioxane, though they have rather different values of $g_\parallel$ and $|A_\parallel|$ (Table II, Figure 3, and Figure 5, triangle I), are unique among all of those seen in this study in that they are axial and yet have no extra absorption peak. They also have well-resolved nitrogen shfs from up to three and what appears to be equal numbers of coordinated nitrogens. The absence of these features in the case of Cu(sal-gly) and Cu(sal-lysAc) with *N*-MeIm in aqueous dioxane solutions suggests a difference in the coordination sphere of Cu(II) from that in the solutions of Cu(sal-his) and Cu(sal-hm). Possible differences include (a) deprotonation of the histidine imidazole nitrogens of these two multidentate ligands in the presence of *N*-MeIm, a stronger Lewis base ($pK_a(BH^+) = 7.33$[73]) than pyridine, (b) a change in magnetic axis system so as to place three nitrogens in the x-y plane of Cu(sal-his) + *N*-MeIm to interact with the unpaired electrons in the $d_{x^2-y^2}$ orbital, thus placing the phenolate and carboxylate oxygens on the z magnetic axis, and (c) the interchange of imidazole and carboxylate groups in Cu(sal-his) in the presence of *N*-MeIm to provide a "histamine-like" coordination mode of the sal-his ligand, thus placing the carboxylate oxygen on the z axis. The last case should reverse the sense of the CD spectra. Although such reversal was not observed in the CD spectrum of Cu(sal-his) + *N*-MeIm at room temperature, the lability of the coordination sphere of Cu(II) could permit switching of the coordination mode of the sal-his ligand as the solution is frozen, if thermodynamic factors favor the "histamine-like" mode in the presence of *N*-MeIm at low temperatures. Each of these possibilities provides a plausible explanation for the similarity in the appearance of the EPR spectra of Cu(sal-his) and Cu(sal-hm) in the presence of *N*-MeIm, while not requiring them to have identical values of $g_\parallel$ and $|A_\parallel|$. In the absence of other physical data obtained in the same solvent system at the same low temperature, it is not possible to differentiate among these possibilities.

In any case, it appears from these results that the branched tetradentate nature of the sal-his ligand, together with the unique coordinating properties of its imidazole nitrogen, confers special reactivity upon its complex with Cu(II) and the further adducts formed with Lewis bases such as pyridine and *N*-methylimidazole. These spectral features are different from those present in tridentate vitamin B_6 model complexes, those either lacking the imidazole (Cu(sal-gly)) or containing it in place of the carboxylate (Cu(sal-hm)), and therefore must be considered in further studies of these models.

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Circular Dichroism and Magnetic Circular Dichroism Spectra of Tetrahedral Cobalt(II) Complexes of Thiophenolate, *o*-Xylene-α,α'-dithiolate, and L-Cysteine-Containing Oligopeptides

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Mononuclear Co(II) complexes of *o*-xylene-α,α'-dithiolate and thiophenolate, $[Co(S_2\text{-}o\text{-}xyl)_2]^{2-}$ and $[Co(SPh)_4]^{2-}$, which possess *terminal* thiolate to Co(II) bonds only, have been compared with a tetranuclear thiophenolate complex, $[(CoSPh)_4(\mu\text{-}SPh)_6]^{2-}$, possessing *bridging* as well as *terminal* thiolate ligations, by absorption and MCD spectra in order to elucidate the core structure of Co(II)-thiolate complexes. The cluster complex has shown a characteristic MCD spectrum for the $^4A_2(F) \rightarrow {}^1T_1(P)$ transition having weak negative Faraday effects at 600-900 nm in addition to a strong absorption due to the charge-transfer transition from *bridging* thiolates to the Co(II) ion at 500-600 nm. The CD and MCD spectra of Co(II) complexes of Z-Cys-Ala-Ala-Cys-OMe and Z-Ala-Cys-OMe (Z = benzyloxycarbonyl and OMe = methoxy) have been interpreted to indicate the presence of *terminal* and *bridging* cysteine thiolate ligands. The results have been compared to the CD and MCD spectra of Co(II)-substituted metallothionein, which has been known to have polynuclear clusters with *bridging* cysteine thiolates.

Introduction

The spectroscopic properties of Co(II) have been utilized as a spectroscopic probe for metal-binding sites in Co(II)-reconstituted metalloenzymes.[2] Among many spectroscopic methods, circular dichroism (CD) and magnetic circular dichroism (MCD) spectroscopies are powerful techniques for this purpose because they reflect electronic and chiroptical properties and, hence, the coordination geometry around the metal ion more sensitively than other methods.[3] Several authors have investigated CD and MCD spectra of Co(II)-

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