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Cytochrome Oxidase Models. 2. μ -Bipyrimidyl Mixed-Metal Complexes as Synthetic Models for the Fe/Cu Binuclear Active Site of Cytochrome Oxidase¹

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Abstract: μ -Bipyrimidyl (bipym) mixed-metal complexes, with [Fe^{II}(bipym)M^{II}] cores (M^{II} = Cu and Zn), have been synthesized to model the proposed imidazolate-bridged [Cyt a_3^{3+} (imid)CuU²⁺] active site structure of cytochrome oxidase where $-J(Fe^{III}-Cu^{II}) \gtrsim 200$ cm⁻¹. The binuclear compounds have been prepared from a six-coordinate [Fe^{II}(C₁₈H₁₈N₆)-(bipym)]²⁺ species (B) (C₁₈H₁₈N₆ = a folded macrocycle) by reaction with the appropriate bis(acetylacetonato)M(II) compound in CH₂Cl₂ to yield [Fe^{II}(C₁₈H₁₈N₆)(bipym)Cu^{II}(acac)₂]²⁺ (C) and [Fe^{II}(C₁₈H₁₈N₆)(bipym)Zn^{II}(acac)₂]²⁺ (D) as ClO₄⁻ salts. Compound B contains low-spin iron(II), whereas C and D are high-spin species in both the solid and solution states at room temperature. Comparative variable-temperature (10-300 K) magnetic susceptibility measurements for C and D indicate C to contain magnetically isolated S = 2 (Fe^{II}) and $S = \frac{1}{2}$ (Cu^{II}) centers with $J \approx 0$ through the bipym bridge. The ⁵⁷Fe Mössbauer spectra of C and D and the Cu^{II} EPR spectrum of C at 8 K are also supportive of this interpretation. In solution, the redox activity of B, C, and D has been examined by cyclic voltammetry in CH_2Cl_2 where $E_{1/2}$ for the Fe^{II}/Fe^{III} couple of high-spin C and D are identical at +0.60 V (SCE) and 700 mV lower in potential than for the low-spin monomer compound (B). The Cu^{II}/Cu^I couple for C appears to occur at $E_{1/2} = -0.24$ V (SCE). Finally, the μ -bipyrimidyl Cu₂ compound, $[(hfa)_2Cu^{II}(bipym)Cu^{II}(hfa)_2]$ (hfa⁻ = hexafluoroacetylacetonato anion), has been prepared and found to exhibit an antiferromagnetic exchange interaction with -J = 7.9 cm⁻¹. The ramifications of these results as they pertain to the magnetically coupled [Cyt a_3^{3+} -Cuu²⁺] active site of resting cytochrome oxidase are discussed, and an oxo-bridged alternative to the imidazolate-bridge possibility is also considered in view of the findings from the present model study.

Introduction

One of the most complex and enigmatic of metalloenzymes is cytochrome oxidase, the terminal oxidation/reduction enzyme in mitochondrial respiration. The enzyme catalytically reduces 1 mol of molecular oxygen to 2 mol of water, with the concomitant release of energy which is stored in the ADP-ATP cycle.⁴ The protein contains four metal centers (two irons and two coppers) per functioning enzyme unit.⁵ Furthermore, through various spectroscopic (EPR, MCD, and UV-vis) and other studies, it is now known that the enzyme contains one isolated iron heme unit (cytochrome a) which is low spin in both the oxidized and reduced forms and one isolated copper center (Cu_D; D for EPR detectable), while at the active or oxygen binding site there is a high-spin iron heme (cytochrome a_3) and a second copper center (Cu_U; U for EPR undetectable).^{6,7} In a full-temperature magnetochemical study, we have recently shown for the fully oxidized or resting form of the protein that the iron centers of Cyt a_3^{3+} and Cu_U^{2+} are strongly coupled antiferromagnetically $(-J \ge 200 \text{ cm}^{-1})$ through a bridge which was suggested to be imidazolate (imid) from a histidine residue.^{8,9} This proposed structure for the active site is shown schematically in Figure 1. The observed "strong" magnetic exchange interaction for oxidase is also manifested in the EPR spectrum, where there appears only one g = 2 signal which is assigned to magnetically isolated Cu_D and quantitates to only approximately 40% of the total copper present.¹⁰ This information, in conjunction with the magnetic susceptibility data, appears to rule out any other possible combinations of metal spin-state and exchange interaction as highly unlikely.⁸ It does not, however, contribute any information concerning the nature of the possible bridge which mediates the exchange between the iron and copper centers. However, there are two pieces of circumstantial evidence that point to imidazolate as the bridge: (1) EPR measurements indicate that the iron center of the a_3 heme has a nitrogen atom as one of its apical donor atoms¹¹ and (2) superoxide dismutase, for which a crystal structure is available, contains a mixed-metal binuclear [Cu^{II}(imid)Zn^{II}] site with an imidazolate bridge from histidine.^{12,13}

There are now several examples of synthetic complexes which contain imidazolate-bridged metal centers.¹⁴⁻¹⁸ Thus, there is no question of the bridging capabilities of the imidazolate anion. The controversy that has arisen is over the ability of imidazolate to foster an exchange of the magnitude $(-J \ge$ 200 cm⁻¹) present for the [Fe^{III}-Cu^{II}] pair of cytochrome oxidase.^{8,14,18} For a derivative of superoxide dismutase, with the Zn^{II} ion replaced by a second Cu^{II}, the strength of the antiferromagnetic exchange between the two Cu^{II} centers is



Figure 1. Schematic of the proposed imidazolate-bridged structure for the active site of resting cytochrome oxidase.

only 26 cm⁻¹.¹³ Furthermore, all other examples of synthetic $[Cu^{II}(imid)Cu^{II}]$ and related systems exhibit antiferromagnetic exchange interactions in the 0–90-cm⁻¹ range,¹⁴⁻¹⁸ or far less than the 200-cm⁻¹ value displayed by oxidase. To date, there has been only one measurement on a mixed-metal binuclear complex in which the bridging ligand is known to be imidazolate, where a Co^{II} substituted derivative of superoxide dismutase appears to be a case of strong exchange between a $[Cu^{II}(imid)Co^{II}]$ pair with $-J \ge 300$ cm⁻¹.¹⁹

The construction of synthetic model compounds represents one of the most viable approaches toward testing the proposed structure for the oxidase active site as shown in Figure 1. At present, however, there are no known examples of such synthetic, mixed-metal binuclear complexes with imidazolate bridges.^{20a} In fact, very few binuclear complexes containing different transition metals have been reported,^{20b} and there appears to be only one authenticated example of a synthetic [Fe-(B)-Cu] complex with a direct ligand bridge of any nature.^{20c} Toward systematically producing such compounds to model cytochrome oxidase, we recently communicated²¹ initial results reporting the first N-heterocyclic-bridged [Fe-(B)-Cu] species to be isolated where B = 2,2'-bipyrimidine (bipym). The present work reports an extended investigation of this study which further comments on the likelihood of a [Cyt a_3 (imid)Cu₁₁] structure for the active site of cytochrome oxidase. Finally, an alternative O₂-bridged model for the site is also considered in light of the information obtained by this initial modeling study.

Experimental Section

Physical Measurements. Variable-temperature magnetic susceptibility measurements in the solid state were performed by the Faraday technique using equipment previously described.²² Solution magnetic susceptibilities were performed by Evans' method²³ on a Varian EM-390 NMR spectrometer. Pascal's constants were used to correct for ligand and anion diamagnetism in cgsu for 2,2'-bipyrimidine, -96.3×10^{-6} ; acac⁻, -55×10^{-6} ; hfac, -70.2×10^{-6} ; $C_{18}H_{18}N_6$, -203.2×10^{-6} ; ClO_4^- , -32×10^{-6} .

Mössbauer spectra were obtained using a previously described spectrometer and computer analyzed by the program of Chrisman and Tumolillo.²⁴ Temperatures were monitored by a copper vs. constantan thermocouple imbedded in the sample. Computer-generated plots of the Mössbauer spectra were obtained using a Calcomp plotting program.

Osmometry measurements were obtained using an HP 302 B vapor pressure osmometer. Solution conductivities were measured on a Model 31 YSI conductivity bridge. UV-vis spectra were obtained on a Cary 17 recording spectrometer using quartz cells.

X-Band EPR spectra were obtained as CH_2Cl_2 glasses on a Varian E-line EPR spectrometer using standard quartz cells. Field positions were referenced relative to DPPH; the copper content of the samples was quantified by computer signal integration using known concentrations of CuSO₄ as standards.

Electrochemical measurements were obtained in CH_2Cl_2 and CH_3CN at room temperature using an EG & G PAR Model 173 potentiostat/galvanostat driven by a Model 175 universal programmer



Figure 2. Synthetic scheme for the preparation of the mononuclear (B) and binuclear $[Fe^{II}(bipym)Cu^{II}]$ (C) iron(II) complexes. (Compound D in the text is the corresponding $[Fe^{II}(bipym)Zn^{II}]$ derivative.)

or a Model 174 A polarographic analyzer. The current/voltage curves were recorded on a Houston Omnigraphic 2000 X-Y recorder at scan rates between 0.02 and 0.50 V/s and a Tektronix Model 5111 storage oscilloscope at scan rates between 0.10 and 50.0 V/s. A three-electrode geometry was employed; a platinum button served as the working electrode for cyclic voltammetric studies and a dropping mercury electrode (DME) for polarographic studies, a platinum wire as the counter electrode, and a Metrohm K901 saturated calomel electrode (SCE) as the reference electrody. The reference electrode was partially separated from the bulk solution by means of a fritted-glass bridge containing supporting electrolyte solution. All solutions were 10^{-3} M in the complex and 0.1 M in tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte.

Chemical analyses were performed by the microanalytical laboratory of the School of Chemical Sciences, University of Illinois.

Materials and Syntheses. 2,6-Diacetylpyridine, hydrazine, 2-aminopyrimidine, acetylacetone, and hexafluoroacetylacetone were obtained from Aldrich Chemical Co. and used without further purification. All solvents used in syntheses were reagent grade and spectroquality for the spectral measurements.

2-Bromopyrimidine was prepared from 2-aminopyrimidine by the method of Bly and Mellon²⁵ and purified by sublimation.

2,2'-Bipyrimidine (bipym) was prepared by the coupling of 2-bromopyrimidine in the presence of a copper-bronze catalyst. Equal weights of 2-bromopyrimidine and the copper-bronze catalyst were thoroughly mixed and sealed in an evacuated flask. After reaction at 110 °C for 12 h, the flask was opened and the products were worked up according to the procedure of Bly and Mellon²⁵ and Westcott.²⁶ The final product was purified by sublimation at 95 °C (1 mmHg): yields varied from 10 to 20%; mp 110–113 °C; mass spectrum Mp⁺ 158.

[Fe^{II}(C₁₈H₁₈N₆)(CH₃CN)₂)(ClO₄)₂ was prepared by the procedure of Goedkin et al.²⁷ The compound was recrystallized from CH₃CN and EtOH, washed with EtOH and Et₂O, and dried over P₂O₅ in vacuo at room temperature. Anal. Calcd for FeC₂₂H₂₄N₈Cl₂O₈: C, 40.32; H, 3.69; N, 17.10; Fe, 8.52. Found: C, 40.02; H, 3.69; N, 16.90; Fe, 8.79.

[Fe^{II}(C₁₈H₁₈N₆)(bipym)](ClO₄)₂ was prepared by the addition of 0.1 mmol of [Fe^{II}(C₁₈H₁₈N₆)(CH₃CN)₂](ClO₄)₂ dissolved in a minimum amount of warm CH₃CN to 0.1 mmol of 2,2'-bipyrimidine dissolved in 10 mL of CH₃CN. The solution immediately changed from blue-green to red upon mixing. The CH₃CN was removed under reduced pressure at room temperature to yield a red solid which was recrystallized from a CH₂Cl₂/heptane mixture. The resulting red crystals were collected by filtration and dried over P₂O₅ in vacuo at room temperature for 12 h. Anal. Calcd for FeC₂₆H₂₄N₁₀Cl₂O₈. CH₂Cl₂: C, 39.76; H, 3.21; N, 17.17; Fe, 6.84. Found: C, 40.09; H, 3.37; N, 17.18; Fe, 6.76.

 $[Fe^{II}(C_{18}H_{18}N_6)(bipym)M^{II}(acac)_2)(CIO_4)_2$, where $M^{II} = Cu$ or Zn, was prepared by the addition of 0.1 mmol of $[M^{II}(acac)_2]$ to a solution containing 0.1 mmol of $[Fe^{II}(C_{18}H_{18}N_6)(bipym)](CIO_4)_2$ dissolved

in CH₂Cl₂. The solution changed from red to purple upon mixing. The solvent was removed in vacuo at room temperature and the purple solid recrystallized from a CH₂Cl₂/heptane mixture. The resulting purple crystals were collected by filtration and dried over P₂O₅ in vacuo at room temperature for 12 h. Anal. Calcd for FeCuC₃₆H₃₈. N₁₀O₁₂Cl₂·0.5CH₂Cl₂: C, 42.34; H, 3.80; N, 13.58; Fe, 5.39; Cu, 6.14. Found: C, 42.58; H, 4.00; N, 13.42; Fe, 5.63; Cu, 6.16. Anal. Calcd for FeZnC₃₆H₃₈N₁₀O₁₂Cl₂·0.5C₇H₁₆: C, 45.40; H, 4.45; N, 13.34; Fe, 5.31; Zn, 6.22. Found: C, 45.23; H, 4.38; N, 13.54; Fe, 5.53; Zn, 6.34.

[(hfa)₂Cu^{II}(bipym)Cu^{II}(hfa)₂] was prepared by the addition of 0.2 mmol of [Cu(hfac)₂(H₂O)] dissolved in 25 mL of CH₂Cl₂ to 0.1 mmol of 2,2'-bipyrimidine also dissolved in 25 mL of CH₂Cl₂. The solvent was removed in vacuo at room temperature and the light green solid was recrystallized from Et₂O. The resulting light green crystals were collected by filtration and dried over P₂O₅ in vacuo at room temperature for 12 h. Anal. Calcd for Cu₂C₂₈H₁₀N₄O₈F₂₄·0.5(C₂H₅)₂O: C, 25.06; H, 1.43; N, 5.31; Cu, 12.05. Found: C, 25.20; H, 1.28; N, 5.11; Cu, 11.10. Mol wt in chloroform by osmometry: theoretical, 1017; found, 1090.

Results and Discussion

Synthesis and Characterization of the Complexes in Solution. Synthesis of the green macrocyclic [Fe^{II}($C_{18}H_{18}N_6$)-(CH₃CN)₂]²⁺ complex, A in Figure 2, involves a Schiff-base condensation of 2,6-diacetylpyridine with hydrazine, as first described by Goedkin et al.²⁷ The macrocyclic ligand is of special interest here in that with axial CH₃CN ligands the macrocycle is planar, while with strong-field, bidentate ligands such as 2,2'-bipyridine²⁷ [or in this study, 2,2'-bipyrimidine (bipym)] the macrocycle "folds" to accommodate the bidentate ligand as shown by B in Figure 2. Thus, when bipym is added to a CH₃CN solution containing [Fe^{II}($C_{18}H_{18}N_6$)- $(CH_3CN)_2$ (ClO₄)₂, a red, crystalline solid is isolated which has been characterized as the mononuclear [Fe^{II}($C_{18}H_{18}N_6$)-(bipym)](ClO₄)₂ species. Further reaction of the $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ cation with $[Cu^{II}(acac)_2]$ in a noncoordinating solvent such as CH₂Cl₂ yields a purple, microcrystalline product which has been characterized as the μ -bipyrimidyl species, $[Fe^{II}(C_{18}H_{18}N_6)(bipym) Cu^{II}(acac)_2](CIO_4)_2$ (C). Substitution of $[Zn^{II}(acac)_2]$ in the reaction yields the corresponding [Fe^{II}(bipym)Zn^{II}] analogue, D. Compounds C and D are the first μ -bipyrimidyl mixedmetal species to be reported, although pure-metal binuclear complexes of Pt_2 and Ru_2 are known.²⁸⁻³⁰ Both of the mixedmetal compounds are soluble and stable in noncoordinating solvents such as CH₂Cl₂ or CHCl₃ for short periods of time (1 h); however, in CH₃CN, the binuclear complexes dissociate into the original [Fe^{II}(C₁₈H₁₈N₆)(CH₃CN)₂]²⁺ species and [Cu^{II}(acac)₂] or [Zn^{II}(acac)₂]. For this reason solvent studies in the present work have been largely limited to CH₂Cl₂. Attempts to isolate a mononuclear [Cu^{II}(acac)₂(bipym)] complex were unsuccessful, indicating that the nitrogen atoms of free base bipym are less basic than the backside, uncoordinated nitrogens of the $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ complex. When complexes with "more acidic" copper centers, such as $[Cu^{II}(hfac)_2]$ (hfac⁻ = hexafluoroacetylacetonate anion), are reacted with B, the bipym ligand is stripped from the iron center and the resulting [Cu^{II}(hfac)₂(bipym)] complex can be isolated. Although no bipym adduct of [Cu^{II}(acac)₂] could be isolated, both mononuclear and binuclear complexes of $[Cu^{II}(hfac)_2]$ are obtainable. The binuclear $[(hfac)_2Cu^{II}]$ (bipym)Cu¹¹(hfac)₂] complex is of special interest in the present work and is discussed more fully below.

Electronic absorption spectra of the complexes have been obtained in solution for purposes of comparison and to completely characterize the species. The spectra are shown in Figure 3 with band positions and intensities summarized in Table I. Owing to their intensities, all of the observed bands are most reasonably assigned to charge-transfer transitions, and a careful search at lower energy failed to resolve any low

Table I. Electronic Spectral Data for Complexes in Solution

complex	λ_{max}, nm^a	ϵ , M ⁻¹ cm ⁻¹ a
$[Fe^{II}(C_{18}H_{18}N_6)(CH_3CN)_2](C O_4)_2^b$	380	3180
	565	1400
	640	1840
	695	2700
	770	2880
$[Fe^{II}(C_{18}H_{18}N_6)(bipym)](ClO_4)_2^{c}$	440	3700
• • • • • • • • • • • • • • • • • • • •	470	3600
	540	3780
	740	480
$[Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2]-(CIO_4)_2^{c}$	550	3870
、	725	2220
$[Fe^{II}(C_{18}H_{18}N_6)(bipym)Zn^{II}(acac)_2]-$ $(ClO_4)_2^{c}$	550	1870
	735	1290

^a Band positions and intensities have been estimated without deconvolution procedures. ^b CH₃CN solution. ^c CH₂Cl₂ solution.

intensity bands which could be assigned as purely "d-d" in nature. Such "d-d" bands have only rarely been detected for six-coordinate Fe^{II} complexes containing strong-field, α -diimine ligands such as those in the present study.³¹ Spectra for the binuclear [Fe^{II}(bipym)Cu^{II}] and [Fe^{II}(bipym)Zn^{II}] complexes, shown in Figures 3c and 3d, appear very similar with both species having intense absorption bands in the 550and 750-nm regions. However, the relative intensities of the bands differ substantially for the two complexes, with those for the Cu^{II} derivative being approximately twice as intense as for the Zn^{II} complex. Finally, Figure 4 displays the comparative absorption spectra in the visible for $[Cu^{II}(acac)_2]$, $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$, and $[Fe^{II}(C_{18}H_{18}N_6)(bipym) Cu^{II}(acac)_2]^{2+}$ in CH₂Cl₂. These spectra serve to conclusively demonstrate that the [Fe^{II}(bipym)Cu^{II}] complex is, indeed, a discrete species in solution in that its spectrum is not simply an addition spectrum of the $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ and [Cu^{II}(acac)₂] components.

Solution conductivities of B, C, and D obtained in CH₂Cl₂ indicate strong ion pairing in solution, with the values being \lesssim 7 mho M⁻¹ cm⁻¹. The instability of the binuclear complexes in coordinating solvents such as CH₃CN, MeOH, or Me₂SO is likely due, in part, to the iron(II) spin state. The monomeric $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ cation possesses a magnetic moment of 1.60 $\mu_{\rm B}$ in CH₂Cl₂ at 298 K. This value, although somewhat higher than normal for low-spin Fe^{II}, is indicative of an essentially low-spin center. Solid-state magnetic and Mössbauer data, given below, also support a low-spin assignment for [Fe¹¹(C₁₈H₁₈N₆)(bipym)](ClO₄)₂. However, the $[Fe^{II}(C_{18}H_{18}N_6)(bipym)M^{II}(acac)_2]^{2+}$ cations are both high spin in solution with magnetic moments of 5.89 ($M = Cu^{II}$) and 4.80 μ_B (M = Zn^{II}) in CH₂Cl₂ at 298 K. Hence, the high-spin binuclear complexes do not benefit from the added CFSE of a low-spin center, which could be a contributory factor leading to dissociation into low-spin mononuclear species. Finally, the binuclear complexes also probably suffer some instability (relative to the low-spin bis-CH₃CN complex) due to "strain energy" required in folding their macrocyclic unit, as well as due to the rather close placement (6-7 Å) of two positively charged metal centers separated by only a neutral bipym bridge. Studies in progress employing negatively charged 2-(pyrimidyl)imidazolate and 2,2'-biimidazolate bridges suggest the latter factor to be of considerable importance.32

The difference in the Fe^{II} spin state between the mononuclear and binuclear complexes is also reflected in the Fe^{II} \rightleftharpoons Fe^{III} redox behavior, as determined electrochemically. Cyclic



 $\begin{array}{l} \textbf{Figure 3. Room temperature electronic absorption spectrum of (a) [Fe^{II}(C_{18}H_{18}N_6)(CH_3CN)_2](ClO_4)_2 in CH_3CN; (b) [Fe^{II}(C_{18}H_{18}N_6)(bipym)](ClO_4)_2 in CH_2Cl_2; (c) [Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2](ClO_4)_2 in CH_2Cl_2; (d) [Fe^{II}(C_{18}H_{18}N_6)(bipym)Zn^{II}(acac)_2](ClO_4)_2 in CH_2Cl_2; (d) [Fe^{II}(C_{18}H_{18}N_6)(bipym)Zn^{II}(acac)_2](C$

voltammograms for the three complexes are shown in Figure 5. As seen in the figure, the low-spin $[Fe^{II}(C_{18}H_{18}N_6)-(bipym)]^{2+}$ complex has $E_{1/2}$ of ca. +1.30 V (SCE) for the Fe^{II}/Fe^{III} couple in CH₂Cl₂. At scan rates of ≤ 0.10 V/s, the reduced $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ complex did not yield a cathodic wave (Figure 5a). However, the anodic peak current is proportional to $V^{1/2}$ at high scan rates and $|E_p - E_{p/2}| = 60 \pm 10$ mV, suggesting a reversible, one-electron transfer followed by a rapid chemical reaction (EC mechanism).³³ Reversible peaks were obtained at scan rates > 1.0 V/s. Cyclic voltammograms for the high-spin binuclear complexes (Figures 5b and 5c) show a large 700-mV decrease in $E_{1/2}$ for the Fe^{II}/Fe^{III} couple and give an *identical* value of +0.60 V

 $(\pm 0.01 \text{ V}$ by differential pulse polarography) for both the Cu¹¹ and Zn¹¹ derivatives. Both cyclic voltammograms also appear reversible at scan rates of >0.10 V/s, with no indication of compound decomposition. These electrochemical results are of particular significance since they (1) further establish that the [Fe¹¹(bipym)M¹¹] compounds are, indeed, discrete entities in solution with Fe¹¹ = Fe¹¹¹ redox behavior differing from the mononuclear precursor, and (2) serve to suggest that the paramagnetic Cu¹¹ center in C has little, if any, electronic interaction (i.e., magnetic exchange) with its Fe¹¹ partner across the bipym bridge. It is tempting to attribute the difference in $E_{1/2}$ between the low-spin compound (B) and its high-spin binuclear relatives (C and D) to the difference in the Fe¹¹ spin



Figure 4. Comparative electronic absorption spectra in CH_2Cl_2 for (a) $[Cu^{II}(acac)_2];$ (b) $[Fe^{II}(C_{18}H_{18}N_6)(bipym)](ClO_4)_2;$ (c) $[Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2](ClO_4)_2.$



Figure 5. Cyclic voltammograms of the Fe^{II}/Fe^{III} couple for the complexes obtained in CH_2Cl_2 at platinum button electrodes. Positions are referenced relative to SCE. (Mac = $C_{18}H_{18}N_6$ in the text.)

state; however, available electrochemical data on the variable-spin $[Fe^{II}(x-py)_3 tren]^{2+}$ and $[Fe^{III}(dtc)_3]$ systems, ^{34,35} as well as more recent variable-temperature data on the $[Fe^{III}(x-sal)_2 trien]^+$ system, ^{36,37} indicate that the spin-state dependency of $E_{1/2}$ will, in general, not exceed 100 mV. This being the case, the 700-mV difference between B and C or D arises mainly from a large substituent effect of the coordinated $[M^{II}(acac)_2]$ moiety. In fact, this same electronic substituent effect is apparently large enough to induce the low-spin \rightarrow high-spin conversion upon formation of the binuclear complexes, since molecular models suggest that the spin conversion is not of steric origin.^{24,38} This interpretation is under investigation by structural work presently in progress on B, C, and D.³⁹ Assuming the [Cyt a_3 (imid)Cu_U] model of Figure 1 to be correct for oxidase, a similar substituent effect due to Cu_U might account for the fact that Cyt a_3 is thought to always be high spin in all of its naturally occurring redox chemistry.⁴⁰ Finally, an attempt has been made to identify a Cu^{II}/Cu^I redox couple for C (Figure 6). Comparison of Figures 6a and 6b indicates that the wave at -0.24 V may be due to the Cu^{II} \rightarrow Cu^I reduction process. The other reduction waves at ca. -0.77, -1.05, and -1.40 V are most likely ligand-centered reductions.

Magnetic Susceptibility Data. The $[Fe^{II}(C_{18}H_{18}N_6)-(bipym)](CIO_4)_2$ compound has a magnetic moment of 1.52



Figure 6. Polarograms and differential pulse polarograms at DME in CH₂Cl₂ for (a) [Fe^{II}(C₁₈H₁₈N₆)(bipym)Cu^{II}(acac)₂](ClO₄)₂; (b) [Fe^{II}(C₁₈H₁₈N₆)(bipym)Zn^{II}(acac)₂](ClO₄)₂. Polarography conditions: scan rate = 10 mV/s, drop time = 0.5 s. Differential pulse polarography conditions: scan rate = 10 mV/s, pulse time = 0.5 s, modulation amplitude = 25 mV.

 μ_B at 298 K, which gradually decreases to 1.30 μ_B by 83 K. While this value is somewhat above that expected for low-spin Fe^{II}, nevertheless, it is in good agreement with the solution moment and indicative of an essentially low-spin center. Upon chelation with $M(acac)_2$ to form the $[Fe^{II}(C_{18}H_{18}N_6)-$ (bipym)M^{II}(acac)₂](ClO₄)₂ binuclear species, the Fe^{II} center converts to fully high spin with $\mu_{eff}(304 \text{ K}) = 5.46 (Cu^{11})$ and $\mu_{eff}(299 \text{ K}) = 5.11 \text{ (Zn}^{II})$. A value of 5.11 μ_B for the [Fe^{II}- $(bipym)Zn^{II}$ case is that expected for an S = 2 iron center with an appreciable orbital contribution. Assuming the same μ_{eff} value for Fe^{II} in [Fe^{II}(bipym)Cu^{II}], the Cu^{II} center contributes $\sim 1.9 \,\mu_{\rm B}$ to the total observed magnetic moment of 5.46 $\mu_{\rm B}$. This is a common value for magnetically dilute Cu^{II}, indicating that the room temperature electronic ground state for the complex is best viewed as *isolated* S = 2 and S = 1/2 spins. Strong antiferromagnetic [Fe^{II}-Cu^{II}] coupling of the mag-nitude present in resting oxidase $(-J \ge 200 \text{ cm}^{-1})$ would, of course, yield a net S = 3/2 state while a strong ferromagnetic interaction would result in an overall S = 5/2 state.

Variable-temperature susceptibility data from 8 to 300 K for the binuclear complexes are displayed in Figure 7 and tabulated in Table II (microfilm). The [Fe^{II}(bipym)Cu^{II}] compound shows a gradual decrease in magnetic moment from 5.46 to 5.15 μ_B by 83 K, but below 80 K the moment decreases rapidly to 4.05 μ_B by 8.3 K. This behavior could be attributed to a moderate antiferromagnetic exchange interaction across the bipym bridge were it not for the fact that the [Fe¹¹-(bipym)Zn¹¹] center exhibits a very similar μ_{eff} vs. temperature pattern where exchange is impossible. In fact, at each temperature, the observed difference in μ_{eff} between the Cu^{II} and Zn^{II} derivatives corresponds almost exactly to that expected due to the additional paramagnetism of an isolated S = 1/2spin. In view of this behavior, the non-Curie behavior at low temperatures for both [Fe¹¹(C₁₈H₁₈N₆)(bipym)M¹¹-(acac)₂](ClO₄)₂ compounds is probably of the same origin, with an Fe^{II} (high-spin) \Rightarrow (low-spin) spin equilibrium and/or zero-field splitting of the S = 2 iron center being likely candidates. While the former explanation is a possibility, especially since the $[Fe^{II}(C_{18}H_{18}N_6)(bipym)]^{2+}$ precursor is low spin. spin-equilibrium systems are relatively rare⁴¹ and available Mössbauer data (vide infra) are inconsistent with this interpretation. Therefore, it appears that zero-field splitting of the isolated S = 2 iron center is the most likely explanation for the observed variable-temperature magnetic behavior. In fact, the data between 10 and 40 K closely resembles that for high-spin



Figure 7. (a) μ_{eff} vs. temperature ($\mathbf{\nabla}$) and $\chi_{M'}$ vs. temperature ($\mathbf{\Theta}$) plots for [Fe^{II}(C₁₈H₁₈N₆)(bipym)Cu^{II}(acac)₂](ClO₄)₂. (b) μ_{eff} vs. temperature ($\mathbf{\nabla}$) and $\chi_{M'}$ vs. temperature ($\mathbf{\Theta}$) plots for [Fe^{II}(C₁₈H₁₈N₆)(bipym)-Zn^{II}(acac)₂](ClO₄)₂.

Table III. Mössbauer Spectral Parameters for the Monomeric and μ -Bipyrimidyl Iron(II) Compounds

compd	δ, mm s ⁻¹ a,b	$\Delta E_{\rm Q}, {\rm mm} {\rm s}^{-b}$
$ \begin{array}{l} [Fe^{II}(C_{18}H_{18}N_6)(CH_3CN)_2](CIO_4)_2 \\ [Fe^{II}(C_{18}H_{18}N_6)(bipym)](CIO_4)_2 \\ [Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2]_{-1} \\ (CIO_4)_2 \end{array} $	0.52 (0.01) 0.60 (0.02) 1.27 (0.03)	1.66 (0.02) 0.66 (0.03) 1.87 (0.04)
$\frac{[Fe^{II}(C_{18}H_{18}N_6)(bipym)Zn^{II}(acac)_2]}{(CIO_4)_2}$	1.26 (0.02)	1.88 (0.03)

^a Spectra were taken at 100 K and referenced relative to a room temperature sodium nitroprusside (SNP) spectrum. ^b Standard deviations in parentheses.

Fe^{II} porphyrin complexes where D (the zero-field splitting parameter) is typically $\gtrsim 5 \text{ cm}^{-1}$.⁴²

Mössbauer Data. The ⁵⁷Fe Mössbauer spectra of A, B, C, and D obtained at 100 K are shown in Figure 8 with the computer-fit isomer shift and quadrupole splitting parameters listed in Table III. Compound A contains low-spin Fe^{II} $(\mu_{eff}(100 \text{ K}) = 0.6 \mu_B)$ and displays an isomer shift of 0.52 which is typical of the spin state.⁴³ The large quadrupole splitting of 1.66 mm s⁻¹ is somewhat unusual, but not necessarily so in view of the tetragonal distortion exhibited by the molecule (Fe-N_(Mac) = 1.90 Å; Fe-NCCH₃ = 1.94 Å).²⁷ Compound B, also low spin, has a spectrum which is more typical of S = 0 iron(11). Both of the μ -bipyrimidyl compounds, C and D, contain high-spin Fe^{II} and possess large δ and $\Delta E_{\rm O}$ values which are characteristic of the S = 2 spin state.⁴³ Furthermore, within experimental error, the [Fe^{II}(bipym)M^{II}] compounds ($M = Cu^{II}$ or Zn^{II}) possess *identical* isomer shift and quadrupole splitting values. This result further collaborates the above electrochemical and magnetic susceptibility data in establishing essentially identical high-spin electronic envi-



 $\begin{array}{l} \label{eq:Figure 8.} \mbox{Figure 8.} {}^{57}\mbox{Fe Mössbauer spectrum at 100 K for (a) [Fe^{II}(C_{18}H_{18}N_6)(CH_3CN)_2](ClO_4)_2; (b) [Fe^{II}(C_{18}H_{18}N_6)(bipym)](ClO_4)_2; (c) [Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_3](ClO_4)_2; (d) [Fe^{II}(C_{18}H_{18}N_6)(bipym)Zn^{II}(acac)_2](ClO_4)_2. \end{array}$



Figure 9. EPR spectrum of $[Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2](ClO_4)_2$ in a CH_2Cl_2 glass at 8 K.

ronments for Fe^{II} within the two compounds, and thus noninteracting $[Fe^{II}-Cu^{II}]$ centers in C. In particular, there is no evidence of a six-line magnetic hyperfine spectrum for the Cu^{II} derivative of the nature that might be present if $[Fe^{II}-Cu^{II}]$ antiferromagnetic exchange were operative.⁴⁴

The Mössbauer spectra for C and D also offer evidence for the absence of high-spin $(S = 2) \rightleftharpoons \text{low-spin} (S = 0)$ spinequilibrium processes which could explain the observed temperature dependency of the magnetic susceptibility. In particular, for the [Fe^{II}(bipym)Zn^{II}] compound, $\mu_{eff}(100 \text{ K}) =$ 4.5 μ_B . Assuming high- and low-spin limiting magnetic moments of 5.1 and 0.5 $\mu_{\rm B}$, respectively, for the two spin states, the low-spin isomer would be $\sim 25\%$ populated for the observed 4.5 µ_B value. Furthermore, assuming that a Mössbauer spectrum similar to that of B would result for a supposed low-spin form of D, it seems likely that the observed spectrum for D would reflect some presence of the low-spin state, 34,45,46 especially by the presence of a peak centered at ~ 0.94 mm s⁻¹. Thus, available information suggests that the observed non-Curie magnetic behavior at low temperature for C and D is not due to a spin-equilibrium process, but lower temperature Mössbauer data are needed to establish this beyond doubt.

EPR Data. The EPR spectrum of C in a CH₂Cl₂ glass at 8 K is shown in Figure 9. The spectrum is typical of magnetically dilute S = 1/2 copper(II), with copper nuclear hyperfine (190 G) seen only on the g_{\parallel} component. In particular, there are no other spectral features identifiable with an S = 3/2 spin state which would be highly populated by 8 K (4.0 μ_B) if an [Fe^{II}-Cu^{II}] antiferromagnetic interaction were responsible for the anomalous low-temperature magnetic susceptibility data. Therefore, when interpreted together, the above susceptibility and Mossbauer data, and the present EPR results, all argue convincingly for noninteracting S = 2 and S = 1/2 spins in C, again leaving the low-temperature magnetic susceptibility behavior of both C and D best rationalized in terms of zerofield splitting of isolated S = 2 iron(II) centers. These S = 2centers are, of course, expected to be EPR silent themselves owing to the relatively large magnitude of $D \ (\gtrsim 5 \ \text{cm}^{-1})$ usually found for high-spin iron(II).42

Finally, it is noted that integration of the EPR signal of Figure 9 accounts for \geq 90% of the copper present in the sample. Thus, there is no appreciable loss of EPR intensity, or relaxation broadening, associated with the presence of the high-spin Fe^{II} center which is possibly capable of a dipolar interaction (⁵T ground state) with the Cu^{II} ion only 6-7 Å away.⁴⁷ Such a dipolar interaction between Cyt a^{3+} and Cu_D²⁺ in cytochrome oxidase has been proposed by Leigh⁴⁸ to be



Figure 10. μ_{eff} vs. temperature ($\mathbf{\nabla}$) and χ_{M}' vs. temperature ($\mathbf{\Phi}$) plots for [(hfac)₂Cu^{II}(bipym)Cu^{II}(hfac)₂]. The solid line is a least-squares fit to the Bleaney-Bowers equation.

responsible for the loss of EPR intensity (without signal broadening or line-shape change)⁴⁹ of the Cu_D^{2+} signal such that the center appears only 80% EPR active. However, as has been recently pointed out by Palmer,⁵⁰ in the case of metalloproteins where EPR resonances are intrinsically broad, due to anisotropy in g, any dipolar field would have to be large with respect to the intrinsic line width if a significant loss in intensity due to the Leigh effect is to be expected. This requirement has frequently led to ridiculously small values of r (the magnitude of the dipolar field is proportional to r^{-3}) for which other effects such as magnetic exchange would almost certainly dominate. The Leigh effect is, therefore, not likely to be as influential for metalloproteins as for simple model compounds with narrower line widths; however, it does not seem to be important for the present [Fe^{II}(bipym)Cu^{II}] complex where the [Fe-Cu] separation is 6-7 Å.47 In contrast, the recently reported nonbridged [Fe^{III}...Cu^{II}] tetrakis(pyridine) capped porphyrin compound of Buckingham et al. is apparently EPR silent for an [Fe-Cu] distance of ~6 Å.51 Whether or not this is due to the Leigh effect or to dipolar relaxation broadening, in general, has yet to be established.

A [Cu^{II}(bipym)Cu^{II}] Center. In order to examine a μ -bipyrimidyl case other than the mixed-metal system, the Cu₂ compound, [(hfac)₂Cu^{II}(bipym)Cu^{II}(hfac)₂], was prepared and characterized (see Experimental Section) to address three questions: (1) Is bipym capable of fostering magnetic exchange in a pure-metal system? (2) If so, how does the magnitude of the exchange compare to that for other known Cu₂ systems with N-heterocyclic bridges, including imidazolate? Finally, (3) with answers to (1) and (2), what can be said about the [Fe^{II}(bipym)Cu^{II}] results as they relate to the possible [Fe^{III}(imid)Cu^{II}] active site structure of cytochrome oxidase?

Variable-temperature (10-300 K) magnetic susceptibility data obtained for $[(hfac)_2Cu^{II}(bipym)Cu^{II}(hfac)_2]$ are shown in Figure 10, with the actual data presented in Table IV (microfilm). As seen from the figure, the $\chi_{M'}$ vs. temperature data are characteristic of an antiferromagnetic exchange interaction. The compound is, of course, an $S_1 = S_2 = \frac{1}{2}$ exchange interacting species and the variation in the molar susceptibility with temperature is represented by the Bleaney-Bowers equation⁵²

$$\chi_{\rm M} = \frac{N\overline{g}^2\beta^2}{kT} \left[\frac{2}{3 + \exp(-2J/kT)} \right] + N\alpha \tag{1}$$

In the equation, N is Avogadro's number, \overline{g} is the average electronic g factor, β is the Bohr magneton, k is the Boltzmann constant, T is the temperature, -J is the parameter which gauges the magnitude of the antiferromagnetic exchange interaction, and $N\alpha$ is the TIP correction. The data in Table IV

Table V. Magnetic Exchange Data for Some Pure- and Mixed-Metal Binuclear Centers with N-Heterocyclic Bridges

compd	bridge	M-M distance, Å	$-J, \mathrm{cm}^{-1}$	ref
$\frac{1}{\left[\operatorname{Cu^{II}(pyz)(NO_3)_2}\right]_2}$	pyrazine	6.71	6	a
$[Cu^{II}(acac)(pz)]^{3+}$	pyrazolyl		35	Ь
$[Cu_{2}^{II}(bpimid)]^{4+}$	imidazolate	6.21 (est)	81.3	15, 16
$[Cu^{II}_{2}(ppimid)(imid)]_{2}$	imidazolate	$6.21 (Cu_1 - Cu_2)$	87.4	15.16
[,(imidazolate	5.91 $(C7_1 - Cu_2)$	30	
$[Cu^{II}(pip)]_{2}(imid)^{3+}$	imidazolate	5.93 (est)	26.1	16
$[Cu^{II}_{2}(Mesdien)_{2}(biimid)](BPh_{4})_{2}$	biimidazolate	5.49	<0.5	18a
$[(bvn)_2ClRu^{[11]}]_2(pvz)$	pyrazine	6.9	~0	с
$[(Cp_2Ti^{III})_2(biimid)]$	biimidazolate	6.02	25.2	18b
$[(Cp_2Ti^{III})_2(bibimid)]$	bibenzimidazolate	6.02	19.5	18b
$[Mn^{11}(TPP)(imid)(THF)]_{\pi}$	imidazolate	6.54	≪8	14
[Fe ^{HI} (TPP)(imid)(THF)] _n	imidazolate	6.5 (est)	<2	14
	Proteins			
superoxide dismutase (Cu ^{II} derivative)	imidazolate	6.0 (est)	26	13
superoxide dismutase (Co ¹¹ derivative)	imidazolate	6.0 (est)	>300	19
cvtochrome oxidase (fully oxidized)	imidazolate (?)	()	≥200	8
cytochrome oxidase (fully oxidized: CN= derivative)	imidazolate (?)		~40	8

^a Juan F. Villa and W. E. Hatfield, J. Am. Chem. Soc., 93, 4081 (1971). ^b C. G. Barraclough, R. W. Brookes, and R. L. Martin, Aust. J. Chem., 27, 1843 (1974). ^c E. C. Johnson, R. W. Callahan, R. P. Eckbag, W. E. Hatfield, and T. J. Meyer, Inorg. Chem., 18, 618 (1979).



Figure 11. An alternative O_2 -bridged structural and catalytic redox model for the active site of cytochrome oxidase.

(mcirofilm) were least squares fit to eq 1, with $N\alpha = 120 \times 10^{-6}$ cgs/mol of dimer, to yield $\overline{g} = 2.12$ and $-J = 7.9 \pm 1.0$ cm⁻¹. This best fit to the susceptibility data is illustrated by the solid lines in Figure 10.

Table V presents a collection of -J values for several synthetic binuclear compounds, many of which contain imidazolate or related bridges with known M-M' distances. For these systems, -J ranges from ~ 0 to 90 cm^{-1} and the present $[(hfac)_2Cu^{II}(bipym)Cu^{II}(hfac)_2]$ compound $(-J = 7.9 \text{ cm}^{-1})$ clearly falls within the lower end of this range. While the exact value of -J is undoubtedly a complicated function of the orbital energies and the geometrical arrangement of the bridge and metal orbitals participating in the exchange interaction, the present [Cu^{II}(bipym)Cu^{II}] results, and most of the data in Table V, imply that heterocyclic bridges such as imid-, biimid²⁻, pyz, and bipym can foster antiferromagnetic exchange interactions, but only with $-J \lesssim 100 \text{ cm}^{-1}$. For the examples in Table V, the only exception appears to be a mixed-metal cobalt(II) SOD derivative where $-J \gtrsim 300 \text{ cm}^{-1}$ has been reported for the [Cu^{II}(imid)Co^{II}] pair; this is a significant result as it relates to cytochrome oxidase (vide infra) and as such it needs to be further substantiated since the work appears to be somewhat ambiguous.¹⁹

An Alternative O2-Bridging Model. Two pieces of magnetochemical evidence support an [Fe^{III}(imid)Cu^{II}] structure for the active site of cytochrome oxidase: (1) the [Cu^{II}(imid)-Co^{II}] SOD result where $-J \gtrsim 300 \text{ cm}^{-1}$ for this mixed-metal imidazolate-bridged species¹⁹ is as large as that measured for the [Fe^{III}-Cu^{II}] exchange interaction in resting oxidase, and (2) the CN⁻ derivative of oxidase has $-J \approx 40 \text{ cm}^{-1,8}$ or a value more in line with the other examples in Table V (cyt a_3^{3+} is low spin in the CN⁻ derivative and high spin in the resting parent enzyme). On the other hand, the present [Fe^{II}-(bipym)Cu^{II}] model compound with $J \approx 0$ is not at all supportive of the imidazolate-bridge structure originally proposed by Palmer et al.⁷ Furthermore, we have preliminary proposed and EPR results in hand⁵³ for an imidazolate-bridged [(TPP)Fe^{III}(imid)Cu^{II}(imidH)DAP]⁺ cation (derived from [Fe^{III}(TPP)Cl]⁺ and [Cu^{II}(imidH)₂DAP]²⁺)⁵⁴ which also indicates -J to be small ($< 20 \text{ cm}^{-1}$) for a case that is a much closer structural analogue of the proposed active site than is the present μ -bipyrimidyl species.

In view of the above conflicting and, as yet, inconclusive evidence for an imidazolate-bridged structure in oxidase, it is important to note that an alternative O_2 -bridged model, as depicted in Figure 11, also possesses considerable merit. This model, as proposed here as a possibility, is not new, but the inorganic precedent in support of such a model has not been fully appreciated until recently.58 Like the imidazolate-bridged model, this O₂-bridging alternative also seems to satisfy all the magnetic and spectroscopic data presently available for the protein,⁴⁰ including some preliminary EXAFS data⁵⁶ which indicated an [Fe···Cu] separation of only ~ 3 Å in resting oxidase (~5 Å is needed for imid⁻). In the fully reduced enzyme in Figure 11, Cyt a_3^{2+} is depicted as a five-coordinate, high-spin Fe^{II} heme with an imidazole ligand located trans to the closely situated Cu_U^+ center with a cavity between the two metals. The parallels between this structure and the heme hydrophobic cavity structure of myoglobin or hemoglobin are both obvious and compelling. Insertion of O₂ between the two metals can then be viewed as producing, via oxidative addition, a transient μ -dioxygen species which further reacts with 2H⁺ to produce H_2O and a stable mixed-metal μ -oxo center. This isolable [Fe^{III}-O-Cu^{II}] form would then be postulated to be the fully oxidized resting form of the enzyme which requires that Cyt a_3^{3+} be high spin and $-J \gtrsim 200 \text{ cm}^{-1}$ for the [Fe^{III}-Cu^{II}] exchange interaction through the oxo (or perhaps hydroxo) bridge. In fact, exchange interactions approaching

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this magnitude are known to be commonplace for synthetic $[Fe^{III}-O-Fe^{III}]$ centers, such as in $[(TPP)Fe]_2O$ where -J is ~150 cm^{-1.59} Furthermore, transient (FeOOFe)* species, reminiscent of the proposed (FeOOCu)* structure for oxidase, are undoubtedly intermediates in the formation of the thermodynamically more stable [Fe¹¹¹-O-Fe¹¹¹] compounds; in fact, one such (FeOOFe)* species has purportedly been detected at low temperatures.⁶⁰ Furthermore, oxyhemerythrin,⁶¹ oxyhemocyanin,⁶² and a recently reported oxyhemocyanin model compound⁵⁴ are also all likely to contain μ -dioxygen centers like that proposed here for the oxidase intermediate. Unfortunately, it is not yet known whether H_2O is a product of the (FeOOFe)* \rightarrow [Fe¹¹¹-O-Fe¹¹¹] oxygen atom extrusion reaction, but in protic media it seems highly likely; by analogy, a similar (FeOOCu)* \rightarrow [Fe^{III}-O-Cu^{II}] extrusion scheme could also produce the required H₂O molecule for oxidase. While Figure 11 shows the imidazole to remain coordinated to iron in the [Fe^{III}-O-Cu^{II}] form, such may not be the case since hematins like [(TPP)Fe]₂O do not strongly react with additional axial ligands, except by cleavage of the [FeIII-O-Fe^{III}] bridge. Upon formation of the CN⁻ derivative, Cyt a_3^{3+} converts to low spin and our susceptibility measurements have shown that -J drops to ~ 40 cm⁻¹ as a function of this change in spin state.^{8,40} In the O₂-bridged model, an attractive structure for this low-spin CN^- derivative would be [(NC)-Fe^{III}-O-Cu^{III}] with a $\dot{C}N^{-}$ ligand substituted for the weaker field imidazole; or alternatively, CN⁻ may actually cleave and replace the oxo bridge, i.e., [Fe¹¹¹-(CN)-Cu¹¹]. Finally, the catalytic redox cycle for the O₂-bridging model would be completed by a concomitant 2e⁻ reduction of the [Fe^{III}-O- Cu^{II}] unit (2e's from reduced Cyt a^{2+} and Cu_{D}^{+}) and a second oxygen atom extrusion-protonation reaction to again generate a H₂O molecule and conclude the observed overall 4e⁻ reduction of $O_2: O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$. This final step in the proposed mechanism is supported by the fact that the 2e⁻ electrochemical reduction of [(TPP)Fe]₂O has been shown to result in reductive cleavage of the oxo bridge with the production of [(TPP)Fe^{II}] and [(TPP)Fe^{II}OH]^{-.63}

Clearly, a modeling approach which places redox-active iron and copper centers in close proximity continues as a strong synthetic challenge, but one that promises major rewards in elucidating mechanistic details of the catalytically important $O_2 \rightarrow H_2O$ reaction of cytochrome oxidase.

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Supplementary Material Available: Variable-temperature magnetic susceptibility data for $[Fe^{II}(C_{18}H_{18}N_6)(bipym)Cu^{II}(acac)_2](ClO_4)_2$ (Table II) and [(hfac)₂Cu^{II}(bipym)Cu^{II}(hfac)₂] (Table IV) (3 pages). Ordering information is given on any current masthead page.

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Volatility and Molecular Structure of Neptunium(IV) Borohydride

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Abstract: The structure of Np(BH₄)₄ was determined by single-crystal X-ray diffraction methods at 130 K. The crystals are tetragonal, space group $P4_2/nmc$; a = 8.559 (9) Å, c = 6.017 (9) Å, Z = 2. R = 0.114 for 352 reflections (Mo K α radiation). The four borohydride ions are disposed tetrahedrally about the Np with Np-B distances of 2.46 (3) Å. The boron atoms are connected to the Np atom with triple hydrogen bridge bonds similar to the terminal borohydrides in uranium borohydride. Hydrogen atoms were observed in the Fourier maps and refined. This molecular structure is similar to that found for hafnium and zirconium borohydride, but the space group is different. The vapor pressure, 10 mmHg at 25°C, was measured in the range of -5 to 25 °C using a Bourdon gauge.

Introduction

The relationship between the physical properties and structures of metal borohydrides has been studied extensively since the first preparation of metal compounds containing the BH_4^- anion.² One property which has attracted attention is the high volatility exhibited by some of them.^{3,4} No univalent metal borohydride is volatile and only a few of the lighter bisand trisborohydrides show volatility below room temperature.5 Tetravalent Th, Pa, and U form borohydrides of low volatility, while Zr, Hf, Np, and Pu give tetrakisborohydrides of greater volatility.

The crystal structures of these seven compounds fall into three types. Thorium,^{3a} protactinium,⁴ and uranium^{6,7} borohydrides show one structure type which has been studied by X-ray and neutron diffraction methods for U(BH₄)₄. The 14-coordinate uranium is surrounded by six BH₄⁻ groups, two of which are triply hydrogen bridge bonded to the metal with a U-B distance of 2.53 Å. The other four borohydride groups are bonded to the uranium by double hydrogen bridges giving a longer U-B distance of 2.87 Å. These four bridging BH₄⁻⁻ units use their remaining two hydrogens to link adjacent U atoms in a polymeric structure of C_2 symmetry at uranium.

Neutron-diffraction results on Hf(BH₄)₄⁸ and an X-ray diffraction study on the isostructural $Zr(BH_4)_4^9$ have shown that these molecules crystallize into a different structure type and are monomeric. The metal atom is bonded to four tetrahedrally arranged borohydride groups by triple hydrogen bridge bonds giving the molecule rigorous T_d symmetry. The coordination sphere around Zr or Hf consists of only 12 hydrogen atoms. The corresponding Zr-B and Hf-B distances are 2.34 (3) and 2.28 (1) Å, respectively.^{8a,9}

The third type,⁴ displayed by $Np(BH_4)_4$ and $Pu(BH_4)_4$, is somewhat similar to that of zirconium and hafnium borohydrides and is discussed here in detail.

In order to put the volatility of the third structure type on a quantitative basis, vapor-pressure measurements of $Np(BH_4)_4$ were carried out as a function of temperature, yielding various thermodynamic quantities. An X-ray crystallographic investigation of Np(BH₄)₄ at 130 K was undertaken to determine its detailed molecular structure.

Experimental Section

Vapor-Pressure Measurement. ²³⁷Np(BH₄)₄ was prepared and purified as described previously.⁴ The extreme chemical reactivity of Np(BH₄)₄ precluded the use of a mercury manometer and only an all-glass-and-Teflon Bourdon gauge was found satisfactory for these measurements. The exposed surfaces of the gauge were passivated with the neptunium borohydride vapor prior to use to minimize a decomposition reaction which evolves hydrogen and diborane.⁴ Excess $Np(BH_4)_4$ was condensed into the passivated gauge and the gauge was pumped out at -78 °C. At various temperatures, the compound was allowed to vaporize and deflect a pointer, whose initial position was precisely determined by a cathetometer. Shortly after equilibrium was established, the pointer was nulled to its original position by pressurizing the outer chamber of the gauge with argon. The argon pressure, as measured by a standard pressure gauge, then equaled the vapor pressure of the $Np(BH_4)_4$. Care was taken to remove all traces of H_2 and B_2H_6 before and after each measurement. Vapor-pressure readings were taken at room temperature and below until the vapor pressure of the compound approached the sensitivity of the Bourdon gauge.

Crystal Preparation. A sample of the dark green liquid $Np(BH_4)_4$ was sealed into a 0.3 mm i.d. quartz capillary and stored in liquid nitrogen prior to use. The capillary was mounted on a Picker FACS-I