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Coordination Chemistry of Microbial Iron Transport Compounds. 13.[1] Preparation and Chirality of the Rhodium(III) Enterobactin Complex and Model Tris(catecholato)rhodate(III) Analogues

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Complexes of Rh(III) with catechol (1,2-dihydroxybenzene) and the bacterial iron transport chelate enterobactin have been prepared and characterized by optical, circular dichroism, infrared, and proton NMR spectroscopy. The ^{1}H NMR of $[Rh(cat)_3]^{3-}$ consists of two multiplets centered at approximately 6.68 and 6.50 ppm. The ^{1}H NMR spectrum of rhodium enterobactin (δ (relative to Me_4Si) 7.11 (d), 6.61 (d), 6.28, 5.22, 3.86 (d), and 11.25 (d) ppm) is similar to that previously reported for the Ga(III) complex and confirms the spectroscopic results which established coordination of enterobactin to Rh(III) via the three catechol rings to give an octahedral complex. The optical spectrum of the $[Rh(cat)_3]^{3-}$ ion has maxima at 442 nm (ϵ 250) and 307 nm (ϵ 12 600), which are assigned as the $^1A_{1g} \rightarrow {}^1T_{1g}$ transition on Rh(III) and an intraligand π–π^* transition, respectively. Resolution of $[Rh(cat)_3]^{3-}$ yielded the Λ and Δ enantiomers with CD extrema at 420 nm ($\Delta\epsilon = -1.41$ for the Δ isomer), which is assigned as such by analogy to $[Rh(ox)_3]^{3-}$. Rhodium(III) enterobactin is found to exist predominantly as the Δ-cis isomer by comparison of its CD spectrum (λ_{max} 418 nm, $\Delta\epsilon \simeq -1.9$) with that of the Δ-$[Rh(cat)_3]^{3-}$ ion. Both are compared to the corresponding Cr(III) complexes (the original report of which included an error in the assignment of transitions).

Introduction

Enterobactin is the extracellular iron scavenger used by enteric bacteria and is one of a class of bacterial iron chelators called siderophores.[2,3] In order to satisfy their nutritional requirement for iron, bacteria produce and excrete siderophores, which complex iron as octahedral high-spin Fe(III). Ferric siderophore complexes then transfer iron from the environment into the cell against a large concentration gradient, by mechanisms which can involve either transport of the entire complex or extracellular release of Fe followed by transport of only Fe^{3+}.

Enterobactin is the cyclic triester of *N*-(2,3-dihydroxybenzoyl)-L-serine (Figure 1). Nuclear magnetic resonance[4] and optical spectra[5] have shown that chelation of metal ions occurs through the oxygens of the catechol moieties. However, space-filling models suggest that the existence of two diastereomers of metal enterobactin complexes, the Δ-cis and Λ-cis isomers, is possible, with no obvious steric preference for either. Determination of the absolute configuration of ferric siderophores may be crucial to understanding the very highly specific cellular transport mechanisms employed by bacteria. A recent report of the synthesis and characterization of chromium enterobactin[5] gave strong evidence that the predominant isomer of that complex has the Δ-cis absolute configuration, which is opposite that of all other siderophores whose chirality has been determined.[3] Unfortunately, the chromium complexes of enterobactin and catechol are quickly oxidized in air. Since uptake of metal–enterobactin complexes occurs in vivo only under aerobic conditions, the ease of oxidation of the chromium enterobactin complex vitiates attempts to transport a kinetically inert metal enterobactin complex into a living bacterial cell.

In an effort to characterize further the absolute configuration of metal complexes of enterobactin and to obtain a suitably air-stable, kinetically inert metal enterobactin complex for in vivo transport experiments, the synthesis and resolution of the model tris(catecholato)rhodate(III) complex as well as the synthesis of rhodium enterobactin have been undertaken. This paper presents the results of those studies.

Experimental Section

Catechol, 99+% purity (a generous gift of Crown Zellerbach Corp.), was used as supplied. Rhodium(III) trichloride trihydrate (Alfa), potassium carbonate, and potassium bicarbonate (Mallinckrodt AR grade) were used without further purification. The complexes $[Cr(en)_3]Cl_3$ (en = ethylenediamine) and Λ- and Δ-$[Co(en)_3]I_3$ were prepared and resolved using literature procedures.[6] The resolved compounds were repeatedly recrystallized until the measured optical purity agreed with the reported values. Rhodium(III) perchlorate hexahydrate was prepared according to the method of Ayres and Forrester.[7]

Enterobactin was extracted from culture media of *Aerobacter aerogenes* grown under low iron concentrations as described by Neilands et al.[8] Yields between 20 and 30 mg/L of culture media were reproducibly obtained. To avoid slow air oxidation, the white enterobactin powder was stored in a vacuum desiccator.

Thin-Layer Chromatography. Kieselgel D-O silica gel was used for thin-layer chromatography on coated glass plates. Potassium salts of iron and rhodium complexes of enterobactin were chromatographed using 6:5 chloroform–methanol. The spots were stained with iodine vapor.

Physical Measurements. Visible and ultraviolet spectra were measured on a Cary Model 118 spectrophotometer. Circular dichroism spectra were measured using a Jasco J-20 automatic recording spectropolarimeter. Infrared spectra were recorded using a Perkin-Elmer Model 337 spectrophotometer. Fourier transform (FT) ^{1}H NMR spectra of rhodium enterobactin were recorded at 220 MHz on a Varian HR-220 spectrometer using a homemade pulse FT system interfaced with a Nicolet NIC-80 data system. Fourier transform ^{1}H NMR spectra of $[Rh(cat)_3]^{3-}$ were obtained at 180 MHz on a homemade instrument based on a Bruker Instruments 42 kG superconducting magnet and a Nicolet 1180 data system. The rhodium enterobactin spectrum was run at ~40 °C in $(CD_3)_2SO$ using Me_4Si as internal reference, while that of $[Rh(cat)_3]^{3-}$ was obtained at 25 °C in D_2O made alkaline with K_2CO_3, using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal reference. X-ray powder patterns were determined by Helena Rubin, Lawrence Berkeley Laboratory.

Microanalysis. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

Preparation of $K_3[Rh(cat)_3]\cdot H_2O$. The preparation of $K_3[Rh(cat)_3]$ (cat = *o*-dihydroxybenzene dianion) was carried out using degassed solvents in an atmosphere of oxygen-free nitrogen in a recirculating atmosphere glovebox. Rhodium(III) trichloride trihydrate, 0.5 g (1.9 mmol), was dissolved in 3 mL of water and added to a solution of catechol, 1.1 g (10 mmol), in 75 mL of pH 10, 1 M potassium carbonate–1 M potassium bicarbonate buffer. The solution was stirred for 3 days. The $[Rh(cat)_3]^{3-}$ anion was isolated from the reaction mixture as the insoluble $[Co(en)_3]^{3+}$ salt by adding a solution of 0.75 g (2 mmol) of $[Co(en)_3]Cl_3\cdot H_2O$ in 5 mL of water to the reaction mixture. The complex salt $[Co(en)_3][Rh(cat)_3]$ was removed by filtration and washed with water before conversion to the potassium salt.

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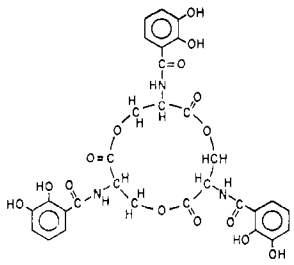

Figure 1. Structure of enterobactin.

Anal. Calcd for $[Co(C_2H_8N_2)_3][Rh(C_6H_4O_2)_3]\cdot 2H_2O$ (mol wt 702.54): C, 41.03; H, 5.75; N, 11.97; Co, 8.39. Found: C, 42.36; H, 5.31; N, 11.99; Co, 8.05.

Conversion to the potassium salt was effected by stirring $[Co(en)_3][Rh(cat)_3]$ in a slurry of 10 g of Bio-Rad AG 50W-X4 cation-exchange resin in the K^+ form for several days. The resin was removed by filtration and the filtrate evaporated to dryness to yield $K_3[Rh(cat)_3]\cdot H_2O$.

Anal. Calcd for $K_3[Rh(C_6H_4O_2)_3]\cdot H_2O$ (mol wt 562.53): C, 38.43; H, 2.51; K, 20.85; Rh, 18.30. Found: C, 36.95; H, 2.59; K, 20.2; Rh, 18.7.

Resolution of Δ-$K_3[Rh(cat)_3]$. To a solution of 0.04 g (0.01 mmol) of $K_3[Rh(cat)_3]\cdot H_2O$ in 8 mL of pH 10 potassium carbonate–potassium bicarbonate buffer was added a solution of 0.019 g (0.03 mmol) of Λ-$[Co(en)_3]I_3\cdot H_2O$ in 2 mL of degassed water. The solid $[Co(en)_3][Rh(cat)_3]$ was removed by filtration using a 0.22 μm Millipore filter, and 0.005 mmol of Λ-$[Co(en)_3]I_3\cdot H_2O$ in 1 mL of degassed water was added to the filtrate. Again the precipitated $[Co(en)_3][Rh(cat)_3]$ was removed by filtration and the CD spectrum recorded. Resolution was not improved by adding successive stoichiometric amounts of the resolving agent in repeating the above procedures. Since this represents only the limit of solubility difference between Λ- and Δ-$[Rh(cat)_3]^{3-}$ salts of Λ-$[Co(en)_3]^{3+}$, this resolution may not be complete. Similar procedures using Δ-$[Co(en)_3]I_3\cdot H_2O$ were used to prepare Λ-$K_3[Rh(cat)_3]$.

Preparation of K_3[Rh(enterobactin)]. A solution of 60 mg of enterobactin dissolved in 20 mL of 95% ethanol was added to a 10% molar excess of $Rh(ClO_4)_3\cdot 6H_2O$ dissolved in 20 mL of 95% ethanol. To this reaction mixture was added 5 mL of 0.5 M aqueous pH 7 phosphate buffer. The solution was stirred for 24 h in an evacuated Schlenk tube. The solution was then evaporated to dryness at 40 °C and the crude product extracted with water. The solution was dried once again; the product was dissolved in a minimum amount of 20% methanol/water and passed through an alumina column (ICN Pharmaceuticals) using 20% methanol/water as eluant. The solution was dried, and the residue was dissolved in methanol and passed through a Sephadex LH-20 column. The material was eluted with methanol to yield greenish gold $[Rh(ent)]^{3-}$. Removal of the methanol was accomplished by freeze-drying. The complex was characterized by UV–visible, NMR, and circular dichroism spectroscopy and by thin-layer chromatography (see below).

Results and Discussion

NMR Results and Discussion. The ^1H NMR data for rhodium enterobactin, the free ligand, and its Ga complex[4] are collected in Table I. Coordination of the Rh(III) to the enterobactin is clearly indicated by the shift in the amide proton resonance to 11.25 ppm (11.72 ppm in gallium enterobactin) from the 9.06 ppm value observed in the free ligand. Similarly, the shifts of the ring proton resonances are indicative of coordination to the Rh(III) ion. Doublets centered at 7.06 and 6.55 ppm (the latter poorly resolved) in the rhodium enterobactin spectrum correspond to those of 6.84 and 6.44 ppm in the spectrum of the Ga analogue. Following Llinás, Wilson, and Neilands,[4] these peaks are assigned to the ortho and para protons of the catechol moiety. Similarly, an absorption at 6.13 ppm for gallium enterobactin, assigned to the meta proton, corresponds to a peak at 6.29 ppm for rhodium enterobactin. All of the ring proton resonances are shifted upfield from their position in the free ligand upon coordination to Ga; similar shifts are observed for the Rh case, consistent with Rh coordination, although the shifts are smaller in magnitude. The upfield shifts in coordination may be attributable to decreased electron density in the catechol ring due to the electron-withdrawing metal ions, leading to reduced ring currents and shielding of the ring protons. In this light the greater shielding observed for Ga than Rh is consistent with the greater Lewis acidity of Ga^{3+}. Thus both the ring and amide proton regions confirm catechol coordination in the rhodium enterobactin complex. The $C_\alpha H$ and $C_\beta H_1$ resonances of the seryl group, found at 5.14 and 5.22 ppm for gallium enterobactin, are observed as a large poorly resolved peak at 5.21 ppm for rhodium enterobactin. Similarly, the 3.80 ppm peak of gallium enterobactin, assigned to the $C_\beta H_2$ seryl protons, appears as a poorly resolved doublet at 3.86 ppm.

Table I. Proton NMR Data for Enterobactin,[a] Gallium Enterobactin,[a] and Rhodium Enterobactin (in Me_2SO-d_6, Me_4Si Internal Reference, ~40 °C)

	enterobactin	gallium enterobactin	rhodium enterobactin
		Catechol	
o,p-CH	7.34, 6.98	6.84, 6.44	7.11, 6.61
m-CH	6.73	6.13	6.28
o-OH	11.56		
m-OH	9.34		
		Seryl	
$C_\alpha H$	4.94	5.14	5.22
$C_\beta H_1$	4.66	5.22	
$C_\beta H_2$	4.41	3.80	3.86
NH	9.06	11.72	11.25

[a] From ref 4.

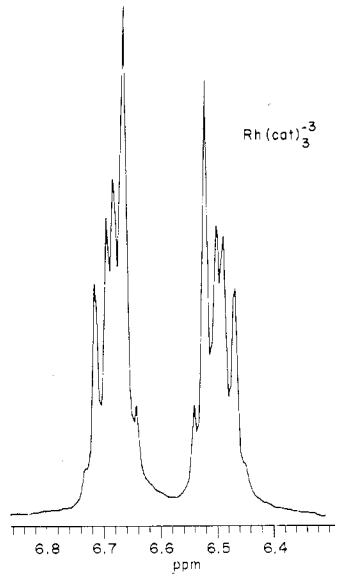

Figure 2. ^1H NMR spectrum of $K_3[Rh(cat)_3]\cdot H_2O$ in D_2O/K_2CO_3, DSS internal reference, 25 °C: δ (relative height) 6.688 (4), 6.668 (5), 6.656 (5), 6.638 (8), 6.618 (2), 6.514 (2), 6.495 (70), 6.475 (5), 6.464 (5), 6.444 (4).

The $[Rh(cat)_3]^{3-}$ spectrum exhibits two multiplets centered at approximately 6.68 and 6.50 ppm, as shown in Figure 2. The spectrum is not first order since the difference in δ of the 1,4 and 2,3 protons is comparable to J_{H-H} between adjacent protons, as in the case of free catechol, which it greatly resembles. No evidence for coupling to this Rh nucleus (^{103}Rh $I = {}^1/_2$, 100% abundance) was found.

Potassium Tris(catecholato)rhodate(III). Because of the sensitivity of alkaline solutions of catechol to air oxidation, all preparations were carried out under inert atmosphere. Comparison of the infrared spectra of catechol and $K_3[Rh-$

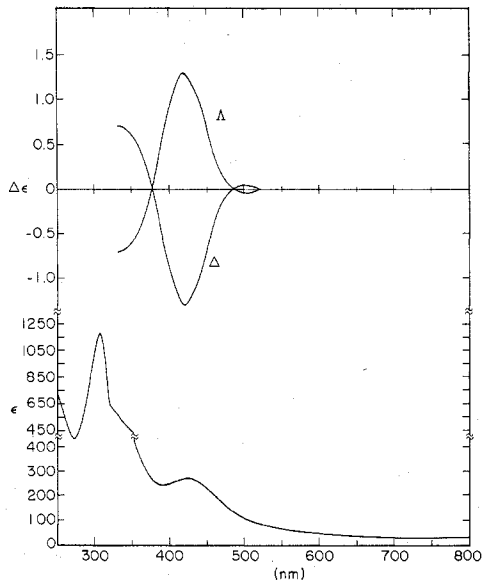

Figure 3. Electronic absorption spectrum of $K_3[Rh(cat)_3]\cdot H_2O$ in aqueous basic solution (lower curve) and circular dichroism spectra of Δ- and Λ-$K_3[Rh(cat)_3]\cdot H_2O$ solutions (upper curves).

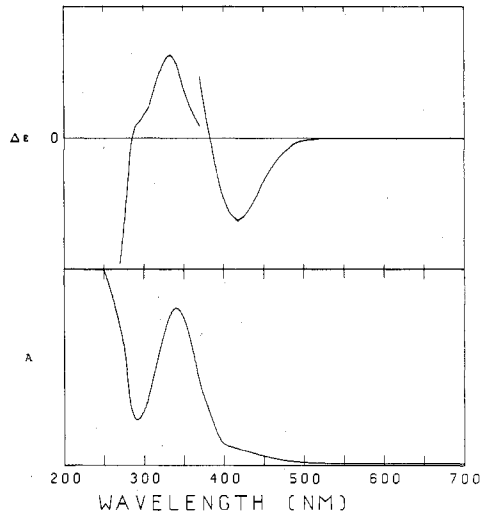

Figure 4. Electronic absorption spectrum of $K_3[Rh(ent)]$ in aqueous solution (lower curve) and circular dichroism spectrum of Δ-*cis*-$K_3[Rh(ent)]$ in methanol solution (upper curve). Extinction coefficients are approximate (see text).

$(cat)_3]$ shows the disappearance of several catechol bands in the complex (1526, 1514, 1365, 1280, 1190 cm^{-1}) and shift of several other bands, all attributable to CO and OH vibrations being altered upon complex formation. In addition, the infrared spectrum is very similar to that of other $[M(cat)_3]^{3-}$ ions (M^{3+} = Al^{3+}, Cr^{3+}, Fe^{3+}).[9]

The UV–visible spectrum of the $[Rh(cat)_3]^{3-}$ anion (Figure 3) may be compared with that of the tris(oxalato)rhodate(III) anion. The bands at 422 nm (ε 250) and at 307 nm (ε 12600) are assigned to the spin-allowed $^1A_{1g} \rightarrow {}^1T_{1g}$ d–d transition and to a ligand-based π–π^* transition, respectively. The latter, intense transition obscures the higher energy $^1A_{1g} \rightarrow {}^1T_{2g}$ transition seen in the oxalate and hexaaquo complexes at about 300 nm.[10]

The assignment of absolute configuration of the Λ and Δ isomers of $[Rh(cat)_3]^{3-}$ is based on three lines of reasoning: (1) The CD spectrum can be compared with that of the tris(oxalato)rhodate(III) species of known absolute configuration. The Λ-$[Rh(ox)_3]^{3-}$ complex shows a positive CD maximum at 400 nm ($\Delta\epsilon$ = +2.85) assigned to the E_a transition.[11] By analogy, this same transition occurs at 420 nm in $[Rh(cat)_3]^{3-}$ and is positive for the Λ isomer ($\Delta\epsilon$ = +1.41). (2) The empirical rule for assigning the absolute configuration of d^3, d^8, and low-spin d^6 transition metal complexes with D_3 point group symmetry can be used. This rule states that for complexes with the Λ configuration, the transitions with E and A_2 symmetry have positive and negative CD curves, respectively.[12] (3) An analogy is drawn between the $[Rh(cat)_3]^{3-}$ species and the $[Cr(cat)_3]^{3-}$ species previously prepared.[5] (*Note*: In an earlier paper in this series on the $[Cr(cat)_3]^{3-}$ and chromium enterobactin complexes an error appeared in the table of CD data for Λ-$[Cr(cat)_3]^{3-}$. The CD band at 663 nm is assigned to the $^4A_2 \rightarrow {}^4A_1$ transition and the 582-nm band to the $^4A_2 \rightarrow {}^4E_a$ transition; these labels were reversed in the table.) X-ray powder patterns of the complex salts $[Co(en)_3][M(cat)_3]$ (M = Rh, Cr), formed by addition of Λ-$[Co(en)_3]I_3\cdot H_2O$ to 2 equiv of a solution of $[M(cat)_3]^{3-}$, show these to be isostructural—indicating the same absolute configuration for both anions.

The Rhodium Enterobactin Complex. That enterobactin complexes rhodium in a manner strictly analogous to that for iron is demonstrated by a number of points. The ultraviolet spectra of the two complexes are identical (Figure 4). The ultraviolet spectra show marked change from that of the uncomplexed ligand,[13] which has maxima at 311 and 243 nm.

The visible and CD spectra of $[Rh(ent)]^{3-}$ (Figure 4) are similar to those of $[Rh(cat)_3]^{3-}$. This again confirms that coordination of enterobactin to Fe(III) and similar ions occurs through the catechol oxygens and does *not* involve the carbonyl of the amide (to give a salicylate type of coordination). The low-energy d–d transition in the visible spectrum of $[Rh(ent)]^{3-}$ appears at approximately 420 nm as a shoulder on the tail of the strong π–π^* absorption in the enterobactin complex and is assigned as the $^1A_{1g} \rightarrow {}^1T_{1g}$ transition, which may be seen explicitly at 422 nm in the $[Rh(cat)_3]^{3-}$ spectrum. This assignment is supported by the presence of the CD absorption band since in $[Rh(cat)_3]^{3-}$ the higher energy $^1A_{1g} \rightarrow {}^1T_{2g}$ transition is lost under the π–π^* transition. The π–π^* transition maxima are at lower energy in the enterobactin complexes compared to the case of the catechol complexes, probably because of the ortho acyl substitution on the catechol rings.

No satisfactory elemental analysis of $K_3[Rh(ent)]$ could be obtained since only small amounts of the complex, which proved to be extremely hygroscopic, were ever available. Approximate extinction coefficients for $[Rh(ent)]^{3-}$ were obtained by assuming that the extinction coefficients for $[Rh(ent)]^{3-}$ in the ultraviolet region of the spectrum (the region dominated by localized ligand transitions) are identical with those for $[Fe(ent)]^{3-}$ over the same region. On the basis of these approximate extinction coefficients, $\Delta\epsilon$ at 418 nm is estimated to be −1.9.

The CD spectrum of $[Rh(ent)]^{3-}$ demonstrates that one isomer of that complex predominates in solution. By comparison with the CD spectra of the Λ and Δ isomers of $[Rh(cat)_3]^{3-}$, we conclude that the $[Rh(ent)]^{3-}$ favors Δ-cis coordination. Furthermore, because the optical activity of $[Rh(ent)]^{3-}$ is comparable to that which has been obtained for $[Rh(cat)_3]^{3-}$, it is probable that the $[Rh(ent)]^{3-}$ complex exists as only one optical isomer. The CD spectrum obtained in the ultraviolet region is assigned to ligand localized transitions and is very similar to the UV CD spectrum of the ferric enterobactin complex.[14] The existence of only one $[Rh(ent)]^{3-}$ isomer is supported by the observation of only one sharp spot on silica gel TLC plates, whereas diastereoisomers might be expected to be separated by TLC (as we have done in similar systems[17]). In addition, the R_f value of $K_3[Rh(ent)]$ eluted by a 6:5 mixture of chloroform and methanol is identical with the R_f

value of K_3[Fe(ent)], 0.75, eluted on the same system.

Thin-layer chromatography of the $[Rh(ent)]^{3-}$ indicates that it is much more stable to air than the $[Cr(ent)]^{3-}$ complex. After 3 h of exposure at room temperature only one sharp spot is observed using the TLC procedure previously described. The vis–UV spectra also remain constant. At 0 °C, $[Rh(ent)]^{3-}$ can be stored for at least 1 week with no apparent change. The chromatographic behavior on silica gel TLC plates of $[Cr(ent)]^{3-}$, $[Rh(ent)]^{3-}$, and $[Fe(ent)]^{3-}$ are very similar. Since the ionic radius of Fe^{3+} (0.645 Å) lies between those of Cr^{3+} (0.615 Å) and Rh^{3+} (0.665 Å) and is within 0.03 Å of each and because the $[Cr(ent)]^{3-}$ and the $[Rh(ent)]^{3-}$ absolute configurations have been reliably assigned, the configuration of the $[Fe(ent)]^{3-}$ may be given as Δ-cis with a good degree of certainty. Thus, assignment of the absolute configuration of $[Fe(ent)]^{3-}$ is confirmed as opposite to that known for all other siderophores with the exception of the recently characterized rhodotorulic acid complex.[18]

In summary: (1) The enterobactin complexes of Rh(III), Cr(III), and Fe(III) are identical in structure and are formed by octahedral coordination of the catechol oxygen atoms. (2) There is one isomer which is preferred, the Δ absolute configuration, which is opposite in chirality to the ferrichromes and mycobactin.

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Registry No. $[Co(en)_3][Rh(cat)_3]$, 67577-16-6; $K_3[Rh(cat)_3]$, 67577-17-7; Δ-$K_3[Rh(cat)_3]$, 67597-64-2; Λ-$K_3[Rh(cat)_3]$, 67597-65-3; Δ-*cis*-K_3[Rh(ent)], 67577-18-8.

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Binuclear Metal Complexes. 1. Dicopper(II) Complexes with Binucleating Ligands Derived from 2-Hydroxy-5-methylisophthalaldehyde and 2-(2-Aminoethyl)pyridine or Histamine

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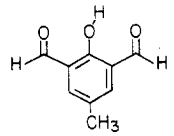
Dicopper(II) complexes are described with the binucleating Schiff-base ligands derived from the condensation of 2-hydroxy-5-methylisophthalaldehyde, **1**, and 2-(2-aminoethyl)pyridine or histamine and the corresponding secondary amine ligands produced by chemical reduction of the Schiff bases. Analytical data and conductance, spectral, and magnetic studies support the binuclear formulations **2–5** of these complexes. The antiferromagnetic interaction between the copper(II) ions is dependent on the bridging anion with values of $2J$ ranging from −385 to −545 cm^{-1} for the hydroxo-bridged species and from −156 to −230 cm^{-1} for the chloro-bridged species. Electrochemical reductions of the complexes are irreversible and ill-defined and do not lead to stable dicopper(I) complexes of the binucleating ligands. Reaction of the hydroxo-bridged binuclear copper(II) complexes with sodium ascorbate yields an ascorbate adduct characterized by intense charge-transfer bands.

Introduction

Interest in binuclear copper centers has focused primarily on the magnetic exchange (spin–spin) interaction between the two paramagnetic ($S = {}^1/_2$) cupric ions[1-3] as determined by bulk susceptibility or electron spin resonance (ESR) measurements. Since binuclear copper centers are proposed[4,5] to be part of the active site of several multicopper-containing proteins, the redox behavior of binuclear copper chelates assumes increased importance. Malmström[4] originally proposed that the type 3 (ESR nondetectable) copper in laccase consists of a pair of antiferromagnetically coupled Cu(II) ions which are capable of acting as a two-electron oxidant. More recently, Mason[5] has reviewed the evidence for binuclear copper centers in a variety of proteins. Since little information is known about the nature of these binuclear centers, it is appropriate to examine the properties of simple binuclear chelate systems in order to provide a basis for understanding the biological analogues.

Complexes with two metal ions in close proximity can result from the association of two monomeric units via an appropriate bridging group or from the incorporation of two metal ions into a single binucleating ligand. The latter route offers the advantage that the presence of the binuclear form in solution is not governed by a monomer ⇌ dimer equilibrium, and all of the complexes reported here are based on binucleating ligands. Robson[6] and Okawa and Kida[7] have introduced 2-hydroxy-5-methylisophthalaldehyde, **1**, as an important



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building block for binucleating macrocyclic and nonmacrocyclic ligands and have reported extensively on the

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